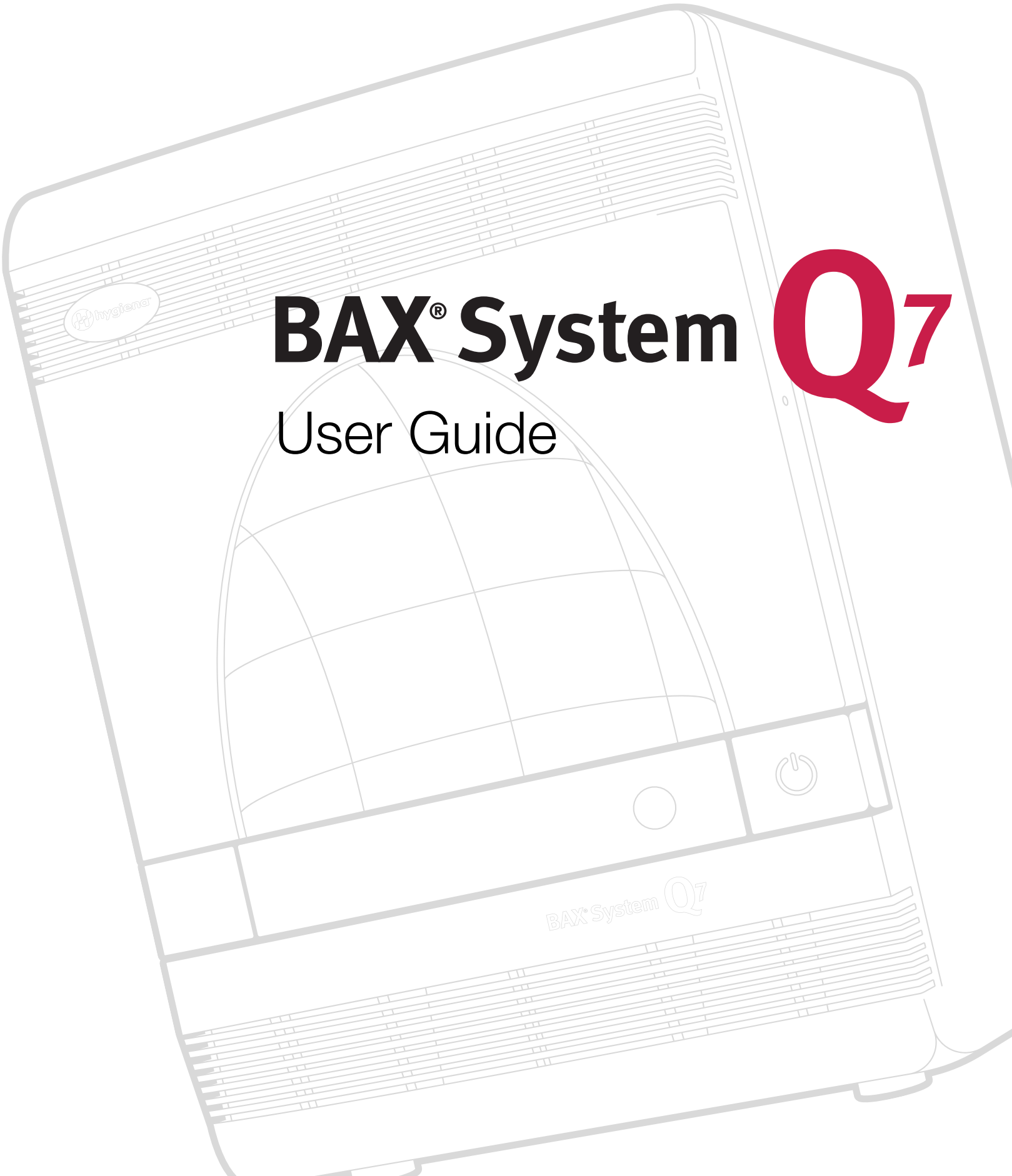
hygiena™

BAX® System Q7

User Guide



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FIELD OF USE
Data obtained from the BAX® System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The BAX® System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Use for research and development, quality assurance and quality control under supervision of technically qualified persons. Read the Limitation of Warranty and Liability before using product.

LICENSING

MATHWORKS
MATLAB 1984-2005 The MathWorks, Inc. is deployed with the BAX® System application and governed by a certain license agreement between Hygiena and MathWorks. License agreement files (MathWorks or Third-Party) that are included with the MCR Libraries ("MCR Library License") may not be modified or removed.

APPLIED BIOSYSTEMS
BAX® SYSTEM Q7 INSTRUMENTS ARE SOLD UNDER LICENSING ARRANGEMENT WITH APPLIED BIOSYSTEMS LLC FOR FOOD TESTING, ENVIRONMENTAL TESTING AND INDUSTRIAL MICROBIOLOGY APPLICATIONS.

The BAX® System real-time PCR assays for Campylobacter, Staphylococcus aureus and Vibrio are licensed 5' nuclease application kits. Their purchase price includes a limited, non-transferable right to use only this amount of the product in the practice of the 5' nuclease process solely for food testing, environmental testing and industrial microbiology applications. No right under any other patent claim (such as apparatus or system claims) and no right to use this product for any other purpose, is hereby granted expressly, by implication or by estoppel. Further information on purchasing licenses may be obtained by contacting outlicensing@lifetech.com.

THERMO FISHER SCIENTIFIC
The SYBR® Green I stain in some BAX® System PCR assays is sold under license from Life Technologies Corporation, a Thermo Fisher Scientific Company, for use only in homogenous polymerase chain reaction assays in combination with the DNA detection technology of Hygiena, and is covered by pending and issued patents.

DXS LTD.
The Scorpion®® Technology in some BAX® System real-time PCR assays is sold under licensing arrangement with DXS Ltd. for food and beverage testing.

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PROMEGA CORPORATION
GoTaq® Hot Start Polymerase in some BAX® System PCR assays is sold under distribution agreement with Promega Corporation. GoTaq® Hot Start Polymerase is manufactured by and licensed to Promega Corporation under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents and sold to Hygiena for distribution.

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Preface

The BAX® System and this *User Guide* have been designed for use in quality control/assurance laboratories of food processing and related industries.

User Assumptions

- Users are qualified lab personnel who follow good microbiology laboratory practices, including the safe handling and disposal of potentially pathogenic materials.
- Users are familiar with Microsoft® Windows® operating system, including navigation and backup procedures.

Text Conventions

This manual uses the following text conventions to indicate software commands/selections/messages:

- Menu selections appear in UPPER CASE separated by > to indicate sequence.
- Field names and screen names appear in **bold lower case**.
- Screen buttons appear in upper case in a **BOX**
- System messages appear in “quotes.”
- *Notes appear in italics*.
- Safety symbols are detailed in Chapter I, but the main categories are as follows:

General alert or warning

Live power may still be available even with power off or failed fuse.

Hot surface! Avoid touching.

I. BAX® System Method Overview

About the BAX® System

The BAX® System is an automated molecular method for detecting microbes in food and environmental samples. The BAX® System combines speed and ease of use with unprecedented performance to give you fast, accurate and reliable results.

Traditional screening methods are based on bacterial traits or behavior, such as antibody response, which can present problems with cross-reactivity of related organisms. The BAX® System, however, focuses on the actual genetic structure of bacteria by detecting a unique DNA fragment found only in the target organism.

The BAX® System was the first molecular food testing method to use Polymerase Chain Reaction (PCR) technology, which rapidly creates millions of copies of the targeted DNA fragment, if present. Thus, you get clearly detectable "yes-or-no" answers within hours of starting the assay, without the need for expert interpretation. The BAX® System Q7 series provides additional capabilities for more information with selected tests, such as faster processing time, multi-target analysis in the same tube, species differentiation and quantitative results.

The BAX® System simplifies PCR in your lab. All necessary reaction reagents (primers, polymerase, nucleotides and positive control) are already combined into a single tablet, conveniently packaged inside the PCR tubes you receive with each assay. This eliminates the multiple liquid transfers used in other methods and effectively reduces the potential for errors caused by operator technique. The proprietary tablets also allow for efficient processing of large numbers of samples, up to 96 tests in a single batch.

The automated BAX® System combines PCR with fluorescent detection to significantly reduce hands-on time, minimize the potential for cross-contamination, and provide for consistent results based on computerized algorithms for analysis. You simply load your prepared samples, run the program and read the results on screen.

This *User Guide* describes how to use the BAX® System when screening food or environmental samples for targeted organisms. We would like to hear from you and welcome comments on these products or this *User Guide*.

About the Polymerase Chain Reaction (PCR)

PCR is an analytical tool for quickly replicating a targeted DNA fragment. In a typical application, sample DNA is combined with DNA polymerase, nucleotides and primers that are specific for a given nucleotide sequence. This mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognize and anneal (bind) to the targeted DNA sequence. Taq polymerase then uses the nucleotides to extend the primers, thus creating two copies of the targeted DNA fragment (amplification). Repeating the cycle of denaturing, annealing, and extending produces an exponential increase in the number of target DNA fragments, creating millions of copies in a very short time. If the target sequence is not present, no detectable amplification takes place.

About the BAX® System Q7 Instrument

Your purchase of the BAX® System includes the Q7 instrument with a computer workstation operating on a Microsoft Windows® platform. The workstation includes removable media drives, monitor, keyboard, mouse and cables. The BAX® System application is already loaded and ready for use.

Note: *Although the instrument and its peripherals can be connected to a network, Hygiena cannot provide technical support for problems that arise from using this workstation on a network. Furthermore, Hygiena cannot warrant BAX® System results if this computer is used with other, potentially incompatible, software.*

BAX® System Q7 hardware

Item	Specification
Dimensions	14" wide x 18" deep x 20" high (34 x 45 x 49 cm)
Weight	Approx. 75 pounds (34.1kg)
Power usage	950 watts
Nominal current draw	8 A
Power requirements	100-240 VAC ±10%, 50-60 Hz ±1%, 15 amp circuit, grounded outlet
Fuses	Two 12.5 A, 250 V, 5 x 20 mm
Filters	5 excitation, 5 emission
Halogen bulb	12 V, 75 W
Thermal range	4-100°C
Thermal homogeneity	±1.0°C well-to-well within 30 seconds of reaching 60°C
Thermal accuracy	±0.5°C (35°C - 95°C) 3 minutes after reaching set point temperature
Ramp speed	±1.6-3.5°C per second
Warm up time	5 minutes or less from 25°C start
Sound	65 decibels maximum
Room environment	Altitude at or below 2000 m (6500 ft) above sea level Temperature 10-35°C (50-95°F) Relative humidity 20-80% (non-condensing) Locate away from heaters, cooling ducts, and out of direct sunlight. Protect from accidental spills. Non-conductive pollutants only
Sample throughput	1-96 tests per batch, time dependent on assay
Delivered with system	Computer workstation with CD read/write drive, monitor, keyboard, mouse and cables Microsoft® Windows® operating system and BAX® System application BAX® System documentation package

Symbols and Labels

Electrical Symbols

Symbol	Description
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels

English	Francais
CAUTION Hazardous chemicals. Read the Safety Data Sheets (SDS) before handling.	ATTENTION Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	ATTENTION Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to SDS and local regulations for handling and disposal.	ATTENTION Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	AVERTISSEMENT Lampe brûlante
WARNING This instrument is designed for 12V, 75W Halogen lamps only.	AVERTISSEMENT Cet instrument est concu pour des lampes d’halogéne de 12V et 75W seulement.
CAUTION Hot surface.	ATTENTION Surface brûlante.
DANGER High voltage.	DANGER Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Hygiena qualified service personnel.	AVERTISSEMENT Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L’instrument ne contient aucune pièce réparable par l ’ utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Hygiena.
CAUTION Moving parts.	ATTENTION Parties mobiles.

Safety Considerations

General Instrument Safety

Moving and lifting the instrument	**CAUTION - PHYSICAL INJURY HAZARD.** If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.
Moving and lifting stand-alone computers	**WARNING** - Do not attempt to lift or move the computer without the assistance of others. Depending on the weight of the computer, moving it may require two or more people. Things to consider before lifting the computer: • Make sure that you have a secure, comfortable grip on the computer when lifting. • Make sure that the path from where the object is to where it is being moved is clear of obstructions. • Do not lift an object and twist your torso at the same time. • Keep your spine in a good neutral position while lifting with your legs. • Participants should coordinate lift and move intentions with each other before lifting and carrying. • Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.
Operating the instrument	Ensure that anyone who operates the instrument has: • Received instructions in both general safety practices for laboratories and specific safety practices for the instrument. • Read and understood all applicable Safety Data Sheets (SDS). **WARNING - PHYSICAL INJURY HAZARD**. Use this instrument as specified by Hygiena to avoid personal injury or damage to the instrument

Electrical Safety

Moving and lifting the instrument	**DANGER - ELECTRICAL SHOCK HAZARD**. Severe electrical shock can result from operating the BAX® System Q7 without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
Fuses	**DANGER - FIRE HAZARD**. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory. **WARNING - FIRE HAZARD**. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.
Power	**DANGER - ELECTRICAL HAZARD**. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected. **DANGER - ELECTRICAL HAZARD**. Use properly configured and approved line cords for the voltage supply in your facility. **DANGER - ELECTRICAL HAZARD**. Plug the system into a properly grounded receptacle with adequate current capacity
Over-voltage rating	The BAX® System Q7 has an installation (over-voltage) category of II and is classified as portable equipment.

Physical Hazard Safety

Moving parts	**WARNING - PHYSICAL INJURY HAZARD**. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument. **DANGER - PHYSICAL INJURY HAZARD**. Do not operate the instrument with its door open. Keep hands out of the sample block area when the instrument is running.
Lamp	**WARNING - PHYSICAL INJURY HAZARD**. The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective gloves before handling it.
Ultraviolet Light	**WARNING - ULTRAVIOLET LIGHT HAZARD**. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing

Safety and Electromagnetic Capability (EMC) Standards

U.S. and Canadian Safety Standards	This instrument has been tested to and complies with standard UL 3101-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements."
c UL US	This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."
Canada EMC Standard	This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.
European Safety and EMC Standards CE	**Safety** This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements," and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials." **EMC** This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."
Australian EMC Standards	This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Required Materials

BAX® System Kits	Contents
Standard and Real-Time PCR Assays (96-test kits)* *STEC Panel 1 and 2 assays are sold as 48-test kits. **_Cronobacter_ assay uses its own ES Lysis Buffer.	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
Real-Time PCR Assays for Genus *Listeria* and *L. monocytogenes* (96-test kits)	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
24E PCR Assays for Genus *Listeria* and *L. monocytogenes* (96-test kits)	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 1 (3 mL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
PCR Assay for Yeast and Mold (96-test kit and supplement kit)	• YM Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96) **Supplement Kit:** • Yeast and Mold disrupter tubes (96) • DNA stabilizer (2 x 1 mL)
BAX® System Media	**Contents**
BAX® System MP Media *(Previously BAX® System Media for E. coli O157:H7 MP)*	Available in 2.5-kg tubs or StatMedia™ soluble packets (33.75g)
BAX® System Media for *Listeria*	Available in 2.5-kg tubs
24 LEB enrichment broth for 24E assays	24 LEB Complete* - available in 2.5-kg tubs (Oxoid CM1154 or MED2005 or D14654989) Buffer supplement - available in packages of 20 tubes each (Oxoid BO1204K or MED2000 or D15407304) or 10 bottles of 100 mL each (Oxoid BO1204M) **This media can also be purchased as 24 LEB base (500-g bottles; Oxoid CM1107 or MED2004 or D13921126) and selective supplement (10-vial packs; Oxoid SR0243 or MED2015 or D13921133)*
Buffered Peptone Water	Buffered Peptone Water - available in 2.5-kg MED2010 (D15452608) and 500 gram tubs MED2011 (D15452596)

Required Materials (Continued)

BAX® System Equipment/Supplies	Contents
Start-Up Package (equipment and supplies for 192 tests)	**Equipment:** • BAX® System Q7 instrument • Computer workstation • Heating blocks with inserts capable of maintaining temperatures within ±2°C or ±3°C (2)* • Cooling blocks with inserts (3)* • Capping/decapping tools (2) • Adjustable mechanical pipettes (2) • Repeating pipette (1) • Multi-channel pipette (1) **Supplies:** • Cluster tubes with caps and racks • Pipette tips with barriers (100 µL, 250 µL, 5.0 mL) • Powder-free nitrile gloves
Additional equipment and supplies – customer-supplied	• Homogenize to mix samples between 200-260 rpm (or equivalent) • Filtered homogenize bags (unless otherwise specified) • Incubators capable of maintaining temperatures within ±2°C** • Cell disrupter device (2.0 mL) for yeast and mold • Hypochlorite bleach (10% household solution) • Standard solutions, consumables and media *Uninterruptible power source for BAX® System instrument and/or computer – 1980 Watts / 2200 VA output (optional)* *Line noise filter - 2.2 kVa (optional)* *Surge protector – 10 kVa (optional)* • Refrigerator for storing kits (ability to hold temperature from 2-8°C) • Laboratory balance for weighing media and samples - 5kg capacity • pH measurement device for measuring media pH

** The BAX® System Start-Up Package is available with either separate heating and cooling blocks or the Automated Thermal Block.*
*** Health Canada and AFNOR Certifications standards require an incubator capable of maintaining ±1°C*

A Note on Heating Block Temperature Stability

The heating blocks provided with the BAX® System Start-Up Package demonstrate a temperature stability of ±2°C across the block when set at 37-55°C and a stability of ±3°C across the block when set at 95°C. These heating blocks have been used reliably in both internal and third-party validation studies and are approved by Hygiena and third-party organizations such as AOAC as part of the BAX® System method.

A Note on Heating Block Temperature Stability (Continued)

These temperature variances mean that when the temperature of the heating block is set to 37°C (or 55°C) according to the BAX® System method, the actual temperature of a single well in the heating block may read between 35-39°C (or 53-57°C) without affecting the method performance.

When the temperature of the heating block is set to 95°C, the actual temperature of a single well in the heating block may read between 92-98°C without affecting the method performance.

Before using any heating blocks with the BAX® System method, verify that the blocks are capable of maintaining a temperature stability of ±2°C when set at 37-55°C and a stability of ±3°C when set at 95°C.

Materials Handling, Storage and Disposal

Precautions

Cycler/Detector

The instrument requires a constant supply of air that is 31°C or cooler in order to remove heat generated by operation. If the air supply is inadequate or too hot, the machine can overheat, causing performance problems, software error messages, and even automatic shutdowns. Please see the guidelines for installation in Chapter VII: BAX® System Hardware.

The cycler/detector can generate enough heat to inflict serious burns and can deliver strong electrical shocks if not used according to the directions in this manual. Please read the safety considerations in Chapter I: BAX® System Method Overview before using this instrument for the first time.

Reagents and Supplies

The BAX® System method includes sample preparation enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria. In particular, individuals who are pregnant or immunocompromised should avoid any potential contact with *Listeria monocytogenes*.

Reagents used with the BAX® System assays should pose no hazards when used as directed. Before using this product, please review the Safety Data Sheets (SDS), available on Hygiena's website. Refer to your site practices for safe handling of materials at extreme temperatures.
www.hygiena.com

Storage

Reagents should be used by the expiration date stamped on the individual labels.
Reagent packages should be kept refrigerated at 2–8°C. Do not freeze.
If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the mylar bag of PCR tubes into a larger bag with desiccant or store at 4°C in a desiccation unit, if possible.

Note: *Storage of PCR tubes with desiccant is particularly important for real-time assays.*

After protease has been added to the lysis buffer, shelf life of the solution is two weeks when stored at 2-8°C.

Diluted Lysing Agent 1, used with the 24E assays, can be stored up to 6 months at 20-30°C.

After combining diluted Lysing agent 1 and Lysing agent 2, the mixture must be used within 4 hours.

After protease and Lysing Agent 2, used with the real-time Listeria assays, have been added to the lysis buffer, shelf life of the solution is one week when stored at 2-8°C.

Cooling blocks should be kept refrigerated at 2-8°C and used within 30 minutes of removal from refrigerator.

Pipettes should be calibrated to deliver within 10% of required volumes. Barrier tips are recommended for all pipettes.

Please see the manufacturer's documentation for handling, disposal and storage of the pipettes, computer system and other equipment.

Disposal

Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations.

For additional recommendations about preventing, identifying and removing PCR contamination, see Appendix B: PCR Contamination Control.

Technical Assistance

If you have any questions or comments on the BAX® System, contact your distributor for technical assistance.

You can also contact Hygiena Diagnostics Support directly by phone at +1 800-863-6842 or by email at diagnostics.support@hygiena.com.

II. Protocols for Standard Assays

II. Protocols for Standard Assays

About End-Point Detection

The BAX® System PCR tablets in standard assays contain fluorescent dye, which binds with double-stranded DNA and emits a fluorescent signal in response to light. After amplification, the BAX® System begins a detection phase where the fluorescent signal is measured. During detection, the temperature of the samples is raised to the point where the DNA strands separate (denature), releasing the dye and lowering the signal. This change in fluorescence can be plotted against temperature to generate a melting curve, which is interpreted by the BAX® System software as positive or negative results.

Assay Validation

The protocols in this chapter have been validated by Hygiena and certified by organizations such as AOAC, AFNOR, Health Canada or NordVal, for the sample types listed. Many food types use standard enrichment protocols. However, certain food types have been validated using specific media or enrichment protocol modifications.

In order to follow the methods approved by AOAC or AFNOR Certification, the validated enrichment protocols described in this user guide and the kit instructions insert must be followed.

For many sample types, reference enrichment protocols (such as the USDA-FSIS, FDA-BAM or ISO methods) may also be used. These enrichment protocols should be internally validated before use with the BAX® System, as these methods have not been validated by Hygiena or any third-party organizations. To test a sample type that is not listed, please contact Hygiena Diagnostics Support for recommendations on enrichment and sample preparation.

The reference method enrichment protocols described in this chapter reflect those in use at the time of the assay's validation. Because these may have changed since that time, you should always refer to the appropriate reference method source for the most current enrichment protocol.

Reference method source	Link
USDA-FSIS Microbiology Laboratory Guidebook	http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
FDA Bacteriological Analytical Manual	http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
Health Canada Compendium of Analytical Methods	http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php
AOAC	http://www.aoac.org
International Organization for Standardization (ISO)	http://www.iso.org

Before You Begin

Review all best practices, tips and techniques described in Chapter VI: BAX® System Best Practices before beginning any BAX® System protocol.

Protocol Overview Flowchart

Enrichment

- Combine sample with enrichment media
- Incubate samples for the appropriate enrichment time
- If necessary, transfer sample to pre-warmed secondary enrichment media and incubate samples for the appropriate enrichment time

Sample Prep

- Prepare lysis reagent (150 µL protease + 12 ml lysis buffer)
- Transfer 200 µL prepare lysis reagent to cluster tube
- Add 5 ul sample to cluster tube of prepared lysis reagent*

*For *E.coli* MP assay using MP media, add 20 µl sample

(Lysing steps can be performed using the automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions)

Lysis

- Heat tubes at 37°C for 20 minutes*
- Heat tubes at 95°C for 10 minutes
- Cool tubes for 5 minutes

*For *Listeria* and *L.mono* assays, heat tubes at 55°C for 60 minutes

Processing

- Place PCR tubes into a chilled cooling block*
- Transfer 50 µL lysate to PCR tube
- Load samples into BAX® system cycler and run full process
- Read results in about 3.5 hours

*For *E. coli* MP assay, use frozen (-18 to -22°C) cooling block

E. coli O157:H7 MP Assay

1. Enrich Samples

Part No. KIT2004

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 050501

*The **AOAC Research Institute** has certified the BAX® System for detecting E. coli O157:H7 in raw ground beef, beef trim, spinach and lettuce.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Raw ground beef	**For 25 g** – Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours. **For 65 g** – Homogenize 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.
Beef trim	Gently massage 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 8-24 hours.
Spinach and lettuce	Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.

1. Enrich Samples (Continued)

QUA 18/04-03/08
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System MP assay according to the NF VALIDATION rules for detecting E. coli O157:H7 in raw beef, raw pork, ovine and chicken meats, raw milk, fruits and vegetables ready to eat and ready to reheat dishes.*

Sample type	Enrichment
Raw beef meats	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.
Raw milk, fruits and vegetables, ready to eat and ready to reheat dishes, raw pork, ovine and chicken meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) mTSB broth supplemented with novobiocin (20 mg/L). Incubate at 41.5°C for 18-24 hours.

Health Canada has certified the BAX® System MP assay as MFLP-30 for detecting *E. coli* O157:H7 in raw beef, dairy, meat, fruits, vegetables, dry foods, and animal feed with a modified enrichment protocol. See the Health Canada website to request details and protocols.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details). There are two target choices when testing for *E. coli* O157:H7 using the MP assay:
   - *E. coli* O157:H7 MP – for single or mixed target batches.
   - *E. coli* O157:H7 MP Express – for faster processing of single target batches.

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.
   a. For samples enriched in BAX® System MP media, transfer 20 µL enriched sample to cluster tubes.
   b. For all other samples, transfer 5 µL enriched sample to cluster tubes.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a frozen (-20°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the freezer.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue; remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

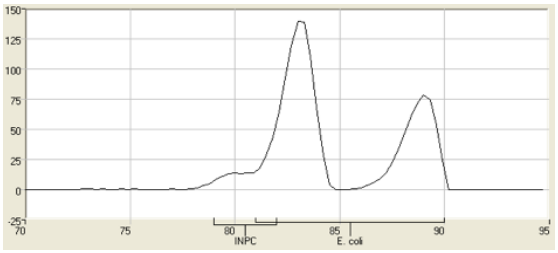
5. Review Results – Standard MP Protocol

Note: *Results were obtained using a frozen (-20°C) cooling block.*

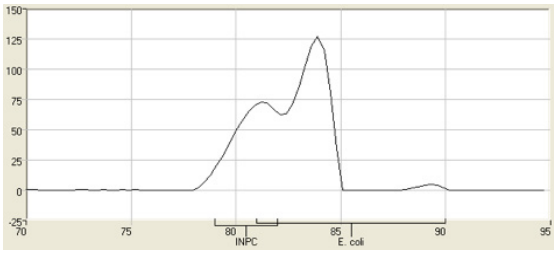
Positive *E. coli* O157:H7 MP Melting Curve Profiles

- Two target peaks: one at range 82 to 84°C and the other at range 87 to 89.8°C.
- Target peak range 81 to 90°C.

Control peak range 79 to 82°C. A small peak may be seen at 75-76°C; its presence does not affect results.



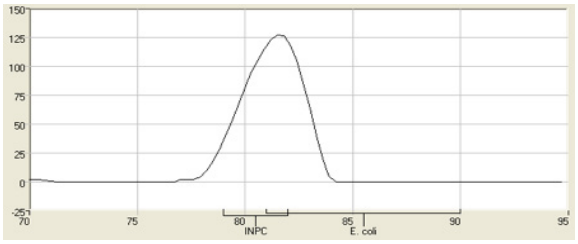
Strong *E. coli* O157:H7 MP positive



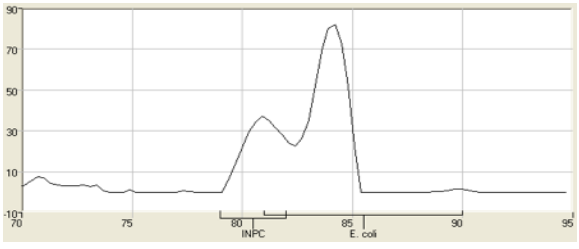
Weak *E. coli* O157:H7 MP positive

Negative *E. coli* O157:H7 MP Melting Curve Profiles

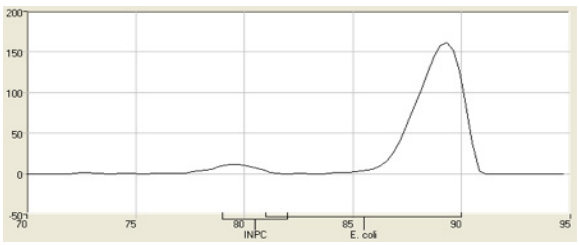
Results for the MP assay are negative when either or both of the peaks in the target range are missing; control peak is present.



Peak 1	Positive
Peak 2	Negative
Peak 3	Negative



Peak 1	Positive
Peak 2	Positive
Peak 3	Negative



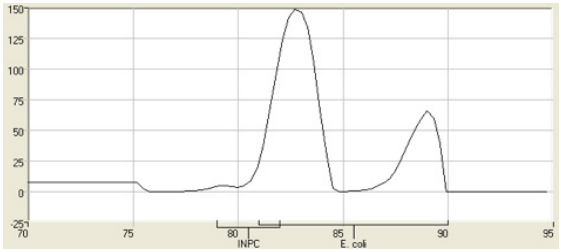
Peak 1	Positive
Peak 2	Negative
Peak 3	Positive

5. Review Results - MP Express Protocol

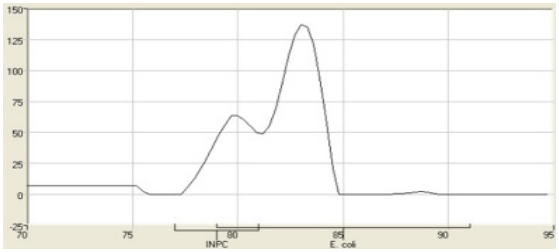
Note: *The melt curve will sometimes show a dip before the control peak; this dip is an artifact of the software algorithm and has no effect on results.*

Positive *E. coli* O157:H7 MP Express Melting Curve Profiles

- Two target peaks: one at range 82 to 84°C and the other at range 87 to 89.8°C.
- Target peak range 81 to 90°C.
- Control peak range 79 to 82°C. A small peak may be seen at 75°C; its presence does not affect results.



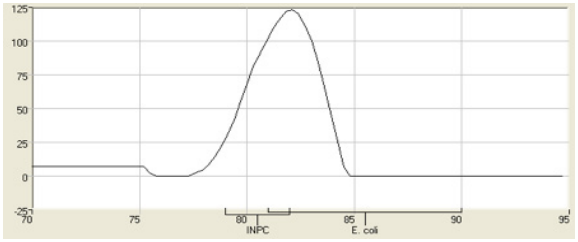
Strong *E. coli* O157:H7 MP Express positive



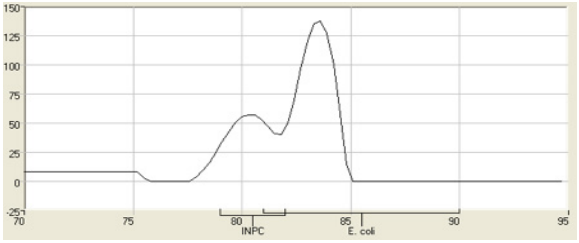
Weak *E. coli* O157:H7 MP Express positive

Negative *E. coli* O157:H7 MP Express Melting Curve Profiles

Results for the MP Express assay are negative when either or both of the peaks in the target range are missing; control peak is present.

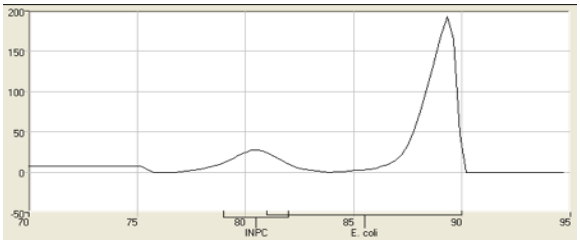


Peak 1	Positive
Peak 2	Negative
Peak 3	Negative



Peak 1	Positive
Peak 2	Positive
Peak 3	Negative

Note: *The melt curve will sometimes show a dip before the control peak; this dip is an artifact of the software algorithm and has no effect on results.*



Peak 1	Positive
Peak 2	Negative
Peak 3	Positive

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Cronobacter (*E. sakazakii*) Assay

The organism *E. sakazakii* has been reclassified as the genus *Cronobacter*. Internal studies were performed at the time of reclassification to demonstrate that this assay accurately detects organisms now classified as *Cronobacter* species.

1. Enrich Samples

Sample type	Primary Enrichment	Secondary Enrichment
Powdered infant formula*	Homogenize samples 1:10 with pre-warmed (37°C) BPW. Incubate at 37°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Powdered infant formula*, dry dairy and soy ingredients, environmentals	Homogenize samples 1:10 with pre-warmed (45°C) mLST broth with vancomycin. Incubate at 45°C for 20-22 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Environmental sponges	Homogenize sponge with 190 mL pre-warmed (35°C) BPW. Incubate at 35°C for 18 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

* Although either enrichment protocol may be used for powdered infant formula, primary enrichment in BPW is recommended for this food type.

Health Canada has certified this BAX® System assay as MFLP-27 for detecting *Cronobacter* in selected foods, including powdered infant formula, dry dairy and soy ingredients, and food production environmental samples after enrichment. See the Health Canada website to request details and protocols.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of ES lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

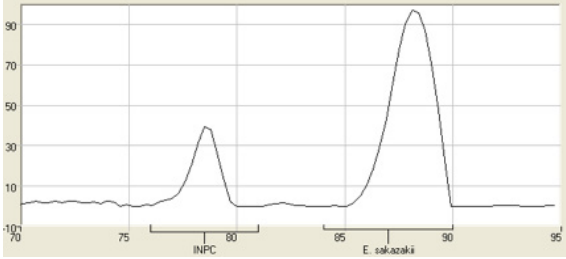
3. Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

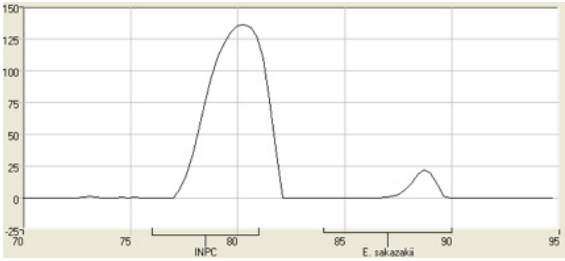
5. Review Results

Positive *Cronobacter* Melting Curve Profiles

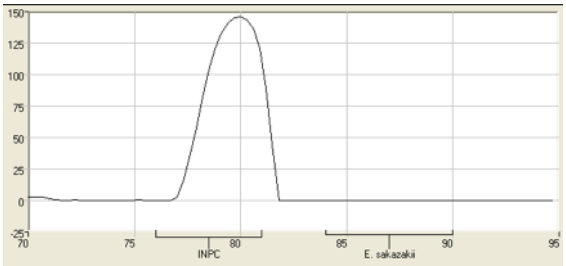
- One target peak at approximately 87°C.
- Target peak range 84 to 90°C.
- Control peak range 76 to 81°C.



Strong *Cronobacter* positive



Weak *Cronobacter* positive



Negative *Cronobacter* Melting Curve Profile

No target peaks; large control peak is present.

Cronobacter negative

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual.

To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Genus *Listeria* Assay

1. Enrich Samples

Part No. KIT2016

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 030502

*The **AOAC Research Institute** has certified the BAX® System for detecting Genus Listeria on environmental surfaces (plastic, ceramic tile, rubber, painted and unpainted wood, sealed concrete, cast iron, and air filter material) and on foods (dairy, meat, fish and vegetables).*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System Listeria media		
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with 10 mL D/E Neutralizing Broth or equivalent. Homogenize sponge with 190 mL pre-warmed (36°C) BAX® System *Listeria* media broth. Incubate at 36°C for 26-30 hours.	None
Environmental swabs	Sample a 1 x 1 in (2.5 x 2.5 cm) environmental area with a swab pre-moistened with 300-500 µL D/E Neutralizing Broth or equivalent. Add swab to 10 mL pre-warmed (36°C) BAX® System *Listeria* media broth. Incubate at 36°C for 22-26 hours.	None
Standard Media		
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with D/E Neutralizing Broth. Homogenize sponge with 190 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Frankfurters	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

1. Enrich Samples (Continued)

Standard Media		
Smoked salmon	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Spinach and other vegetables	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Cheese	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

Health Canada has certified this BAX® System assay as MFLP-15 for detecting *Listeria* species from environmental surfaces. See the Health Canada website to request details and protocols.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

3. Perform Lysis (Continued)

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 60 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

Note: *These steps can also be performed using the Automated Thermal Block. See the Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

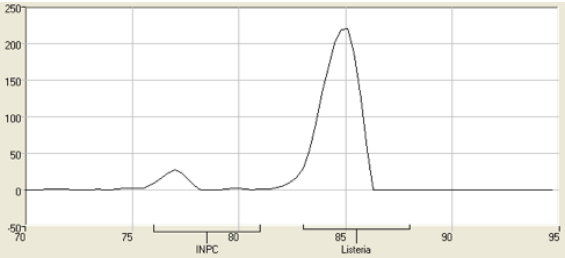
3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

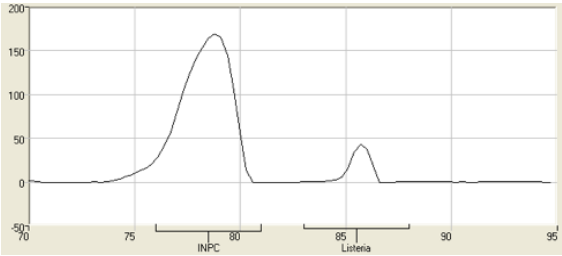
5. Review Results

Positive Genus *Listeria* Melting Curve Profiles

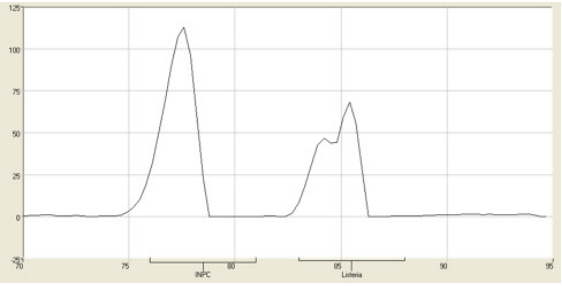
- One target peak at approximately 85.5°C.
- Target peak range 83 to 88°C.
- Control peak range 76 to 81°C.



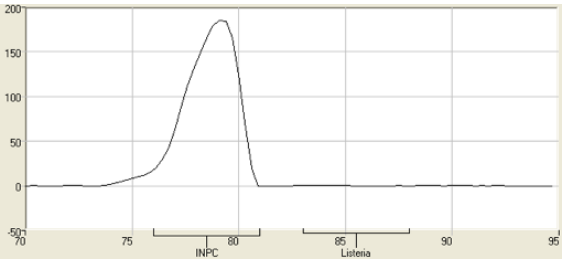
Strong Genus *Listeria* positive



Weak Genus *Listeria* positive



Genus *Listeria* Positive (with double target peaks)
Occasionally, you may see double target peaks for the Genus *Listeria* assay. This result is strain specific.



Genus *Listeria* Negative
No target peak; large control peak is present.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Listeria monocytogenes Assay

1. Enrich Samples

Part No. KIT2017

*The **AOAC Research Institute** has certified the BAX® System for detecting Listeria monocytogenes in a wide variety of foods, including raw meats, fresh produce/vegetables, processed meats, seafood, dairy cultured/non-cultured, and fruit juices.*

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 070202

***AOAC International** has also certified the BAX® System on dairy products, fruits and vegetables (except radishes), seafood, raw and processed meats and poultry as AOAC International Official Method℠ 2003.12.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
Raw meat and poultry	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-24 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed MOPS-BLEB (35°C). Incubate at 35°C for 18-24 hours.
Processed meats	Homogenize 25 g sample with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 22-24 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed MOPS-BLEB (35°C). Incubate at 35°C for 18-24 hours.
Dairy products	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Complete Selective enrichment broth. Incubate at 30°C for 22-24 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed MOPS-BLEB (35°C). Incubate at 35°C for 18-24 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Smoked fish	Homogenize 25 g sample with 225 mL pre-warmed (35°C) Universal pre-enrichment broth. Incubate at 35°C for 22-26 hours.	Transfer 1 mL enriched sample to 9 mL pre-warmed (35°C) MOPS-BLEB (1:10 dilution). Incubate at 35°C for 18-24 hours.
Other seafood, fresh vegetables, apple juice and orange juice	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for 20 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Environmental sponges (Plastic)	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with D/E Neutralizing Broth. Homogenize sponge with 225 mL pre-warmed (30°C) UVM. Incubate at 30°C for 20-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C). MOPS-BLEB Incubate at 35°C for 18-24 hours.

The **USDA Food Safety and Inspection Service (USDA-FSIS)** has adopted the BAX® System for detecting *Listeria monocytogenes* in processed meat and poultry products, environmental sponge samples and pasteurized liquid eggs with a modified enrichment protocol. See FSIS Microbiology Laboratory Guidebook (MLG) Method #8A for details and protocols. Please note that the enrichment and sample preparation steps in the MLG may differ from those in the BAX® System documentation.

Health Canada has certified this BAX® System assay as MFLP-28 for detecting *Listeria monocytogenes* in a variety of foods and environmental surfaces. See the Health Canada website to request details and protocols.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 60 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

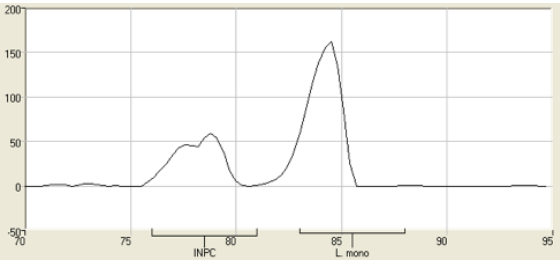
   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

5. Review Results

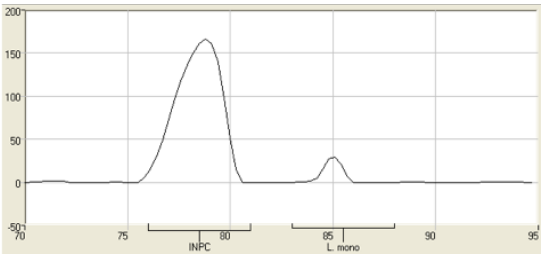
Positive *L. monocytogenes* Melting Curve Profiles

- One target peak at approximately 85°C.
- Target peak range 83 to 88°C.
- Control peak range 76 to 81°C.

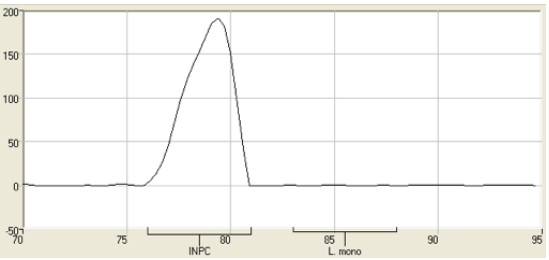
Often, the control peak for *L. monocytogenes* appears as a double peak.



Strong *L. monocytogenes* positive with double control peak



Weak *L. monocytogenes* positive



***L. monocytogenes* negative**

Negative *L. monocytogenes* Melting Curve Profile
No target peak; large control peak is present.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Salmonella Assays

1. Enrich Samples

PCR Assay for *Salmonella* 2 – Part No. KIT2011

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 100201

*The **AOAC Research Institute** has certified the BAX® System for detecting Salmonella in meat, poultry, fruit and vegetable products, dairy products, chocolate/bakery products, pasta, dry pet food and environmentals.*

***AOAC International** has also certified the BAX® System on raw ground beef, raw ground chicken, raw frozen fish, mozzarella cheese, frankfurters and orange juice as AOAC International Official Method℠ 2003.09.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
Standard media		
Meat and poultry	Homogenize (if ground or processed) or gently massage (if not ground) 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours.	None
Mozzarella cheese	Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Liquid eggs	Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Peanut butter	Blend 25 g sample with 225 mL pre-warmed LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Standard media		
Alfalfa sprouts	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BPW with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Raw frozen fish	Homogenize 25 g sample with 225 mL room temperature LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	None
Orange juice	Homogenize 25 g sample with 225 room temperature mL Universal Pre-enrichment broth. Let stand at room temperature 55-65 minutes. Do not adjust pH. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Milk chocolate	Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Add 0.45 mL 1% brilliant green dye solution. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Nonfat dry milk	Pour 25 g sample slowly over 225 mL Brilliant Green Water (2 mL 1% brilliant green dye solution/L deionized water). Let stand at room temperature 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Black pepper	Homogenize 25 g sample with 225 mL room temperature TSB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Standard media		
Custard, 2% milk, chilled ready meal, cooked fish, prawns, macaroni, pizza dough, frozen peas, cream cheese and dry pet food	Homogenize 25 g sample with 225 mL pre-warmed (35°C LB). Let stand at room temperature for 55-65 minutes. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Soy protein flour	Pour 25 g sample slowly over 225 LB in a flask. Let stand for 55-65 minutes at room temperature. Cap loosely. Incubate at 37°C for 18-22 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Frankfurters, chipped ham and cooked chicken	Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	None
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with 10 mL D/E Neutralizing Broth or equivalent. Finished Product Areas – Homogenize sponge with 225 mL pre-warmed (35°C) LB. Incubate at 35°C for 22- 26 hours. Raw Materials Areas – Homogenize sponge with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Sample type	**Primary Enrichment**	**Secondary Enrichment**
BAX® System MP media		
Ground beef	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.	None
Beef trim	Gently massage 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.	None
Spinach and lettuce	Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.	None

1. Enrich Samples (Continued)

The **USDA Food Safety and Inspection Service (USDA-FSIS)** has adopted the BAX® System for detecting *Salmonella* in meat, poultry, pasteurized egg, and catfish products and carcass and environmental sponges with a modified enrichment protocol. See FSIS Microbiology Laboratory Guidebook (MLG) Method #4C for details and protocols. Please note that the enrichment and sample preparation steps in the MLG may differ from those in the BAX® System documentation.

The USDA National Poultry Improvement Plan (USDA-NPIP) has developed an alternative protocol for detecting *Salmonella* in environmental samples, including drag swabs, fecal samples, dust, chick pads and meconium. Contact Hygiena Diagnostics Support for details and protocols.

The **U.S. Food and Drug Administration (US-FDA) Egg Safety Action Plan** has determined the BAX® System to be equivalent to the FDA-BAM method (Chapter 5: *Salmonella*) for detecting *Salmonella* Enteritidis in pooled liquid eggs, without the 96-hour hold time recommended by the BAM. See the FDA Testing methodology for *Salmonella* Enteritidis (SE) for details or contact Hygiena Diagnostics Support for protocols.

Health Canada has certified this BAX® System assay as MFLP-29 for detecting *Salmonella* in foods and environmental surface samples. See the Health Canada website to request details and protocols.

1. Enrich Samples (Continued)

QUA 18/03-11/02
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Salmonella in all human food products, animal feed and environmental samples (except primary production stage environmental samples).*

Sample type	Primary Enrichment	Secondary Enrichment
Raw meats and poultry (without spices)	Homogenize 25 g sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 16-20 hours.	None
Dairy (except powdered milk)	Homogenize 25 g sample with 225 mL room temperature BPW supplemented with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.	None
Other foods and environmental samples including composite foods, vegetables, seafood, animal feed and egg products	Homogenize 25 g sample with 225 mL room temperature BPW. Incubate at 37°C for 16-20 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Raw beef meat (including seasoned and frozen) in MP media	Homogenize 25 g sample in 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.	None
Other raw meat (including seasoned)	Homogenize 25 g sample in 225 mL room temperature BAX® System MP media. Incubate at 42°C for 24 hours.	None

1. Enrich Samples (Continued)

NordVal International *has certified the BAX® System according to the NordVal Validation Protocol for detecting Salmonella in all human food products, animal feed and environmental samples (except primary production stage environmental samples).*

Sample type	Primary Enrichment	Secondary Enrichment
Raw meats and poultry (without spices)	Homogenize 25 g sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 16-20 hours.	None
Dairy (except powdered milk)	Homogenize 25 g sample with 225 mL room temperature BPW supplemented with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.	None
Other foods and environmental samples including composite foods, vegetables, seafood, animal feed and egg products	Homogenize 25 g sample with 225 mL room temperature BPW. Incubate at 37°C for 16-20 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Raw beef meat (including seasoned and frozen) in MP media	Homogenize 25 g sample in 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.	None
Other raw meat (including seasoned)	Homogenize 25 g sample in 225 mL room temperature BAX® System MP media. Incubate at 42°C for 24 hours.	None

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

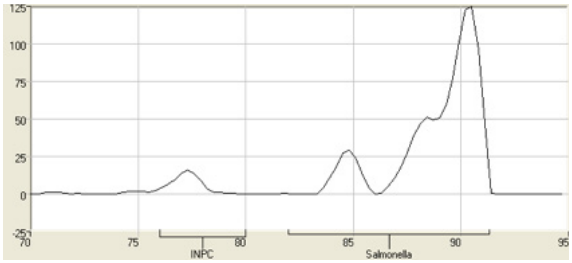
   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

5. Review Results

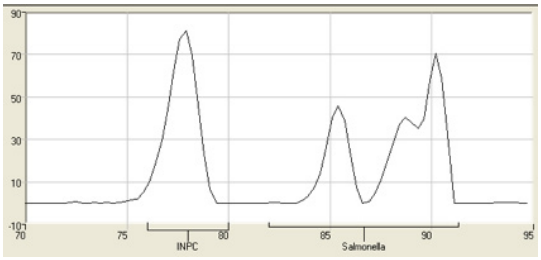
Positive *Salmonella* Melting Curve Profiles

- Three target peaks at approximately 85, 88 and 90°C.
- Approximately 5°C distance between the first and the third peak.
- Target peak range 82 to 91°C.
- Control peak range 76 to 80°C.

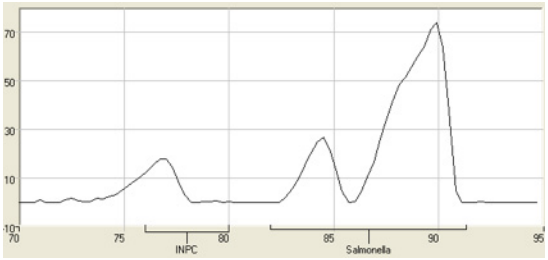
When the *Salmonella* level in a sample is very high, the 88°C and the 90°C peaks may merge, so that you see only two distinct peaks. In this case, the merged peak is very large and the control peak may be very small or absent.



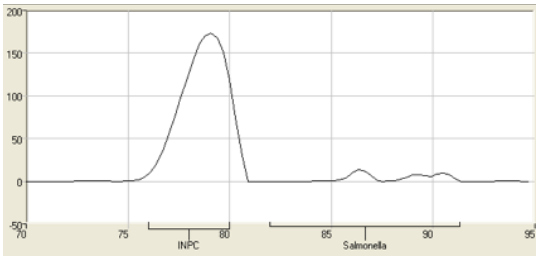
Strong *Salmonella* positive



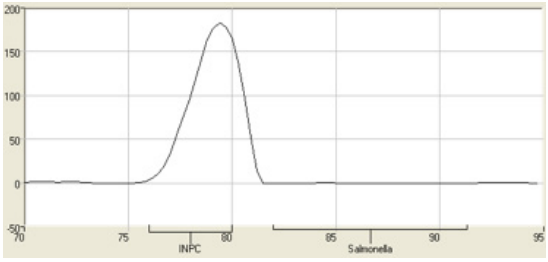
Moderate *Salmonella* positive
(notice the increased height of the control peak)



Strong *Salmonella* positive with merged peaks 2&3



Weak *Salmonella* positive



Negative *Salmonella* Melting Curve Profile

No target peaks; large control peak is present.

In some cases, very small non-specific peaks may be seen between 84-92°C, but they will not appear as described above for positive results.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR and NordVal Certification

In the context of NF VALIDATION samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

III. Protocols for 24E Assays

About End-Point Detection

The BAX® System 24E assays use the same end-point detection technology as the standard assays. The BAX® System PCR tablets in these assays contain fluorescent dye, which binds with double-stranded DNA and emits a fluorescent signal in response to light. After amplification, the BAX® System begins a detection phase where the fluorescent signal is measured. During detection, the temperature of the samples is raised to the point where the DNA strands separate (denature), releasing the dye and lowering the signal. This change in fluorescence can be plotted against temperature to generate a melting curve, which is interpreted by the BAX® System software as positive or negative results.

Assay Validation

The protocols in this chapter have been validated by Hygiena and certified by organizations, such as AOAC or AFNOR Certification, for the sample types listed. Many food types use standard enrichment protocols. However, certain food types have been validated using specific media or enrichment protocol modifications.

In order to follow the methods approved by AOAC or AFNOR Certification, the validated enrichment protocols described in this user guide and the kit instructions insert must be followed.

For many sample types, standard enrichment protocols (such as the USDA-FSIS, FDA-BAM or ISO methods) may also be used. These enrichment protocols should be internally validated before use with the BAX® System, as these methods have not been validated by Hygiena or any third-party organizations. To test a sample type that is not listed, please contact Hygiena Diagnostics Support for recommendations on enrichment and sample preparation.

The reference method enrichment protocols described in this chapter reflect those in use at the time of the assay's validation. Because these may have changed since that time, you should always refer to the appropriate reference method source for the most current enrichment protocol.

Reference method source	Link
USDA-FSIS Microbiology Laboratory Guidebook	http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
FDA Bacteriological Analytical Manual	http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
Health Canada Compendium of Analytical Methods	http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php
AOAC	http://www.aoac.org
International Organization for Standardization (ISO)	http://www.iso.org

Before You Begin

Review all best practices, tips and techniques described in Chapter VI: BAX® System Best Practices before beginning any BAX® System protocol.

Protocol Overview Flowchart

Enrichment

- Combine sample with room-temperature prepared 24 LEB enrichment
- Incubate samples at 37°C for 24-28 hours

Lysis Part One

- Prepare lysing agent for each sample (40 µL diluted lysing agent 1 with 10 µL lysing agent 2)
- Transfer 50 µL combined lysing agents to cluster tubes
- Add 0.5 mL sample to cluster tube with combined lysing agents
- Heat at 37°C for 30 minutes

Lysis Part Two

- Prepare lysis reagent (150 µL protease +12 mL lysis buffer)
- Transfer 200 µL prepared lysis reagent to cluster tube
- Add 5 uL of lysate to prepared lysis reagent in cluster tube
- Heat tubes at 55°C for 30 minutes
- Heat tubes at 95°C for 10 minutes
- Cool tubes for 5 minutes

Processing

- Place PCR tubes into a chilled cooling block
- Transfer 50 µL lysate to PCR tubes*
- Load samples into BAX® System cycler and run full process
- Read results in about 3.5 hours

*For *L. mono* 24E assay, transfer 30 µL

Genus *Listeria* 24E Assay

1. Enrich Samples

Part No. KIT2003

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 050903

*The **AOAC Research Institute** has certified the BAX® System 24E assay for detecting Genus Listeria in frankfurters, spinach, shrimp, queso fresco cheese and stainless steel.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Frankfurters, spinach and shrimp	Homogenize 25 g sample with 225 mL room-temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Queso fresco cheese	Homogenize 25 g sample with 225 mL room-temperature 24 LEB Complete media. Incubate at 37°C for 26-28 hours.
Stainless steel	Homogenize sponge with 90 mL room-temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

***Note:** Some third-party studies suggest that samples that experience a drop in pH during enrichment (such as smoked fish, salami or other charcuteries) may require the addition of buffer supplement to the 24 LEB enrichment broth. However, this addition has not been certified by AOAC. Before testing any food types that have not been certified by AOAC, it is strongly recommended that you internally validate your samples to determine if the buffer supplement is required.*

1. Enrich Samples (Continued)

QUA 18/06-07/08
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Genus Listeria in all human food products and environmental samples (excluding samples from primary production).*

Sample type	Enrichment
Meat, seafood, dairy, vegetables (except smoked fish)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish, raw and cooked delicatessen	Homogenize 25 g sample with 225 mL of room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C, 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C, if necessary. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

The 24E assays require a two-part lysis procedure.

Perform Part One of Lysis

1. Dilute bottle of Lysing agent 1 at room temperature with 1.8 mL sterile water.

   ***Note:** Lysing agent 1 is solid below room temperature.*

2. For each sample, combine diluted Lysing agent 1 (40 µL) with Lysing agent 2 (10 µL) in 4:1 ratio. Prepare slightly more than required (see chart on Ready Reference for 24E PCR assays) to compensate for pipetting loss.

   ***Note:** After combining diluted Lysing agent 1 and Lysing agent 2, the mixture must be used within 4 hours.*

3. Perform Lysis (Continued)

3. Transfer 50 µL combined lysing agents and 0.5 mL sample to cluster tubes.

   Note: *Remove samples from homogenize bag below the fatty layer that may be floating on top, as this layer may interfere.*

4. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 30 minutes.

Perform Part Two of Lysis

1. Label and arrange a second set of cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL heated sample from Part One of lysis to prepared lysis reagent in corresponding cluster tubes, using new pipette tips for each sample.
5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 30 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *If using cooling blocks, minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

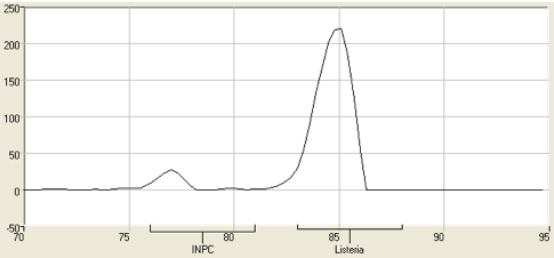
3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

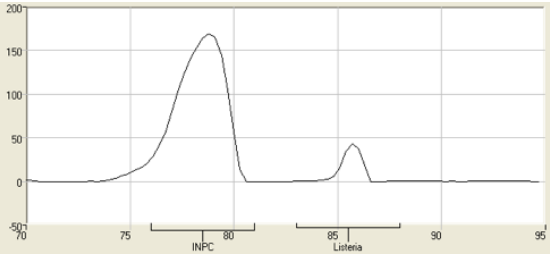
5. Review Results

Positive Genus *Listeria* 24E Melting Curve Profiles

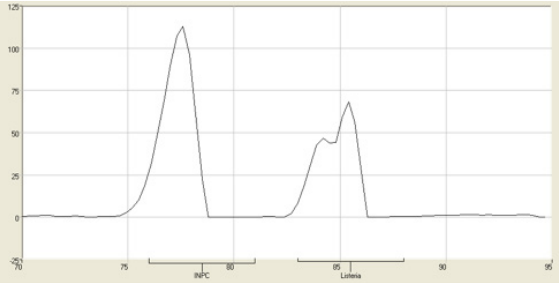
- One target peak at approximately 85.5°C.
- Target peak range 83 to 88°C.
- Control peak range 76 to 81°C.



Strong Genus *Listeria* positive

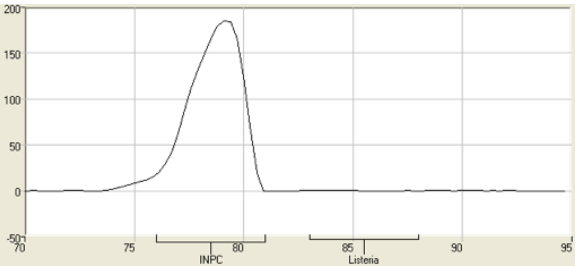


Weak Genus *Listeria* positive



Genus *Listeria* Positive with double target peak

Occasionally, you may see double target peaks for the Genus *Listeria* 24E assay. This result is strain specific.



Genus *Listeria* Negative

No target peak; large control peak is present.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Listeria monocytogenes 24E Assay

1. Enrich Samples

Part No. KIT2002

*The **AOAC Research Institute** has certified the BAX® System 24E assay for detecting Listeria monocytogenes in frankfurters, spinach, shrimp, queso fresco cheese and stainless steel.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Frankfurters, spinach and shrimp	Homogenize 25 g sample with 225 mL **room-temperature 24** LEB Complete media. Incubate at 37°C for 24-28 hours.
Queso fresco cheese	Homogenize 25 g sample with 225 mL **room-temperature 24** LEB Complete media. Incubate at 37°C for 26-28 hours.
Stainless steel	Homogenize sponge with 90 mL **room-temperature 24** LEB Complete media. Incubate at 37°C for 24-28 hours.

***Note:** Some third-party studies suggest that samples that experience a drop in pH during enrichment (such as smoked fish, salami or other charcuteries) may require the addition of buffer supplement to the 24 LEB enrichment broth. However, this addition has not been certified by AOAC. Before testing any food types that have not been certified by AOAC, it is strongly recommended that you internally validate your samples to determine if the buffer supplement is required.*

1. Enrich Samples (Continued)

QUA 18/05-07/08
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Listeria monocytogenes in all human food products and environmental samples (excluding samples from primary production).*

Sample type	Enrichment
Meat, seafood, dairy, vegetables (except smoked fish and charcuteries)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish, raw and cooked delicatessen	Homogenize 25 g sample with 225 mL of room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C, 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

The 24E assays require a two-part lysis procedure.

Perform Part One of Lysis

1. Dilute bottle of Lysing agent 1 at room temperature with 1.8 mL sterile water.

   Note: *Lysing agent 1 is solid below room temperature.*

2. For each sample, combine diluted Lysing agent 1 (40 µL) with Lysing agent 2 (10 µL) in 4:1 ratio. Prepare slightly more than required (see chart on Ready Reference for 24E PCR assays) to compensate for pipetting loss.

   Note: *After combining diluted Lysing agent 1 and Lysing agent 2, the mixture must be used within 4 hours.*

3. Transfer 50 µL combined lysing agents and 0.5 mL sample to cluster tubes.

   Note: *Remove samples from homogenizer bag below the fatty layer that may be floating on top, as this layer may interfere.*

4. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 30 minutes.

Perform Part Two of Lysis

1. Label and arrange a second set of cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL heated sample from Part One of lysis to prepared lysis reagent in corresponding cluster tubes, using new pipette tips for each sample.
5. After all transfers have been completed, secure the caps.

3. Perform Lysis (Continued)

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 30 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

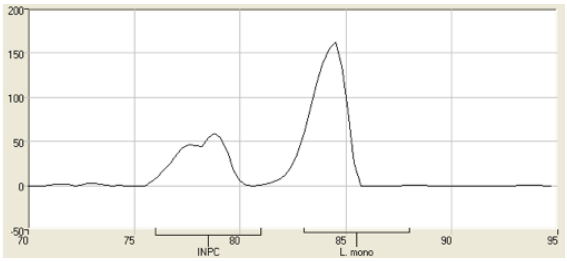
   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

5. Review Results

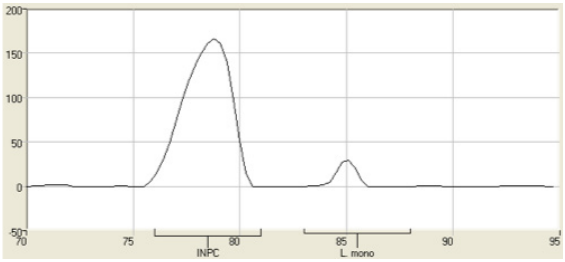
Positive *L. monocytogenes* 24E Melting Curve Profiles

- One target peak at approximately 85°C.
- Target peak range 83 to 88°C.
- Control peak range 76 to 81°C.

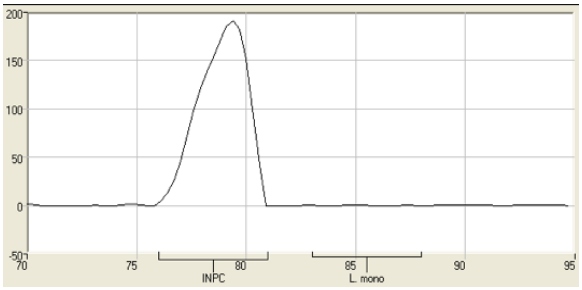
Often, the control peak for *L. monocytogenes* appears as a double peak.



Strong *L. monocytogenes* positive with double control



Weak *L. monocytogenes* positive



***L. monocytogenes* negative**

No target peak; large control peak is present.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

IV. Protocols for Real-Time Assays

About Real-Time Detection

The BAX® System PCR tablets used in real-time assays contain multiple target-specific, dye labeled probes. Probes are short oligonucleotides with quencher dye at one end that greatly reduces fluorescence from the fluorophore dye at the opposite end. During PCR, probes bind to a specific area within the targeted fragment and the fluorophore is separated from the quencher, allowing for increased fluorescent signal. The BAX® System Q7 instrument uses dye-specific filters to measure signal at the end of each cycle and report positive/negative results for each target.

Assay Validation

The protocols in this chapter have been validated by Hygiena and certified by organizations, such as AOAC or AFNOR Certification, for the sample types listed. Many food types use standard enrichment protocols. However, certain food types have been validated using specific media or enrichment protocol modifications.

In order to follow the methods approved by AOAC or AFNOR Certification, the validated enrichment methods described in this user guide and the package instruction insert must be followed.

For many sample types, standard enrichment protocols (such as the USDA-FSIS, FDA-BAM or ISO methods) may also be used. These enrichment protocols should be internally validated before use with the BAX® System, as these methods have not been validated by Hygiena or any third-party organizations. To test a sample type that is not listed, please contact Hygiena Diagnostics Support for recommendations on enrichment and sample preparation.

The reference method enrichment protocols described in this chapter reflect those in use at the time of the assay's validation. Because these may have changed since that time, you should always refer to the appropriate reference method source for the most current enrichment protocol.

Reference method source	Link
USDA-FSIS Microbiology Laboratory Guidebook	http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
FDA Bacteriological Analytical Manual	http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
Health Canada Compendium of Analytical Methods	http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php
AOAC	http://www.aoac.org
International Organization for Standardization (ISO)	http://www.iso.org

Before You Begin

Review all best practices, tips and techniques described in Chapter VI: BAX® System Best Practices before beginning any BAX® System protocol.

Protocol Overview Flowchart

Campylobacter jejuni/coli/lari Assay (Real-Time)

1. Enrich Samples

Part No. KIT2018

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 040702

*The **AOAC Research Institute** has certified the BAX® System for detecting only the presence or absence of Campylobacter in enriched samples of turkey breast and chicken carcass rinses.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Poultry carcass rinses	Homogenize sample 1:1 with pre-warmed (42°C) double strength Bolton broth with supplement (no blood). Volume prepared should be sufficient to allow < 2.5 mm headspace from the top of air-tight tube or other air-tight container. Incubate at 42°C for 24-48 hours.
Processed turkey	Homogenize 25 g sample with 225 mL pre-warmed (42°C) single strength Bolton broth with supplement (no blood). Incubate under microaerobic* conditions at 42°C for 24-48 hours.

***Note:** Campylobacter are microaerophilic and therefore susceptible to environmental stresses, such as exposure to air, drying, low pH and prolonged storage. Sample preparation usually requires a microaerobic gassing (5% O_2, 10% CO_2, 85% N_2) step. See Laboratory Procedure #MLG 41.04 for details.*

1. Enrich Samples (Continued)

NordVal International *has certified the BAX® System according to the NordVal Validation Protocol for detecting Campylobacter jejuni/coli and lari on poultry clocae swabs above 100 cfu/g.*

Sample type	Primary Enrichment
Poultry cloacae swabs	DIRECT TESTING - Collect 2 cloacae swabs (2 cotton swabs are used for cloacae sampling in 25 chickens [1 cotton swab represents 12 or 13 chickens]). Place the swabs into a vial containing 2 mL of 0.9% sodium chloride and 0.1% peptone.

The **USDA Food Safety and Inspection Service (USDA-FSIS)** has adopted the BAX® System for detecting *Campylobacter jejuni/coli/lari* in poultry rinses, sponge and raw product samples. See FSIS Microbiology Laboratory Guidebook (MLG) Method #41.04 for details and protocols. Please note that the enrichment and sample preparation steps in the MLG may differ from those in the BAX® System documentation.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample. (For NordVal poultry cloacae swab testing, transfer 50 µL of sample to corresponding cluster tube.)

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

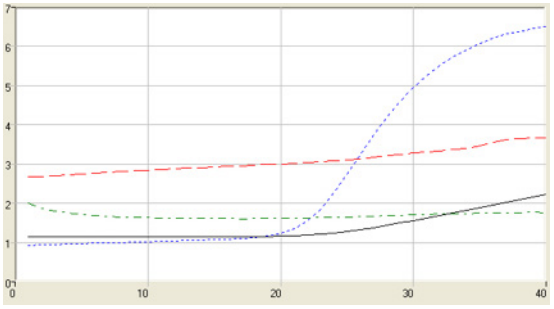
   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

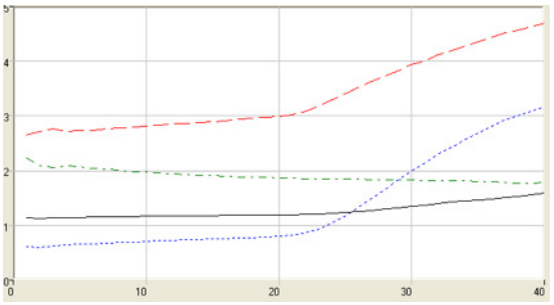
5. Review Results

- **Positive:** if any of the 3 targets create a sigmoid-shaped curve with a Ct value up to 40. The Ct value is usually between 20 and 40.
- **Negative:** if none of the 3 targets creates a sigmoid-shaped curve and the IPC is positive.



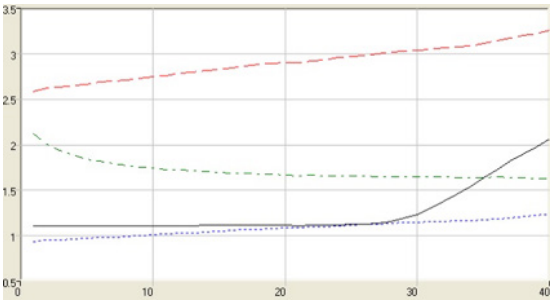
Target	Result	Ct	CFU/ml
C. coli	Negative	0.0	0
C. jejuni	Positive	27.2	1.6E+08
C. lari	Negative	0.0	0
Campy IPC	Positive	32.5	

Campylobacter* positive with 1 target *(C. jejuni)



Target	Result	Ct	CFU/ml
C. coli	Positive	29.1	3.7E+07
C. jejuni	Positive	30.1	1.5E+07
C. lari	Negative	0.0	0
Campy IPC	Positive	30.7	

Campylobacter* positive with 2 targets *(C. coli and C. jejuni)



Target	Result	Ct	CFU/ml
C. coli	Negative	0.0	0
C. jejuni	Negative	0.0	0
C. lari	Negative	0.0	0
Campy IPC	Positive	35.0	

***Campylobacter* negative**

Note: *CFU/mL calculations should only be used when testing Direct Enrichments.*

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

E. coli O157:H7 Assay (Real-Time)

1. Enrich Samples

Part No. KIT2000

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031002

*The **AOAC Research Institute** has certified the BAX® System for detecting E. coli O157:H7 in raw ground beef, beef trim, spinach and lettuce.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Raw ground beef	Homogenize 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.
Beef trim	Gently massage 375 g sample with 1.5 L pre-warmed (45°C) BAX® System MP media (1:5 dilution) for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 10-24 hours.
Spinach and lettuce	Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.

Health Canada has certified this BAX® System real-time assay as MFLP-76 for detecting *E. coli* O157:H7 in raw meat trim and raw ground beef. See the Health Canada website to request details and protocols.

1. Enrich Samples (Continued)

QUA 18/07-07/10
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting E.coli O157:H7 in raw beef meat, raw vegetable samples.*

Sample type	Enrichment
Raw beef meat	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 7-24 hours.
Raw vegetables	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.

QUA 18/07-07/10
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting STEC in raw beef meat, raw dairy products and vegetable samples.*

Sample type	Enrichment
Raw beef meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BPW. Incubate at 41.5°C for 10-24 hours.
Raw beef meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BAX® System MP media. Incubate at 41.5°C for 7-24 hours.
Raw dairy products	Homogenize 25 g/mL sample with 225 mL pre-warmed (41.5°C) double strength (DS) BPW. Incubate at 41.5°C for 20-24 hours
Vegetables	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BAX® System MP media. Incubate at 41.5°C for 8-24 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the freezer.*

3. Place a PCR tube rack over the insert.
4. Remove the required number of PCR tubes from the mylar bag in the refrigerator. Reseal the bag tightly.
5. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.

   Note: *Check PCR tubes to make sure that the tablets are seated in the bottom of the tubes. If tablets are stuck to the cap or sides, gently tap tubes on a counter or other flat surface to move tablets to the bottom of the tubes.*

6. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool. Visually check that each PCR tube contains a white, complete tablet. If tablets have shrunken or appear pink, discard tubes and replace with new ones before proceeding.
7. Transfer 30 µL lysate to PCR tubes. Place new flat optical caps on the strip of tubes, and secure tightly.

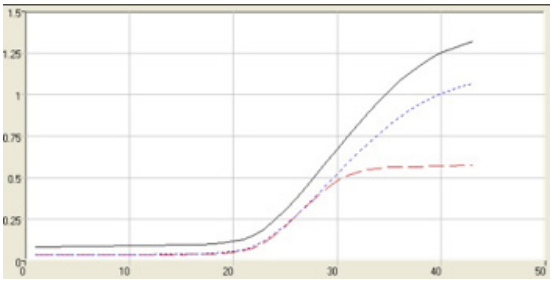
   Note: *PCR tablets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.*

8. Repeat from step 5 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
9. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

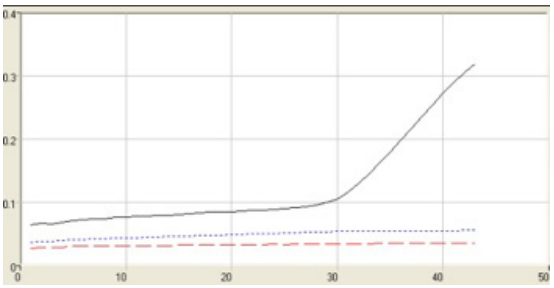
5. Review Results

- **Positive:** if both *E. coli* targets create a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 20 and 43.
- **Negative:** if neither *E. coli* target creates a sigmoid-shaped curve and the IPC is positive.



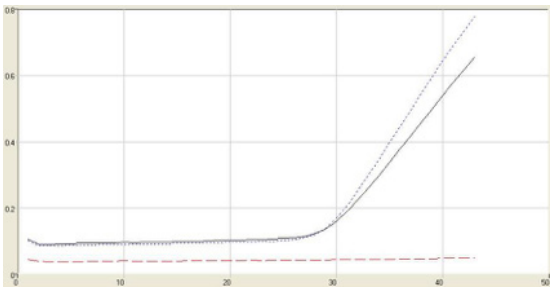
Target	Result	Ct	CFU/ml
Targ S	Positive	26.6	N/A
Targ W	Positive	30.6	N/A
IPC	Positive	30.6	

***E. coli* O157:H7 positive (both targets positive)**



Target	Result	Ct	CFU/ml
Targ S	Negative	0.0	N/A
Targ W	Negative	0.0	N/A
IPC	Positive	37.0	

***E. coli* O157:H7 negative (both targets negative)**



Target	Result	Ct	CFU/ml
Targ S	Negative	0.0	N/A
Targ W	Positive	36.4	N/A
IPC	Positive	36.9	

***E. coli* O157:H7 negative (one target negative, one target positive)**

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

E. coli O157:H7 EXACT Assay (Real-Time)

1. Enrich Samples

Part No. KIT2039

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 102003

*The **AOAC Research Institute** has certifiedthe BAX® System for detecting E. coli O157:H7 in raw ground beef, beef trim, leafy greens and raw milk.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Raw ground beef	**For 25 g:** Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media or mTSB (Modified Tryptic Soy Broth). Incubate at 42°C for 8-24 hours. **For 375 g:** Homogenize 375 g sample with 1.5 L pre-warmed (42°C) BAX® System MP media or mTSB. Incubate at 42°C for 8-24 hours.
Beef trim	**MP media:** Gently massage 375 g sample with 1.5 L pre-warmed (45°C) BAX® System MP media (1:5 dilution). Incubate at 42°C for 8-24 hours. **mTSB:** Gently massage 375 g sample with 1.5 L pre-warmed (45°C) mTSB (1:5 dilution). Incubate at 42°C for 10-24 hours.
Spinach and lettuce	Combine 375 g sample with 1.5 mL pre-warmed (42°C) BAX® System MP media (1:5 dilution). Incubate at 42°C for 6-24 hours.
Raw milk	Swirl 25 mL of sample with 225 mL pre-warmed (41.5°C) double strength BPW. Incubate at 41.5°C for 12-24 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see "Running a Full Process" in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the freezer.*

3. Place a PCR tube rack over the insert.
4. Remove the required number of PCR tubes from the mylar bag in the refrigerator. Reseal the bag tightly.
5. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.

   Note: *Check PCR tubes to make sure that the tablets are seated in the bottom of the tubes. If tablets are stuck to the cap or sides, gently tap tubes on a counter or other flat surface to move tablets to the bottom of the tubes.*

6. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool. Visually check that each PCR tube contains a white, complete tablet. If tablets have shrunken or appear pink, discard tubes and replace with new ones before proceeding.
7. Transfer 30 µL lysate to PCR tubes. Place new flat optical caps on the strip of tubes, and secure tightly.

   Note: *PCR tablets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.*

8. Repeat from step 5 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
9. After the completion of hydration of all PCR tablets, let PCR tubes sit in the cooling block for 10-30 minutes before loading into the BAX® System instrument.

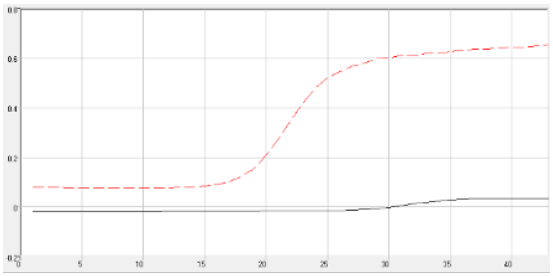
   Note: *Do not let PCR tubes sit for more than 30 minutes.*

10. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

    Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

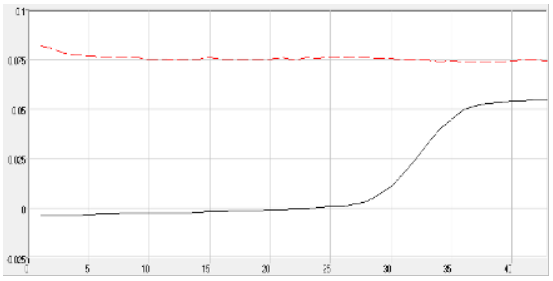
5. Review Results

- **Positive:** if the *E. coli* EXACT target creates a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 20 and 43.
- **Negative:** if the *E. coli* EXACT target does not create a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
E. coli O157:H7	Positive	21.9	1.0E+00
IPC	Positive	31.5	

***E. coli* O157:H7 EXACT positive**



Target	Result	Ct	CFU/ml
E. coli O157:H7	Negative	0.0	0
IPC	Positive	32.3	

***E. coli* O157:H7 EXACT negative**

6. Confirm Positive Results

If following a method approved by AOAC, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

E. coli - STEC Suite (Real-Time)

1. Enrich Samples

Part No. KIT2021 - STEC Screening: *stx* and *eae*
Part No. KIT2008 - STEC Panel 1: *E. coli* O26, O111, O121
Part No. KIT2009 - STEC Panel 2: *E. coli* O45, O103, O145

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 091301

*The **AOAC Research Institute** has certified the BAX® System for detecting these strains of Shiga toxin-producing E. coli (STEC) in raw ground beef, raw ground beef with soy, flour and beef trim.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Raw ground beef (375 g)	Homogenize sample with 1.5 L pre-warmed (46°C) glucose-containing TSB with 2 mg/L novobiocin. Incubate at 41°C* for 12-24 hours.
Raw ground beef with soy (325 g)	Homogenize sample with 975 mL pre-warmed (35°C) glucose-containing TSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 41°C* for 12-24 hours.
Raw beef trim (325 g)	Gently massage sample with 975 mL room-temperature glucose-containing TSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 41°C* for 15-24 hours.
Raw beef trim (375 g)	**TSB Enrichment** – Gently massage sample with 1.5 L pre-warmed (46°C) glucose-containing TSB. Incubate at 41°C* for 12-24 hours. **MP Media Enrichment** – Gently massage sample with 1.5 L pre-warmed (46°C) BAX® System MP media. Incubate at 41°C* for 12-24 hours.
Flour (25 g)	Homogenize sample with 225 mL pre-warmed (42°C) mTSB + 2 mg/L novobiocin. Incubate at 42°C* for 24 hours.
Ground beef (25 g)	**TSB Enrichment** – Homogenize sample with 225 mL pre-warmed (37°C) mTSB + casamino acids. Incubate at 42°C* for 10- 24 hours. **BPW Media Enrichment** – Homogenize sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 10- 24 hours.

**** Note:** Incubation temperature must be maintained between 39°C and 42°C for this assay.*

1. Enrich Samples (Continued)

QUA 18/07-07/10
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting STEC in raw beef meat, raw dairy products and vegetable samples.*

Sample type	Enrichment
Raw beef meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BPW. Incubate at 41.5°C for 10-24 hours.
Raw beef meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BAX® System MP media. Incubate at 41.5°C for 7-24 hours.
Raw dairy products	Homogenize 25 g/ml sample with 225 mL pre-warmed (41.5°C) Double strength (DS) BPW. Incubate at 41.5°C for 20-24 hours.
Vegetables	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BAX® System MP media. Incubate at 41.5°C for 8-24 hours

The **USDA Food Safety and Inspection Service (USDA-FSIS)** has adopted the BAX® System STEC suite for monitoring meat products (including raw ground beef mixed with raw pork and/or raw poultry products) and carcass and environmental sponges. See FSIS Microbiology Laboratory Guidebook (MLG) Method #5B for details and protocols. Please note that the enrichment and sample preparation protocols in the MLG may differ from those in the BAX® System documentation.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

***IMPORTANT NOTE FOR STEC SCREENING WITH "STX ONLY"**: An alternative target drop-down option is available for running the "stx only" program. See the special instructions at the end of this section for details and directions.*

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

***Note:** If preparing a negative control sample, add 20 µL blank enrichment media to cluster tube of lysis reagent.*

***Note:** Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block with insert and allow to cool for at least 5 minutes.

***Note:** Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

***Note:** These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the freezer.*

3. Place a PCR tube rack over the insert.
4. Remove the required number of PCR tubes from the mylar bag in the refrigerator. Reseal the bag tightly.
5. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.

   Note: *Check PCR tubes to make sure that the tablets are seated in the bottom of the tubes. If tablets are stuck to the cap or sides, gently tap tubes on a counter or other flat surface to move tablets to the bottom of the tubes.*

6. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool. Visually check that each PCR tube contains a white, complete tablet. If tablets have shrunken or appear pink, discard tubes and replace with new ones before proceeding.
7. Transfer 30 µL lysate to PCR tubes. Place new flat optical caps on the strip of tubes, and secure tightly.

   Note: *PCR tablets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.*

8. Repeat from step 5 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
9. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

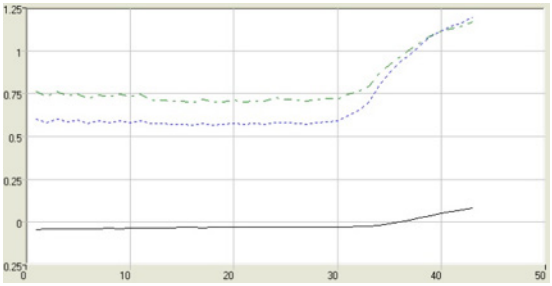
   Note: *If desired, remaining lysate can be sealed and stored for additional testing with other BAX® System STEC suite assays. Lysates may be stored at 2-8°C for up to 7 days or at -20°C for up to 14 days.*

5. Review Results

Screening Assay Results (*stx* and *eae*)

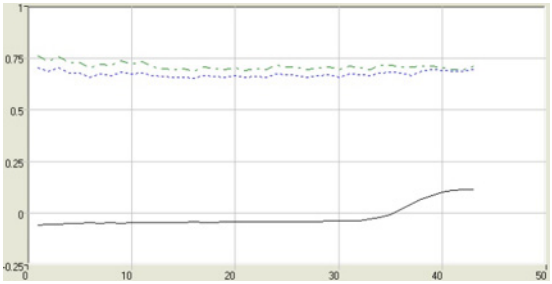
- **Positive:** if both the *stx* and *eae* targets create a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 15 and 43.
- **Negative:** if both the *stx* and *eae* targets are not present in the sample and the IPC is positive.

Note: *If only one of the stx or eae targets is present, the sample is considered negative.*



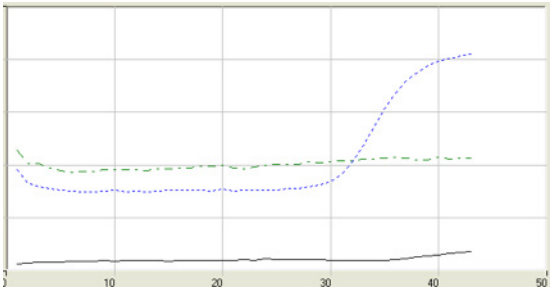
Target	Result	Ct	CFU/ml
stx	Positive	35.4	N/A
eae	Positive	35.5	N/A
IPC	Positive	38.4	

Screening assay positive (*stx* pos /*eae* pos)



Target	Result	Ct	CFU/ml
stx	Negative	0.0	N/A
eae	Negative	0.0	N/A
IPC	Positive	36.9	

Screening assay negative (*stx* neg /*eae* neg)



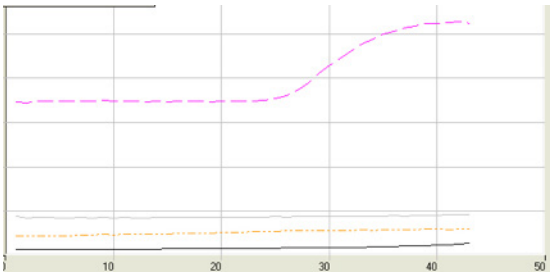
Target	Result	Ct	CFU/ml
stx	Positive	34.3	N/A
eae	Negative	0.0	N/A
IPC	Negative	0.0	

Screening assay negative (*stx* pos /*eae* neg)

5. Review Results (Continued)

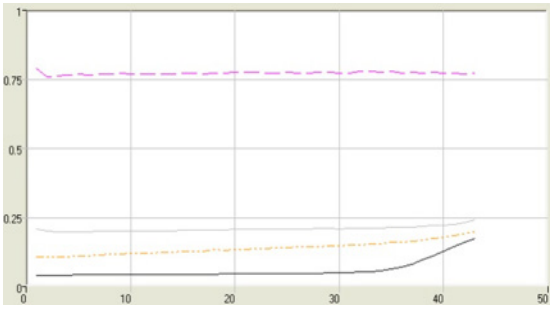
Panel 1 Assay Results (*E. coli* O26, O111, O121)

- **Positive:** if one or more of the Panel 1 targets creates a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 15 and 43.
- **Negative:** if none of the three targets creates a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
O26	Negative	0.0	N/A
O111	Negative	0.0	N/A
O121	Positive	33.3	N/A

Panel 1 assay positive (*E. coli* O121 pos)



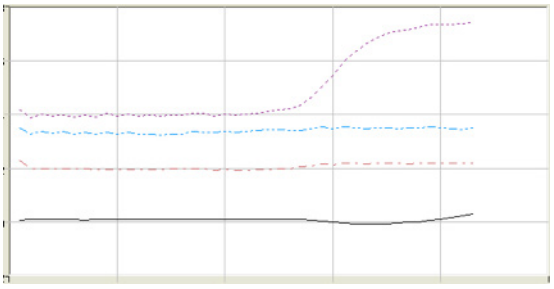
Target	Result	Ct	CFU/ml
O26	Negative	0.0	N/A
O111	Negative	0.0	N/A
O121	Negative	0.0	N/A

Panel 1 assay negative

5. Review Results (Continued)

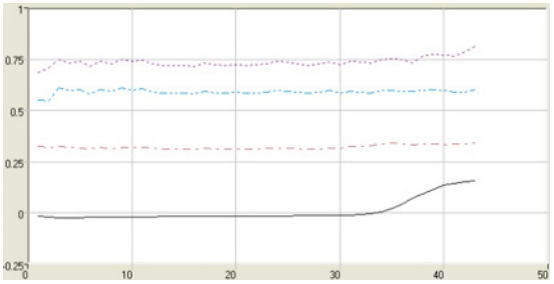
Panel 2 Assay Results (*E. coli* O45, O103, O145)

- **Positive:** if one or more of the Panel 2 targets creates a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 15 and 43.
- **Negative:** if none of the three targets creates a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
O45	Positive	30.5	N/A
O103	Negative	0.0	N/A
O145	Negative	0.0	N/A

Panel 2 assay positive (*E. coli* O45 pos)



Target	Result	Ct	CFU/ml
O45	Negative	0.0	N/A
O103	Negative	0.0	N/A
O145	Negative	0.0	N/A

Panel 2 assay negative

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Note: *Some positive results may be difficult to culturally confirm due to low levels of E. coli target cells, high levels of background flora, or a combination of these factors. Contact Hygiena Diagnostics Support for additional information.*

Add the alternative target called **"Real Time STEC Screen stx ONLY"** to your drop-down menu of available target programs before you begin this assay. See the instructions for Setting Optional Preferences in Chapter VIII: BAX® System Software.

Using the STEC Screening Assay with "stx only" Option

The BAX® System STEC Screening assay can also be used with an alternative target program to return positive/negative results for only the *stx* genes. Although *eae* is also detected, the plus/minus results for this "stx only" program are based only on the response of the *stx* target. Any *eae* amplification is ignored.

Follow the protocol for STEC Screening for *stx* and *eae* with the following exceptions to steps 2 and 5

1. Enrich Samples – No change to the STEC protocol.
2. Prepare Equipment - Prepare as described except, when creating the rack file, select "Real Time STEC Screen stx ONLY" from the Target drop-down menu.

   Note: *If the "Real Time STEC Screen stx ONLY" option does not appear in the Target drop-down menu, see the instructions for Setting Optional Preferences in Chapter VIII: BAX® System Software to add it to the menu.*

3. Perform Lysis - No change to the STEC protocol.
4. Hydrate PCR Tablets with Lysate - No change to the STEC protocol.
5. Review Results - Interpret results as follows:

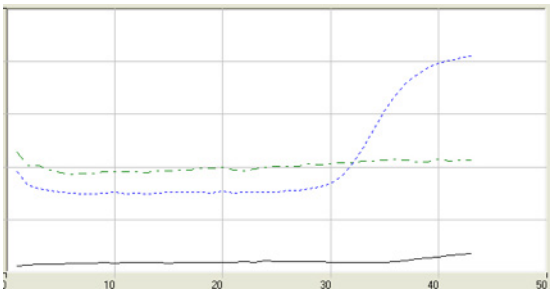
   Positive: if the *stx* target create a sigmoid-shaped curve with a Ct value up to 43, regardless of the result of the *eae* target. The Ct value is usually between 15 and 43.

   Note: *Although the results display an amplification plot for the eae gene, the plus/minus results are based only on the response of the stx target.*

   Negative: if the *stx* target is not present in the sample and the IPC is positive, regardless of the result of the *eae* target.

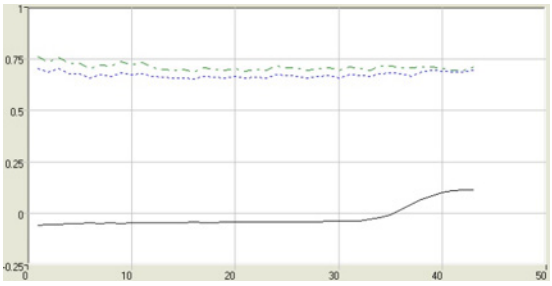
   Note: *In some cases, the IPC response may appear negative in samples with high levels of the stx target; as long as the stx target has amplified, the reaction has taken place.*

Using the STEC Screening Assay with “stx only” Option (Continued)



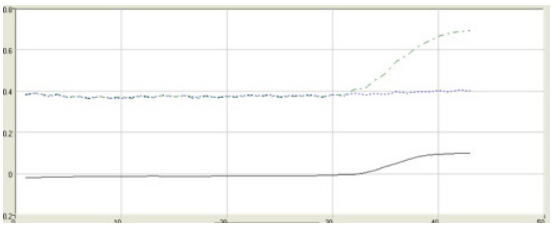
Target	Result	Ct	CFU/ml
stx	Positive	34.3	N/A
eae	Negative	0.0	N/A
IPC	Negative	0.0	

Screening assay positive (*stx pos*)



Target	Result	Ct	CFU/ml
stx	Negative	0.0	N/A
eae	Negative	0.0	N/A
IPC	Positive	36.9	

Screening assay negative (*stx neg* / *eae neg*)



Target	Result	Ct	CFU/ml
stx	Negative	0.0	N/A
eae	Positive	36.0	N/A
IPC	Positive	35.7	

Screening assay negative (*stx neg* / *eae pos*)

6. Confirm Positive Results - No change to the STEC protocol.

Genus *Listeria* Assay (Real-Time)

1. Enrich Samples

Part No. KIT2019

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081401

*The **AOAC Research Institute** has certified the BAX® System for detecting Listeria species in frankfurters (beef), bagged spinach, queso fresco, cooked shrimp, stainless steel, plastic and concrete.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
24 LEB Complete Media		
Frankfurters	Homogenize 125 g sample with 500 mL pre-warmed (35°C) 24 LEB Complete. Incubate at 35°C for 28-48 hours.	None
Spinach and shrimp	Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20-35°C) 24 LEB Complete. Incubate at 35°C for 24-48 hours.	None
Queso fresco	Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20-35°C) 24 LEB Complete. Incubate at 35°C for 26-48 hours.	None
Environmental sponges (stainless steel, plastic and concrete)	Homogenize sponge with 90 mL room-temperature or pre-warmed (20-35°C) 24 LEB Complete. Incubate at 35°C for 24-48 hours.	None
Actero™ Listeria Enrichment Media		
Frankfurters	Homogenize 125 g sample with 750 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 26-28 hours.	None
Spinach, shrimp, smoked salmon and queso fresco	Homogenize 25 g sample with 150 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 22-24 hours.	None
Environmental sponges	Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 20-24 hours.	None

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Reference Method Media		
Frankfurters	Homogenize 125 g sample with 1125 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 23-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Spinach, shrimp and queso fresco (single-stage enrichment)	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth (BLEB) without antibiotics. Incubate at 30°C for 4 hours, and then add antibiotics. Incubate at 30°C for 44-48 hours.	None
Spinach, shrimp and queso fresco (two-stage enrichment)	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth (BLEB) without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for 22-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Environmental sponges (stainless steel, plastic and concrete)	Homogenize sponge with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 20-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

Health Canada has certified this BAX® System assay as MFLP-79 for detecting *Listeria* species in environmental surface samples. See the Health Canada website to request details and protocols.

QUA 18/09-01/19
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Genus Listeria in all human food products and environmental samples (excluding samples from primary production).*

Sample type	Enrichment
Meat, seafood, dairy, vegetables (except smoked fish and raw and cooked delicatessan)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish, raw and cooked delicatessen	Homogenize 25 g sample with 225 mL of room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

Note: *Sample heating and cooling steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Add 150 µL of protease to one 12 mL bottle of lysis buffer.
3. **Add 200 µL of Lysing Agent 2 to the bottle of protease and lysis buffer mixture.**
4. Transfer 200 µL prepared lysis reagent to each of the cluster tubes.
5. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

6. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 30 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see "Running a Full Process" in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.

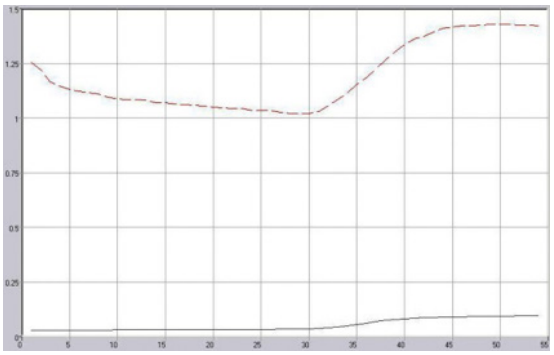
   Note: An optional 10-30-minute hold of the hydrated PCR tablets in the cooling block is recommended for the Real-Time Listeria assays in order to be consistent with the Real-Time Salmonella procedure.

8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

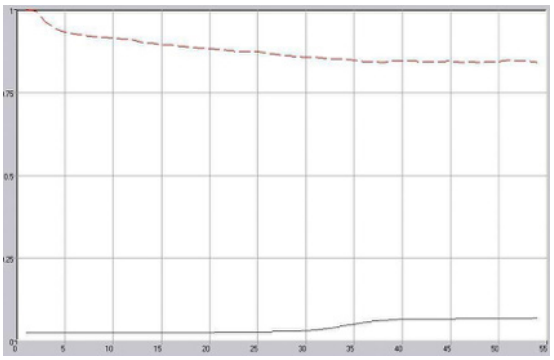
5. Review Results

- **Positive:** if the target shows a sigmoid-shaped curve with a Ct value up to 54. The Ct value is usually between 25 and 50.
- **Negative:** if the target does not show a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
Listeria	Positive	37.2	1.0E+00
RTL IPC	Positive	36.2	

Genus *Listeria* positive



Target	Result	Ct	CFU/ml
Listeria	Negative	0.0	0
RTL IPC	Positive	34.3	

Genus *Listeria* negative

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Listeria monocytogenes Assay (Real-Time)

1. Enrich Samples

Part No. KIT2005

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 121402

*The **AOAC Research Institute** has certified the BAX® System for detecting Listeria monocytogenes in frankfurters (beef), bagged spinach, queso fresco, cooked shrimp, stainless steel, plastic and concrete.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
24 LEB Complete Media		
Frankfurters	Homogenize 125 g sample with 500 mL pre-warmed (35°C) 24 LEB Complete. Incubate at 35°C for 28-48 hours.	None
Spinach	Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20–35°C) prepared 24 LEB Complete. Incubate at 35°C for 24–48 h.	None
Cooked shrimp	Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20–35°C) prepared 24 LEB Complete. Incubate at 35°C for 48 h.	None
Queso fresco	Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20-35°C) 24 LEB Complete. Incubate at 35°C for 26-48 hours.	None
Environmental sponges (stainless steel, sealed concrete and plastic surfaces)	Homogenize sponge with 90 mL room-temperature or pre-warmed (20-35°C) 24 LEB Complete. Incubate at 35°C for 24-48 hours.	None

1. Enrich Samples (Continued)

Actero™ Listeria Enrichment Media		
Frankfurters	Homogenize 125 g sample with 750 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 26-28 hours.	None
Spinach, shrimp, smoked salmon and queso fresco	Homogenize 25 g sample with 150 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 22-24 hours.	None
Environmental sponges	Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 20-24 hours.	None
Reference Method Media		
Frankfurters	Homogenize 125 g sample with 1125 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 23-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Spinach, shrimp and queso fresco	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth (BLEB) without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for 44-48 hours.	None
Environmental sponges (stainless steel, sealed concrete and plastic surfaces)	Homogenize sponge with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 20-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

1. Enrich Samples (Continued)

QUA 18/10-01/19
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Listeria monocytogenes in all human food products and environmental samples (excluding samples from primary production).*

Sample type	Enrichment
Meat, seafood, dairy, vegetables (except smoked fish)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish, raw and cooked delicatessen	Homogenize 25 g sample with 225 mL of room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Add 150 µL of protease to one 12 mL bottle of lysis buffer.
3. **Add 200 µL of Lysing Agent 2 to the bottle of protease and lysis buffer mixture.**
4. Transfer 200 µL prepared lysis reagent to each of the cluster tubes.
5. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

6. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 30 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.

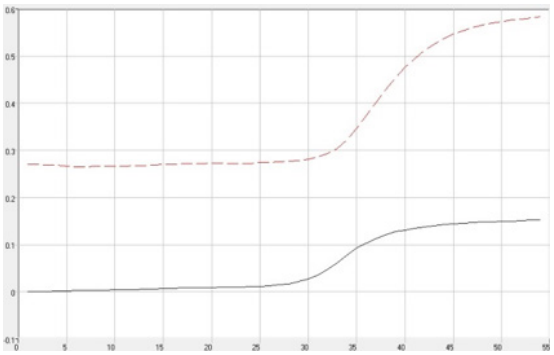
   Note: An optional 10-30-minute hold of the hydrated PCR tablets in the cooling block is recommended for the Real-Time Listeria assays in order to be consistent with the Real-Time Salmonella procedure.

8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

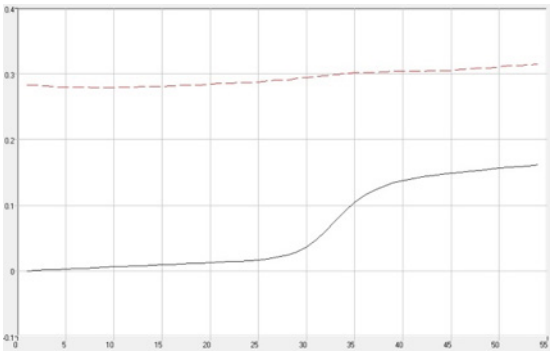
5. Review Results

- **Positive:** if the target shows a sigmoid-shaped curve with a Ct value up to 54. The Ct value is usually between 25 and 50.
- **Negative:** if the target does not show a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
L. monocytogenes	Positive	37.4	1.0E+00
RTL IPC	Positive	33.8	

***L. monocytogenes* positive**



Target	Result	Ct	CFU/ml
L. monocytogenes	Negative	0.0	0
RTL IPC	Positive	33.0	

***L. monocytogenes* negative**

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

IMPORTANT NOTE:

The test protocol for this assay has been revised to specify additional parameters for hydrating PCR tablets. Please read the instructions carefully before using this assay.

Salmonella Assay (Real-Time)

1. Enrich Samples

Part No. KIT2006

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081201

*The **AOAC Research Institute** has certified the BAX® System for detecting Salmonella in raw ground beef, lettuce, cream cheese, dry pet food, poultry rinses, and stainless steel.*

*The **AOAC Research Institute** has certified the BAX® SalQuant™ and BAX® MPN methods.*

AOAC INTERNATIONAL has certified the BAX® System for detecting *Salmonella* in a variety of foods and environmental surfaces as Official Method of Analysis (OMA) #2013.02.

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
Actero™ Elite Salmonella Enrichment Media (validated by Foodchek™ under PTM# 041403)		
Milk chocolate	Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 22-26 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Chocolate liquor	Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 26-30 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Actero™ Elite Salmonella Enrichment Media (validated by Foodchek™ under PTM# 041403)		
Dry pet food	25 g sample - Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 18-22 hours. 375 g sample – Homogenize 375 g sample with approximately one-third to one-half of 2625 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Add the remainder of the pre-warmed media. Incubate at 35°C for 18-22 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Cocoa powder	Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35°C for 16-20 hours.	Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
Shell eggs	Homogenize by hand a 20 egg sample with 1000 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours.	Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
Ground chicken	Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35°C for 14-18 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Ground beef	25 g sample - Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35°C for 16-20 hours. 375 g sample – Homogenize 375 g sample with 1125 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 25 mg/L of malachite green. Incubate at 39 - 42°C for 20-24 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Chicken carcass rinse	Homogenize by hand 30 mL of BPW rinse sample with 30 mL pre-warmed (35°C) Actero™ *Salmonella* Enrichment Media supplemented with 20 mg/L of malachite green. Incubate at 35°C for 16-20 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Actero™ Elite Salmonella Enrichment Media (validated by Foodchek™ under PTM# 041403)		
Dried whole egg	Homogenize by hand 100 g sample with 600 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35°C for 14-18 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Whole liquid egg (pasteurized)	Homogenize by hand 100 g sample with 300 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Adjust pH to 7.0±0.2. Incubate at 35°C for 18-22 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Raw almond	Homogenize 375 g sample with 750 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Dried raisin	Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Peanut butter	Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Whole black pepper	Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Dried parsley	Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 20-24 hours.	Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
Environmental sponges - stainless steel, plastic	Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ *Salmonella* Enrichment Media. Incubate at 35°C for 14-18 hours.	Transfer 40 µL enrichment to 2 mL PBS and mix before processing.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System MP Media and Standard Enrichment Media		
Ground beef	For 25 g – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. For 375 g – Homogenize 375 g sample with 1.5 L pre-warmed (45°C) mTSB with 2 mg/L novobiocin. Incubate at 39-42°C for 22-26 hours.	None
Ground beef with soy	For 25 g – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. For 325 g – Homogenize 325 g sample with 975 mL pre-warmed (35°C) mTSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 35°C for 20-24 hours.	None
Beef trim	For 25 g –Gently massage 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. For 325 g – Gently massage 325 g sample with 1.5 L pre-warmed (41°C) BAX® System MP media. Incubate at 39-42°C for 16-24 hours.	None
Ground turkey, chicken wings	Homogenize (for ground turkey) or gently massage (for chicken wings) 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 16-24 hours.	None
Frankfurters	Homogenize 325 g sample with approximately one-third to one-half of 2925 mL pre-warmed (35°C) BPW. Add the remainder of the pre-warmed media. Incubate at 35°C for 18-24 hours.	None
Shrimp	Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours.	None
Poultry rinses	Homogenize 30 mL BPW sample rinsate with 30 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	None
Eggs, shell	Combine 20 eggs (~1,000 mL) into sterile container with 2 L pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 48 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System MP Media and Standard Enrichment Media		
Peanut butter	LB Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours. BPW Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Lettuce	MP Media Enrichment – Combine 25 g sample with 225 mL pre-warmed (35°C) BAX® System MP media and swirl to soak entire sample. Incubate at 35°C for 10-24 hours. LB Enrichment – Combine 25 g sample with 225 mL pre-warmed (35°C) LB and swirl to soak entire sample. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours.	None
Peas	MP Media Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) BAX® System MP media. Incubate at 35°C for 22-26 hours. LB Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Orange juice	MP Media Enrichment - Swirl 25 mL sample thoroughly with 225 mL pre-warmed (41°C) BAX® System MP media. Incubate at 39-42°C for 22-26 hours. UPB Enrichment - Swirl 25 mL sample thoroughly with 225 mL pre-warmed (35°C) UPB. Let stand at room temperature for 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Cream cheese	MP Media Enrichment – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BAX® System MP media. Incubate at 35°C for 12-24 hours. LB Enrichment – Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	None

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System MP Media and Standard Enrichment Media		
Nonfat dry milk	Pour 25 g sample slowly over 225 mL pre-warmed (35°C) Brilliant Green Water. Let stand at room temperature for 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Ice cream	LB Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours. BPW Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. BGW Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) Brilliant Green Water. Incubate at 35°C for 22-26 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Milk-based infant formula	Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Cocoa	Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature for 55-65 minutes, then swirl thoroughly to mix. Adjust pH to 6.8±0.2, if necessary. Add 0.45 mL 1% brilliant green dye solution and mix well. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL BHI broth before processing. Optional - Incubate BHI broth at 37°C for 3 hours.
White pepper	Homogenize 25 g sample with 225 mL pre-warmed (35°C) TSB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Dry pet food	LB Enrichment – Homogenize 375 g sample with approximately one-third to one-half of 3375 mL pre-warmed (35°C) LB. Add the remainder of the pre-warmed media. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours. BPW Enrichment - Homogenize 375g sample with approximately one-third to one-half of 3375 mL pre-warmed (35°C) BPW. Add the remainder of the pre-warmed media. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System MP Media and Standard Enrichment Media		
Environmental sponges - Stainless steel, ceramic tile, plastic	LB Enrichment – Homogenize sponge with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours. BPW Enrichment – Homogenize sponge with 225 mL pre-warmed (35°C) BPW. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 18-24 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Eggs, dried	LB Enrichment - Add approximately 15 mL pre-warmed (35°C) LB to 25 g sample and stir to smooth. Add 3 additional aliquots of LB of 10 mL, 10 mL, and 190 mL (total media volume 225 mL), stirring after each addition. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2, if necessary. Incubate at 35°C for 22-26 hours. BPW Enrichment - Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	Optional - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Enrichment
BAX® System SalQuant™	
Raw comminuted (ground) turkey and chicken	1. Add 325 g portions to 1625 mL BPW. Homogenize by hand until all clumps have been dispersed. 2. Transfer 30 mL of homogenate to a sterile filtered bag. 3. Add 30 mL of prewarmed (42°C) BAX® MP media with novobiocin (40 mg/L) to each sample bag and hand mix for 30 seconds 4. Incubate samples at 42 ± 1°C for 8 hours. 5. After transferring to BAX® MP with novobiocin for BAX® System SalQuant™ enrichment, incubate the remaining original homogenate sample at 35°C for 24 hours for prevalence testing.
BAX® MPN Method	
Raw comminuted (ground) turkey and chicken	1. Homogenize 65 g samples with 585 mL BPW. 2. Make 3-tube 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: a. For 1 g sample dilution, fill 3 test tubes with 10 mL homogenate. b. For 0.1 g sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL sample homogenate. c. For 0.01 g sample dilution, fill 3 test tubes of 9.9 mL of BPW with 0.1 mL of sample homogenate. d. For 0.001 g sample dilution, add 0.1 mL of sample homogenate to 9.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. e. For 0.0001 g sample dilution, add 0.1 mL of homogenate to 99.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. 3. Incubate tubes at 37°C for 24 hours. Continue with creation of lysates for each incubated tube for prevalence testing.

1. Enrich Samples (Continued)

QUA 18/08-03/15
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

*The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Salmonella in all human food products, animal feed and environmental samples (excluding samples from primary production stage environmental samples).*

Sample type	Primary Enrichment	Secondary Enrichment
Meat products (including meat with spices), seafood, vegetables, pet food, environmental samples)	Homogenize 25 g sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 16-24 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Egg Products	Homogenize 25 g sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 18-24 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Dairy Products (except powdered milk)	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) BPW with 20mg/L novobiocin. Incubate at 41.5°C for 20-24 hours.	None
Chocolate	Homogenize 25 g sample in 225 mL pre-warmed (37°C) reconstituted nonfat dry milk and let stand at room temperature for 55-65 minutes. Add 0.45 mL 1% brilliant green dye solution and mix well. Incubate at 37°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Raw beef (specific short protocol)	Homogenize 25 g sample in 225 mL pre-warmed (41.5°C) BPW. Incubate at 41.5°C for 10-24 hours.	None
Raw meats and raw seafood	Homogenize 25 g sample in 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 16-20 hours.	None

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. **Let PCR tubes sit in the cooling block for 10 minutes before loading into the BAX® System instrument. Do not let PCR tubes sit for more than 30 minutes.**

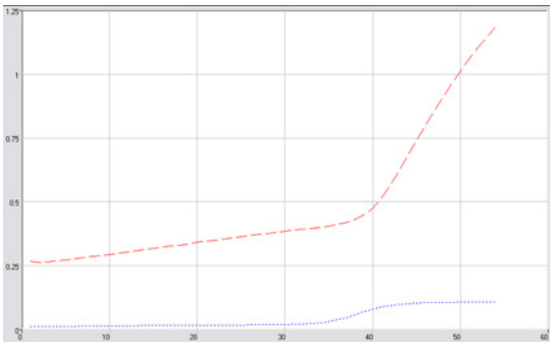
   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

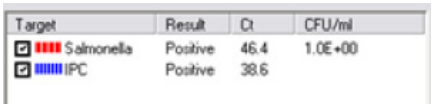
9. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

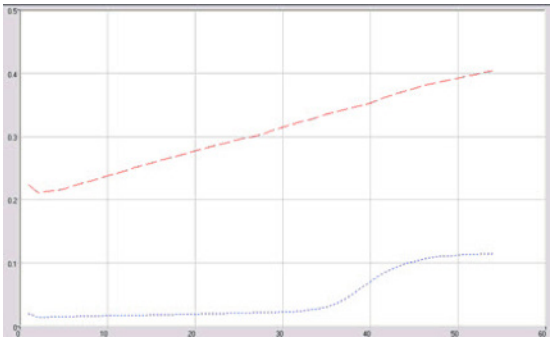
5. Review Results

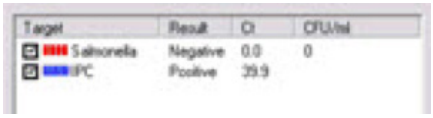
- **Positive:** if the target shows a sigmoid-shaped curve with a Ct value up to 54. The Ct value is usually between 25 and 50.
- **Negative:** if the target does not show a sigmoid-shaped curve and the IPC is positive.





***Salmonella* positive**





***Salmonella* negative**

For BAX® System SalQuant™ only

Transfer CT values generated into Hygiena Quant Calculator to auto-calculate the Log_{10}CFU/g, providing a quantitative result. (Please contact Hygiena representative for training and Hygiena Quant Calculator).

For BAX® MPN method

Utilize MPN tables in USDA-FSIS MLG 2.05 Most Probable Number Procedure and Tables to estimate MPN/g.

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Shigella Assay (Real-Time)

1. Enrich Samples

Part No. KIT2007

Sample type	Enrichment
Frankfurters	Homogenize 25 g sample with 225 mL pre-warmed (42°C) *Shigella* broth with novobiocin. Incubate under anaerobic conditions* for 8-20 hours.
Cooked meats, raw pork, dessert gelatin, ready-to-eat prepared dishes, non-fermented soy products, lunch meals, granulated white sugar	Homogenize 25 g sample with 225 mL pre-warmed (42°C) Shigella broth with novobiocin. Incubate under anaerobic conditions* for 16-20 hours.

* Enrichment in an anaerobic chamber is recommended. If a chamber is not available, a standard incubator can be used with as much air removed from the sample bag as possible; however, incubating without anaerobic conditions may make culture confirmation of *Shigella* difficult.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

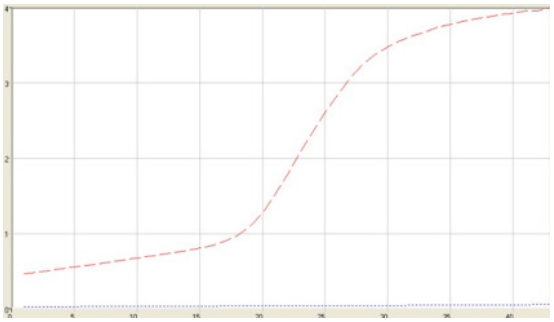
   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

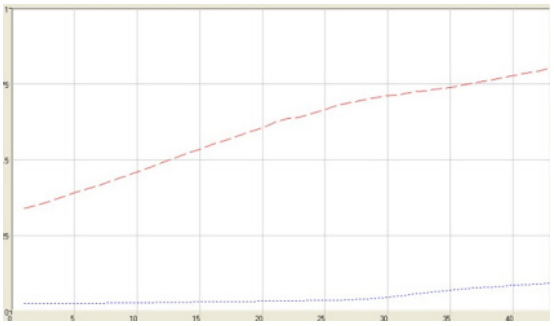
5. Review Results

- **Positive:** if the target shows a sigmoid-shaped curve with a Ct value up to 43. The Ct value is usually between 20 and 43.
- **Negative:** if the target does not show a sigmoid-shaped curve and the IPC is positive.



Target	Result	Ct	CFU/ml
IPC	Negative	0.0	
Shigella	Positive	23.8	N/A

***Shigella* positive**



Target	Result	Ct	CFU/ml
IPC	Positive	33.7	
Shigella	Negative	0.0	N/A

***Shigella* negative**

6. Confirm Positive Results – Required

Positive results obtained with the BAX® System must be confirmed according to one of the following reference culture methods.

For sample types with high levels of background flora, an alternative confirmation may be performed by streaking 10 µL enrichment onto one MacConkey, XLD or HE agar plate AND streaking 10 µL enrichment onto a Rainbow® agar plate for *Shigella/Aeromonas* (Biolog #80302). Incubate all plates at 36°C for 20-48 hours, then confirm suspect colonies with the appropriate serological or biochemical method.

Method approved by China GB

Follow the instructions in GB 4789.5-2012: Microbiological Examination of Food Hygiene-Examination of *Shigella* to streak the BAX® System enrichment onto the specified agars, incubate plates and confirm typical colonies.

Method approved by ISO

Follow the instructions in ISO 21567:2004: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella* spp. to streak the BAX® System enrichment onto the specified agars, incubate plates and confirm typical colonies.

Method approved by U.S. FDA BAM

Follow the instructions in FDA BAM Chapter 6: *Shigella* to streak the BAX® System enrichment onto the specified agars, incubate plates and confirm typical colonies.

Staphylococcus aureus Assay (Real-Time)

1. Enrich Samples

Part No. KIT2020

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 120701

*The **AOAC Research Institute** has certified the BAX® System for detecting Staphylococcus aureus in powdered infant formula, ground beef and soy protein isolate.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Ground beef, soy protein isolate	Threshold testing (10 cfu/g): Homogenize 10 g sample with 90 mL pre-warmed (37°C) Butterfield's Phosphate-Buffered Dilution Water. Transfer 1 mL homogenate to 1 mL of double-strength BHI with 14% (w/v) NaCl. Incubate at 37°C for 20-22 hours (ground beef) or 44-48 hours (soy protein isolate).
Infant formula	Presence/absence testing: Homogenize 10 g sample with 90 mL pre-warmed (37°C) Giolitti-Cantoni Broth with Tween and Tellurite (GCTT). Incubate at 37°C for 22-24 hours.

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 55°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see "Creating a Rack File" in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 55°C for 60 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

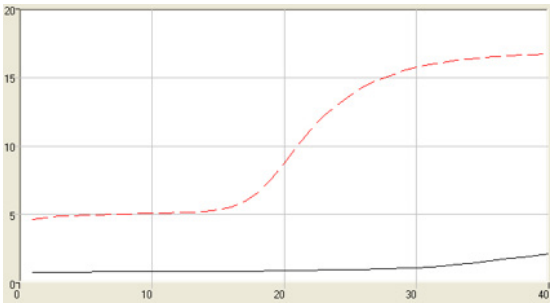
   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 30 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

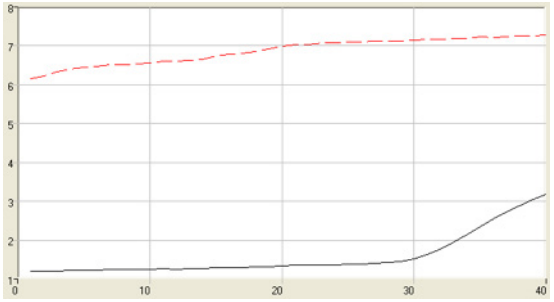
5. Review Results

- **Positive:** if the target shows a sigmoid-shaped curve with a Ct value up to 40. The Ct value is usually between 20 and 40.
- **Negative:** if the target does not show a sigmoid-shaped curve and the IPC is positive



Target	Result	Ct	CFU/ml
S. aureus	Positive	21.8	N/A
Staph IPC	Positive	39.0	

***Staph aureus* positive**



Target	Result	Ct	CFU/ml
S. aureus	Negative	0.0	N/A
Staph IPC	Positive	35.0	

***Staph aureus* negative**

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Vibrio cholerae/parahaemolyticus/vulnificus Assay (Real-Time)

1. Enrich Samples

Part No. KIT2010

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 050902

*The **AOAC Research Institute** has certified the BAX® System for detecting Vibrio cholerae/parahaemolyticus/vulnificus in tuna, raw and cooked shrimp, scallops and oysters.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment
Tuna, shrimp and scallops	Presence/absence testing: Homogenize 25 g sample with 225 mL pre-warmed (35°C) Alkaline Peptone Water*. Incubate at 35°C for 16-20 hours.
Shrimp and scallops	MPN testing** Homogenize 50 g sample with 450 mL Phosphate Buffered Saline (PBS) dilution water to create a 1:10 dilution. From this stock dilution; • Transfer 10 mL dilution to 3 tubes containing 10 mL pre-warmed (35°C) *double-strength* alkaline peptone water* • Transfer 1 mL dilution to 3 tubes containing 10 mL pre-warmed (35°C) *single-strength* alkaline peptone water • Transfer 1 mL dilution to 9 mL PBS and mix, then add 1 mL of new dilution to 3 tubes containing 10 mL pre-warmed (35°C) *single-strength* alkaline peptone water Incubate all samples at 35°C for 16-20 hours. ***Note:** Run all 9 samples in the BAX® System, then use the plus/minus results to perform MPN calculations.*

***Note:** The concentration of alkaline peptone water varies among vendors. Commercial preparations must be diluted to the correct concentration (10 g peptone and 10 g NaCl per L) before use. See Enrichment Recipes for preparation instructions.*

****Note:** For AOAC validation, shrimp and scallops were tested at low spike levels and therefore required only 3 dilutions to determine MPN. Oysters were naturally contaminated at high levels, and therefore a greater number of dilutions were needed to determine MPN.*

1. Enrich Samples (Continued)

Sample type	Enrichment
Oysters	MPN testing** Homogenize sample in 1:2 ratio with Phosphate Buffered Saline (PBS) dilution water for 90 seconds. Combine with additional PBS to create a 1:10 dilution. From this stock dilution; • Transfer 100 mL dilution to 3 tubes containing 100 mL pre-warmed (35°C) *double-strength* alkaline peptone water* • Transfer 10 mL dilution to 3 tubes containing 10 mL pre-warmed (35°C) *double-strength* alkaline peptone water • Serially dilute the stock dilution 6 times into tubes containing PBS, then add 1 mL of each dilution to 3 tubes containing 10 mL pre-warmed (35°C) *single-strength* alkaline peptone water Incubate all samples at 35°C for 16-20 hours. ***Note:*** *Run all 24 samples in the BAX® System, then use the plus/minus results to perform MPN calculations*

***Note:** The concentration of alkaline peptone water varies among vendors. Commercial preparations must be diluted to the correct concentration (10 g peptone and 10 g NaCl per L) before use. See Enrichment Recipes for preparation instructions.*

****Note:** For AOAC validation, shrimp and scallops were tested at low spike levels and therefore required only 3 dilutions to determine MPN. Oysters were naturally contaminated at high levels, and therefore a greater number of dilutions were needed to determine MPN.*

2. Prepare Equipment

1. Turn on the heating blocks, and set temperatures to 37°C and 95°C.
2. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
3. Create a rack file (see “Creating a Rack File” in Chapter VIII: BAX® System Software for details).

3. Perform Lysis

Prepare cluster tubes

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare the lysis reagent by adding 150 µL of protease to one 12 mL bottle of lysis buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 5 µL enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

   Note: *Do not shake or mix the enrichment before transferring samples to cluster tubes.*

5. After all transfers have been completed, secure the caps.

Heat the cluster tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details).
2. Select a chilled (2-8°C) PCR cooling block.

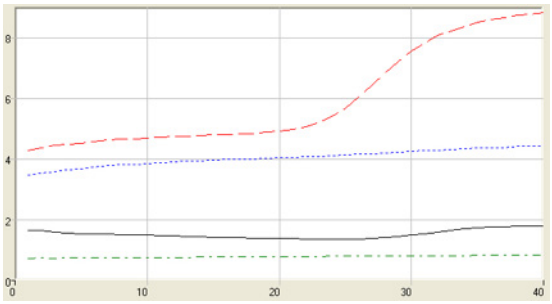
   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Remove the required number of PCR tubes from the mylar bag in the refrigerator. Reseal the bag tightly.
5. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
6. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool. Visually check that each PCR tube contains a white, complete tablet. If tablets have shrunken or appear pink, discard tubes and replace with new ones before proceeding.
7. Transfer 30 µL lysate to PCR tubes. Place new flat optical caps on the strip of tubes, and secure tightly.
8. Repeat from step 5 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
9. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

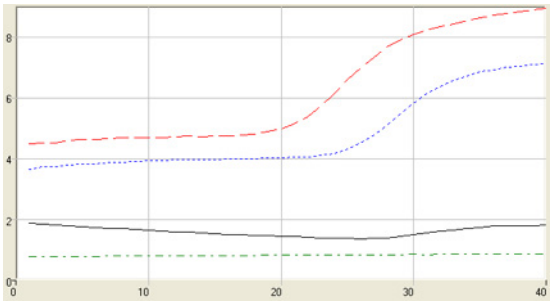
5. Review Results

- **Positive:** if any of the 3 targets create a sigmoid-shaped curve with a Ct value up to 40. The Ct value is usually between 20 and 40.
- **Negative:** if none of the 3 targets creates a sigmoid-shaped curve and the IPC is positive



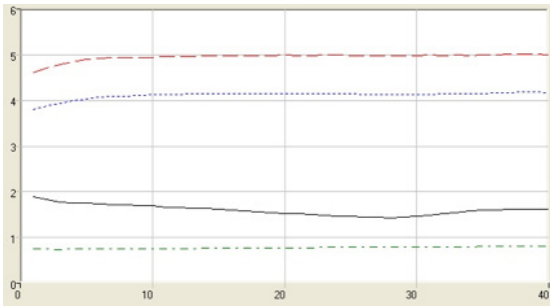
Target	Result	Ct	CFU/ml
V. cholerae	Negative	0.0	N/A
V. parahaem...	Positive	28.0	N/A
V. vulnificus	Negative	0.0	N/A
Vibrio IPC	Positive	31.4	

Vibrio* positive with 1 target *(V. parahaemolyticus)



Target	Result	Ct	CFU/ml
V. cholerae	Positive	29.3	N/A
V. parahaem...	Positive	25.6	N/A
V. vulnificus	Negative	0.0	N/A
Vibrio IPC	Positive	31.8	

Vibrio* positive with 2 targets *(V. cholerae and V. parahaemolyticus)



Target	Result	Ct	CFU/ml
V. cholerae	Negative	0.0	N/A
V. parahaem...	Negative	0.0	N/A
V. vulnificus	Negative	0.0	N/A
Vibrio IPC	Positive	32.6	

***Vibrio* negative**

6. Confirm Positive Results

If following a method approved by AOAC, AFNOR Certification or other third-party organizations, you can confirm results as described below for that particular method. Otherwise, results can be confirmed from the primary enrichment according to your laboratory SOP or the appropriate reference method for your sample type, if desired.

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

V. Protocols for Quality Assays

About Quality Testing

In food quality testing, the emphasis is on spoilage rather than pathogenicity. Food products containing yeast and mold do not usually cause human illness; however, high levels of yeast and mold can cause food products to look, smell or taste bad, diminishing the appeal of the product and the brand.

Labs that test for food quality establish cutoff thresholds (action levels) for yeast and mold concentrations that are the basis for product-release decisions. The BAX® System is designed to detect yeast and mold in food products after 44 hours enrichment at a variety of action levels to meet the needs of your lab. A direct testing protocol for non-enriched samples is also available.

Assay Validation

The protocols in this chapter have been validated by Hygiena and certified by organizations, such as AOAC, for the sample types listed. Many food types use standard enrichment protocols. However, certain food types have been validated using specific media or enrichment protocol modifications.

In order to follow the methods approved by AOAC, the validated enrichment protocols described in this user guide and the kit instructions must be followed.

For many sample types, standard enrichment protocols (such as the FDA-BAM method) may also be used. These enrichment protocols should be internally validated before use with the BAX® System, as these methods have not been validated by Hygiena or any third-party organizations. To test a sample type that is not listed, please contact Hygiena Diagnostics Support for recommendations on enrichment and sample preparation.

The reference method enrichment protocols described in this chapter reflect those in use at the time of the assay's validation. Because these may have changed since that time, you should always refer to the appropriate reference method source for the most current enrichment protocol.

Reference method source	Link
FDA Bacteriological Analytical Manual	http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
AOAC	http://www.aoac.org

Before You Begin

Review all best practices, tips and techniques described in Chapter VI: BAX® System Best Practices before beginning any BAX® System protocol.

Yeast and Mold Assay

Part No. KIT2015

Enriched Protocols

These BAX® System protocols test for yeast and mold in food samples that have been enriched for 44 hours. After adding DNA stabilizer, samples are agitated to disrupt the spores and vegetative cells in preparation for lysis. Results are based on a target concentration that you select during sample preparation. Positive results with the BAX® System reflect the presence of yeast and mold at a concentration above that threshold, while negative results indicate that any yeast and mold in the sample is below that threshold.

Pooled samples: For labs with very low action levels (10-50 cfu/g), this ultra-sensitive protocol prepares each enriched sample in triplicate. Aliquots from three replicate samples are combined in one lysis tube for testing with the BAX® System.

Non-pooled samples: For labs with a range of action levels (25-1000 cfu/g), this protocol prepares each enriched sample as a single test. It is useful for labs where plate counts have historically been far below action levels and can wait for plate count confirmation of positives.

MPN Protocol: For labs with action levels of 25 cfu/g or higher where historic plate counts have been closer to action levels, or labs that cannot wait for plate count confirmation, an alternative protocol that prepares each sample in triplicate can be used. One of the replicates is tested with the BAX® System; if the result is positive, the other two replicates are then tested. This protocol allows you to determine action levels based on the Most Probable Number (MPN) calculation of one to three positive results, without waiting for plate count confirmation.

Direct Protocol (No Enrichment)

The direct testing protocol requires no enrichment, but results are not displayed as positive or negative. Instead, the BAX® System generates a numerical output between 0 and 1, with higher numbers corresponding to higher concentrations of yeast and mold in the sample. Exact correlation varies by product type and plating method, so users must validate the BAX® System output to their plate counts to obtain a quantitative measure for product-release decisions. This method is most useful for labs that accept a relatively high level of target (≥ 500 cfu/g) and wish to ship the same day.

Testing Pooled Samples

This ultra-sensitive protocol for yeast and mold testing uses pooled samples from three disrupter tube enrichment replicates for action levels of 10-50 cfu/g.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 010902

*The **AOAC Research Institute** has certified the BAX® System for detecting yeast and mold in enriched samples of yogurt, corn starch and milk-based powdered infant formula.*

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

1. Enrich Samples

1. Homogenize sample 1:10 with 0.1% peptone water for 2 minutes at 200 rpm.
2. Use Table 1 to determine the appropriate volume of homogenate for the desired action level (target concentration).

***Example:** If your action level is 10 cfu/g, you would use 400 µL homogenate and test 7 µL from all three replicates.*

Table 1: Sample volume calculation for pooled sample protocol

If your action level is:	Then transfer this volume of homogenate to 3 disrupter tubes:	And pool these volumes of disrupted sample for testing:
10 cfu/g	400 µL	7 µL from 3 replicates
20 cfu/g*	200 µL	7 µL from 3 replicates
50 cfu/g	80 µL	7 µL from 3 replicates

**Alternative not submitted to AOAC-RI: If your action level is 20 cfu/g, you can incubate 400 µL of homogenate and pool 10 µL from any 2 replicates.*

3. For each sample, transfer the appropriate volume of homogenate to three disrupter tubes containing disrupter solution.
4. Incubate disrupter tubes at 25°C for 44 hours.

2. Disrupt Samples

1. Add 20 µL DNA stabilizer to each disrupter tube.
2. Place tubes in disrupter device and agitate 15 minutes.
3. Return tubes to the rack. Repeat with the remaining tubes until all samples have been disrupted.

3. Prepare Samples for Lysis

1. Break cluster tubes apart. Label and arrange cluster tubes in rack according to your rack file.
2. Prepare lysis reagent by adding 150 µL of protease to one 12 mL bottle of YM Lysis Buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 7 µL disrupted sample from each triplicate to one cluster tube, using new pipette tips for each sample.
5. After all transfers have been completed, secure the caps.

4. Heat Cluster Tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

5. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details). Select Yeast and Mold Enriched target in BAX® System software.
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

6. Review Results

1. Threshold results:

   A negative icon means that any yeast and mold in the product is below the desired action level.

   Positive icons mean that yeast and mold is present at or above the desired action level.

Testing Non-Pooled Samples

Note: *This protocol was published in the previous BAX® System User Guide as the Standard Enriched Protocol. Although the name for this protocol has changed, the procedure remains the same.*
This protocol for yeast and mold testing can be used without pooling for action levels of 25 cfu/g or above.

1. Enrich Samples

1. Homogenize sample 1:10 according to your lab's standard operating procedure for the food type.

   Example: *Add 10 g sample to 90 mL diluent and homogenize for 2 minutes.*

2. Use Table 2 to select the appropriate volume of homogenate for the desired action level (target concentration).

   Example: *If your action level is 100 cfu/g, a 100* µL *homogenate volume would produce results consistent with plate counts.*

Table 2: Sample volume calculation for alternative enriched protocol (non-pooled)

Action Level	Homogenate Volume
25 cfu/g	400 µL
50 cfu/g	200 µL
100 cfu/g	100 µL
500 cfu/g	20 µL
1000 cfu/g	10 µL

3. Transfer appropriate volume of homogenate to a disrupter tube containing disrupter solution.

   Note: *You can also transfer an additional aliquot of the homogenate prepared in step 1 to an agar plate, according to your lab's standard procedure, if you wish to use plate counts to confirm positive BAX® System results.*

4. Incubate disrupter tubes at 25°C for 44 hours.

2. Disrupt Samples

1. Add 20 µL DNA stabilizer to each disrupter tube.
2. Place tubes in disrupter device and agitate for 15 minutes.
3. Return tubes to the rack. Repeat with the remaining tubes until all samples have been disrupted.

3. Prepare Samples for Lysis

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare lysis reagent by adding 150 µL of protease to one 12 mL bottle of YM Lysis Buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL disrupted sample to the corresponding cluster tubes, using new pipette tips for each sample.
5. After all transfers have been completed, secure the caps.

4. Heat Cluster Tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

5. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details). Select Yeast and Mold Enriched target in BAX® System Software.
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

6. Review Results

1. Threshold results:

   A negative icon means that any yeast and mold in the product is below the desired action level.

   Positive icons mean that yeast and mold is present at or above the desired action level.

Using the MPN Protocol

This protocol prepares samples in triplicate, then tests one replicate. If that result is positive, the other two replicates are then tested and decisions are based on MPN calculations from the total number of positive results for that sample. This protocol can be used for action levels of 25 cfu/g or above, where the product has historically passed quality control testing with plate counts close to the action level, or where the wait for plate count confirmation is unacceptable.

1. Enrich Samples

1. Homogenize sample 1:10 according to your lab's standard operating procedure for the food type.
2. Use Table 3 to determine the MPN concentration you can expect to find for the sample volume selected and number of replicates tested.

Example: *A single 100* µL *sample that returns a positive result corresponds to an MPN of 41 cfu/g. In lieu of plate confirmation, you would test the other two replicates for confidence in the results. If one of the other replicates is positive, the BAX ® results are consistent with a culture-based result of ~110 cfu/g. If all three replicates are positive, your sample probably contains yeast and mold concentrations of >110 cfu/g.*

Table 3: Sample volume calculation for MPN alternative enriched protocol

Homogenate Volume	None Positive	1 of 3 Positive	2 of 3 Positive	3 of 3 Positive
10 µL	<410 cfu/g	410 95% CI 56-2900	1100 95% CI 260-4700	>1100
20 µL	<200 cfu/g	200 95% CI 28-1500	550 95% CI 130-2400	>550
100 µL	<41 cfu/g	41 95% CI 5.6-290	110 95% CI 26-470	>110
200 µL	<20 cfu/g	20 95% CI 2.8-150	55 95% CI 13-240	>55
400 µL	<10 cfu/g	10 95% CI 1.4-73	27 95% CI 6.4-120	>27

3. For each sample, transfer appropriate volume of homogenate to three disrupter tubes containing disruptor solution.
4. Incubate disrupter tubes at 25°C for 44 hours.

2. Disrupt Samples

1. Add 20 µL DNA stabilizer to each disrupter tube.
2. Place tubes in disrupter device and agitate for 15 minutes.
3. Return tubes to the rack. Repeat with the remaining tubes until all samples have been disrupted.

3. Prepare Samples for Lysis

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare lysis reagent by adding 150 µL of protease to one 12 mL bottle of YM Lysis Buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL disrupted sample from one of the triplicate disrupter tubes to the corresponding cluster tubes, using new pipette tips for each sample. Store the other two disrupter tubes at 4°C until test is completed.
5. After all transfers have been completed, secure the caps.

4. Heat Cluster Tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

5. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details). Select Yeast and Mold Enriched target in BAX® System Software.
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument. Load samples as described in Chapter VIII.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

6. Review Results

1. Threshold results:

   A negative icon means that any yeast and mold in the product is below the desired action level.

   Positive icons mean that yeast and mold is present at or above the desired action level.

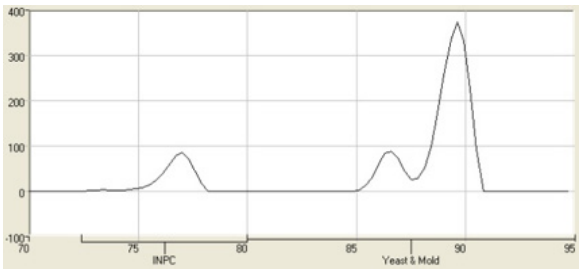
If positive, process the remaining two replicates starting at “Prepare Samples for Lysis”. MPN will vary based on total number of positive replicates, as noted in Table 3.

Threshold Results for Yeast and Mold

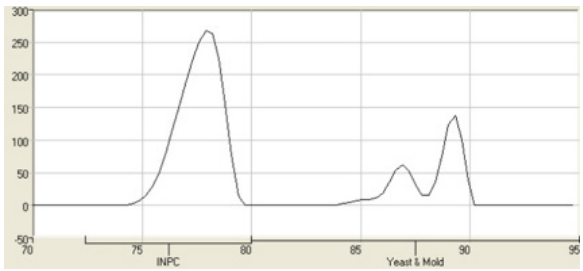
Threshold results: A negative icon means that any yeast and mold in the product is below the desired action level. Positive icons mean that yeast and mold is present at or above the desired action level.

Positive Yeast and Mold Melting Curve Profiles

- One or more target peaks in the range of 81 to 90°C.
- Control peak range 74 to 81°C.



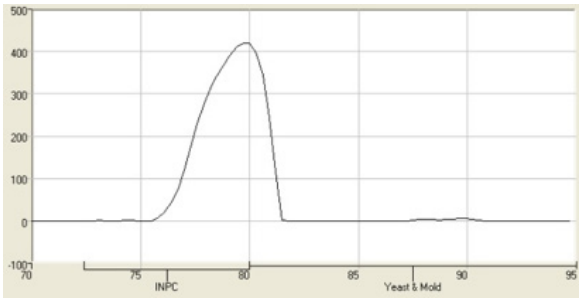
Strong Yeast and Mold positive



Weak Yeast and Mold positive

Negative Yeast and Mold Melting Curve Profile

No target peaks; control peak is present.



Yeast and Mold negative

Using the Direct Protocol

This protocol for yeast and mold testing can be used on non-enriched samples where actions levels are relatively high (≥ 500 cfu/g).

1. Disrupt Sample

1. Homogenize sample 1:10 according to your lab's standard operating procedure for the food type.

   Example: *Add 10 g sample to 90 mL diluent, and homogenize for 2 minutes.*

2. Transfer 200 µL homogenized sample to disrupter tubes containing disrupter solution.
3. Add 20 µL DNA Stabilizer to each disrupter tube.
4. Place tubes in disrupter device and agitate for 15 minutes.
5. Return tubes to the rack. Repeat with the remaining tubes until all samples have been disrupted.

2. Prepare Samples for Lysis

1. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
2. Prepare lysis reagent by adding 150 µL of protease to one 12 mL bottle of YM Lysis Buffer.
3. Transfer 200 µL lysis reagent to each of the cluster tubes.
4. Transfer 20 µL disrupted sample to the corresponding cluster tubes, using new pipette tips for each sample.
5. After all transfers have been completed, secure the caps.

3. Heat Cluster Tubes

1. Place the rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes.
2. Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes.
3. After lysis is complete, place the cluster tubes into a chilled (2-8°C) cooling block and allow to cool for at least five minutes.

   Note: *These steps can also be performed using the Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.*

4. Hydrate PCR Tablets with Lysate

1. Initialize the BAX® System instrument (see “Running a Full Process” in Chapter VIII: BAX® System Software for details). Select Yeast and Mold Direct target in BAX® System Software.
2. Select a chilled (2-8°C) PCR cooling block.

   Note: *Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.*

3. Place a PCR tube rack over the insert.
4. Arrange PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
5. Verify that the instrument has reached the correct load temperature and prompts you to continue, then remove the caps from the first strip of tubes with the decapping tool and transfer 50 µL lysate to PCR tubes.
6. Place new flat optical caps on the strip of tubes, and secure tightly.
7. Repeat from step 4 for the next strip of tubes, continuing until all tubes have been hydrated and resealed.
8. Take the rack of PCR tubes in the cooling block to the BAX® System Q7 instrument.

   Note: *Samples must remain chilled at 2-8°C until loaded into the instrument.*

Direct Testing Results for Yeast and Mold

Instead of the typical positive/negative icons in Rack View, results for Direct testing display as orange circles containing a check mark to indicate the test was successful (Figure 4-1). If the test was not successful, the icons display the standard icons for Indeterminate (yellow circle with question mark) or Error (yellow circle with red slash).

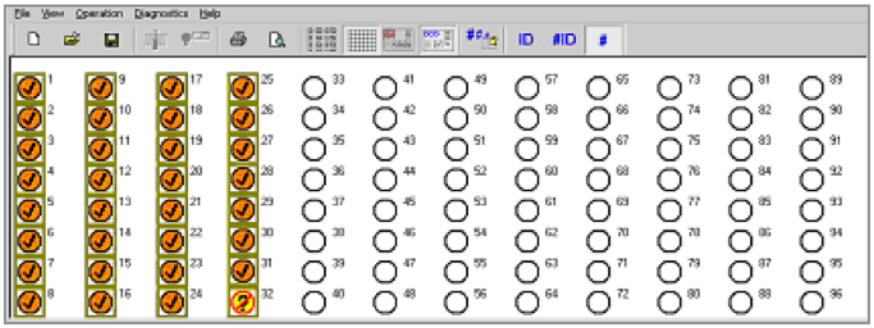
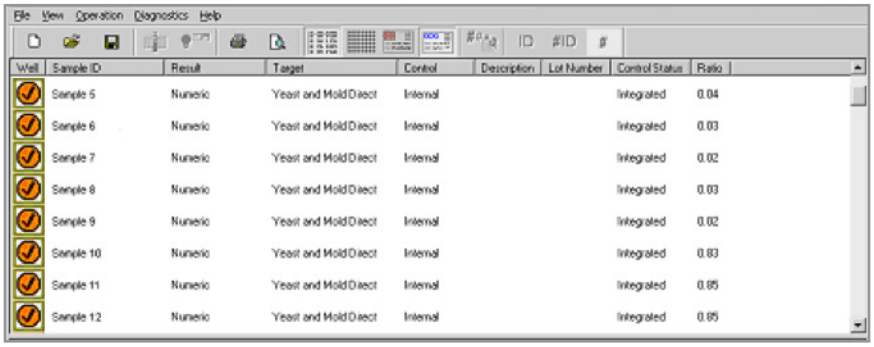


Figure 4-1. Rack View of BAX® System results from direct testing protocol

The Detail View provides a numerical “ratio” value between 0 and 1 in the last column (Figure 4-2). Generally, higher ratio values indicate greater concentrations of yeast and mold cells in the sample. When you begin using the Direct testing protocol, you must validate the correlation of that ratio value to historical plate count data for that product. After mapping the BAX® System ratio results to your own plate counts, you can then use the BAX® System ratio value as an action level measure.



Well	Sample ID	Result	Target	Control	Description	Lot Number	Control Status	Ratio
	Sample 5	Numeric	Yeast and Mold Direct	Internal			Integrated	0.04
	Sample 6	Numeric	Yeast and Mold Direct	Internal			Integrated	0.03
	Sample 7	Numeric	Yeast and Mold Direct	Internal			Integrated	0.02
	Sample 8	Numeric	Yeast and Mold Direct	Internal			Integrated	0.03
	Sample 9	Numeric	Yeast and Mold Direct	Internal			Integrated	0.02
	Sample 10	Numeric	Yeast and Mold Direct	Internal			Integrated	0.83
	Sample 11	Numeric	Yeast and Mold Direct	Internal			Integrated	0.85
	Sample 12	Numeric	Yeast and Mold Direct	Internal			Integrated	0.85

Figure 4-2. Detail View of BAX® System results from direct testing protocol

Manually Setting a Ratio Threshold for Direct Testing (optional)

When selecting the Yeast and Mold Direct protocol, the ratio threshold for samples can be manually entered to set the sensitivity limit for the assay. After validating the correlation of BAX® System ratio values to your plate counts, you can optionally view results as positive or negative icons instead of checkmarks by manually entering this ratio threshold in the lower pane of the Well View (Figure 4-3). The BAX® System then compares the ratio value of the test result to the value you specify in this field. If the test result is equal to or greater than the specified ratio threshold, the well for that sample displays a red positive icon. If the test result is less than the specified ratio threshold, the well displays a green negative icon.

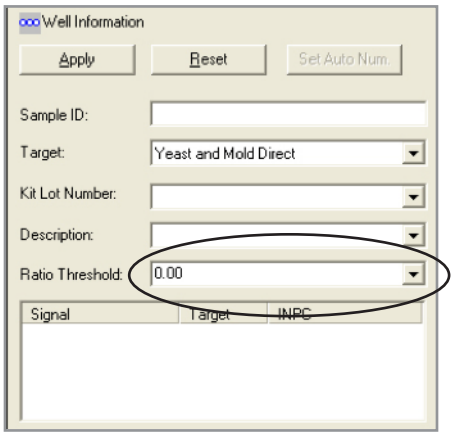


Figure 4-3. Ratio threshold

To apply the ratio threshold before running samples, enter the desired value in the Ratio Threshold field as you create your rack file (see Chapter VIII).

To reanalyze samples previously run with a different ratio threshold:

1. Click FILE > OPEN from the menu bar and select the file to be reanalyzed.
2. Highlight the wells to be reanalyzed.
3. In the Target drop-down menu, make sure the option “Yeast and Mold Direct” is selected.
4. Select or type the correct ratio value in the Ratio Threshold menu.
5. Click the **APPLY** button.
6. Select OPERATION > REANALYZE WELLS from the menu and follow the screen prompts.

VI. BAX® System Best Practices

Tips for Sample Prep Technique

- Always use new pipette tips when transferring samples or hydrating tablets. When dispensing lysis reagent, you can reuse pipette tips.
- Change gloves between each work step. Always use powder-free gloves.
- Use tweezers when removing cluster tubes caps and optical caps from the bag. Once the bag has been opened, store unused caps in a re-sealable container with a lid.
- Do not re-use any caps on cluster tubes or PCR tubes.
- Do not remove cooling block inserts from the refrigerator/freezer until they are needed. Finish using the cooling blocks within 30 minutes of removing inserts from the refrigerator/freezer.
- Use pre-sterilized cluster tubes for all lysis steps. Avoid using bulk cluster tubes that must be autoclaved before use.
- Break cluster tubes apart before use to help prevent cross-contamination between samples during transfers.
- When transferring samples, begin and complete one column at a time as shown in the BAX® System Ready Reference. Cap tubes after finishing a single row of samples to avoid cross-contamination.
- When transferring enriched samples to lysis tubes, check your technique for the following good practices to avoid sporadic indeterminate results:
  - Before transferring any enriched samples, check that all lysis tubes contain the same level of lysis reagent (200 µL).
  - Make sure to transfer the correct amount of enriched sample to the lysis tubes.
  - Slimy samples – Some samples become slimy after enrichment. When transferring these samples, insert just the very end of the pipette tip into the sample to avoid coating the entire tip. Wipe off any excess material by scraping the tip against the side of the sample container. In severe cases, you can use a clean wipe or tissue (fresh for each sample), but care must be taken to avoid cross-contamination.
  - Samples with particulates – Some samples contain particulate matter after enrichment. When transferring these samples, take care to pipette from the upper layer, not the bottom of the enrichment.

Tips for Sample Prep Technique (Continued)

  - Samples with oil/fat layers – Some samples generate an oil/fat layer on top of the enrichment. When transferring these samples, make sure to pipette from the aqueous layer below the oil/fat layer. Wipe off any coating of oil/fat from the tip as with slimy samples (above). If necessary, you can transfer an aliquot from the enrichment to a secondary tube to facilitate a clean transfer to lysis tubes.
- When transferring lysate to PCR tubes, verify the following:
  - Each PCR tube contains one PCR tablet.
  - The position of the PCR tubes in the rack matches the position in the rack file.
- Do not hydrate PCR tablets with lysate until the cycler has reached the correct load temperature and the “Ready for Rack Load” prompt appears.

Tips for Sample Preparation

- According to your laboratory SOP, prepare one positive control, one negative control and/or media blank and run along with your selected samples.
- After lysis has been completed, unopened lysate can be stored at 2-8°C for up 7 days or at -20°C for up to 14 days before hydrating PCR tablets. Stopping the protocol prior to the end of lysis is not recommended, as this may compromise the results for some sample types.
- For re-testing purposes, *enrichments can be stored at room temperature for 4 to 5 hours until results have been reviewed and validated. For longer storage (up to 48 hours) store at 2-8°C, unless validated otherwise internally*.
  For confirmation purposes only, primary enrichments (before regrowth) could be stored at 2-8°C overnight or according to your laboratory SOP. However, these storage conditions have not been validated by Hygiena.
- When preparing enrichment broth, use deionized water that is compatible with PCR testing.
- Use a thermometer to ensure correct incubation temperature before preparing samples. Incubators should be within ±2°C of the specified temperature, unless you are following Health Canada and AFNOR Certifications standards which require an incubator capable of maintaining ±1°C.

Tips for Enrichment Media

- Unless stated otherwise, all solutions should be pre-warmed to the specified temperature before use.
- Media should be warmed in a static incubator overnight. A hot water bath can also be used to warm media if a shorter pre-heating time is needed before preparing samples.
- Use a thermometer to ensure correct media temperature before preparing samples.

A Note on Shaking Samples After Enrichment

After enrichment, samples *should not* be shaken before aliquots are removed for lysis. For many sample types, shaking samples after enrichment increases the chance that larger particulates are transferred to the lysate, which could inhibit the PCR process.

If samples have been agitated after enrichment, let sit for at least 10 minutes before transferring aliquots to cluster tubes for lysis.

Tips for Sample Lysis

- Use a thermometer to ensure set temperatures are correct and within the approved temperature stability ranges before heating samples (see “Note on Heating Block Temperature Stability” in Chapter I).
- When preparing lysis reagent, mix the protease and lysis buffer by capping the bottle and inverting 8-10 times. Do not over-mix lysis reagent, which can cause the mixture to foam.
- If desired, you can make smaller volumes of lysis reagent by keeping the ratio at 12.5 µL protease to 1 mL lysis buffer. Lysis reagent in capped tubes will remain stable for up to two weeks when refrigerated at 2-8°C.
- For steps using a cooling block, minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible.
- Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.
- Unopened lysates may be stored for up to one week for later testing when refrigerated at 4°C.
- Opened lysates may be stored for up to one week for later testing when frozen at -20°C.

Tips for PCR Tablet Hydration

- Remove only the required number of PCR tubes from the bag in the refrigerator. Reseal the bag tightly.
- After placing PCR tubes in the holder, mark the top of each strip to maintain orientation when placing strips in the instrument.
- When hydrating tablets, only remove the caps from one strip of tubes at a time to avoid cross-contamination.
- Visually check that each PCR tube contains a white tablet. If tablets have shrunken or appear pink, discard tubes and replace with new ones before proceeding.
- PCR tablets must be hydrated and re-sealed within 10 minutes after loosening the caps from the PCR tubes.
- Secure flat optical caps on PCR tubes tightly after hydrating tablets, as loose caps can result in PCR failure or invalid detection.
- Keep samples chilled at 2-8°C until immediately before loading them into the instrument.

Note: *After loading samples into the BAX® System instrument, place the racks of lysates and store at 2-8°C until you have finished reviewing the results, then dispose of the waste according to your site practices. Cooling blocks and inserts should be wiped with 10% bleach, rinsed with deionized water and blotted dry. Return the inserts to the refrigerator.*

Tips for Interpretation of Melting Curves

- Only peaks in the expected locations should be used for interpretation. See the melting curve profiles at the end of each BAX® System assay protocol for details.
- A control peak should be visible at approximately 79°C for negative reactions. See the melting curve profiles at the end of each BAX® System assay protocol for details.

Note: *The melting curves for negative E. coli O157:H7 MP reactions are slightly different.*

- In a strong positive reaction, the control peak may be small or absent.
- Target peaks can shift within the specified temperature range. In strong positive reactions, the peaks may shift to slightly lower temperatures. In weaker positive reactions, the peaks may shift to slightly higher temperatures.

VII. BAX® System Hardware

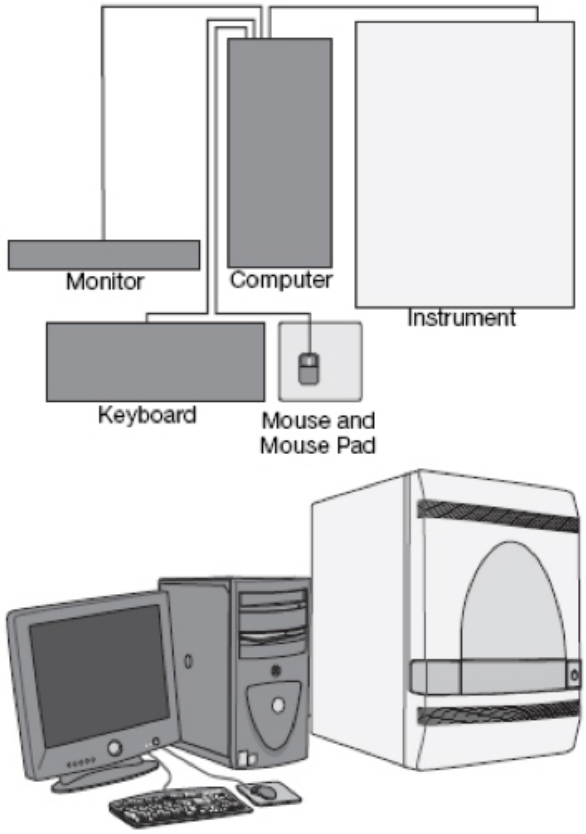
Installing the System

Before placing the system:

- Verify that the designated workbench supports at least 54.5 kg (120 lbs).
- Verify that the pathway to the final position of the instrument is clear of obstructions.
- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting. Coordinate your intentions with your assistant before lifting and carrying.
- Keep your spine in a good neutral position, bend at the knees and lift with your legs. Do not lift an object and twist your torso at the same time.
- When moving the computer, tilt the box on its side, then slide the contents out of the box.

To place the system:

1. Open the crate and remove any protective covers.
2. Lift and place the instrument onto the bench.
3. Unpack the computer boxes and place the monitor, computer, keyboard, and mouse on the bench as shown.



Suggested layout for BAX® System components.

⚠ WARNING - PHYSICAL INJURY HAZARD

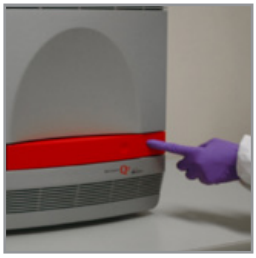
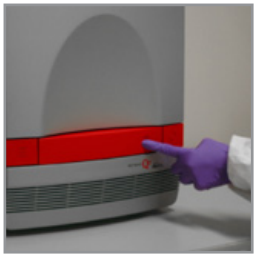
Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the instrument.

Connecting the System

Note: *To connect the BAX® System components, you'll need a thin screwdriver and a power cord.*

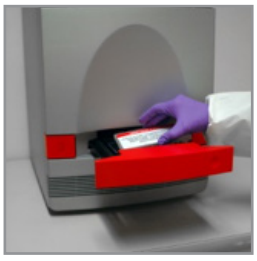
1. Connect the power cord to the instrument.
2. Connect the power cord to a receptacle wall circuit.


3. Press the power button and wait for the system to boot (about 30 seconds).
4. When the instrument displays the 'Power' status light, press the dimpled area on the right side of the drawer to open it.

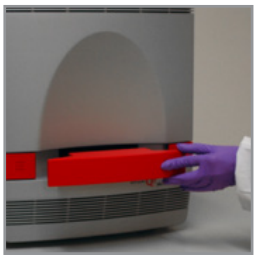
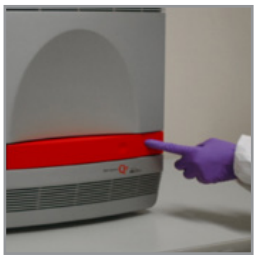
5. Remove the shipping plate from the drawer and set it aside.

Note: *Do not discard the shipping plate. It can be used to store or move the BAX® System Q7.*

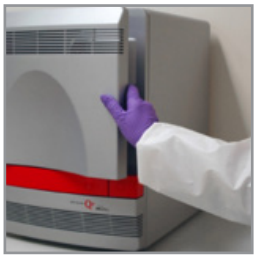



6. Close the drawer.
7. Press the power button to turn off power to the instrument.

Note: *When closing the instrument drawer, apply pressure to the right side and at an angle.*

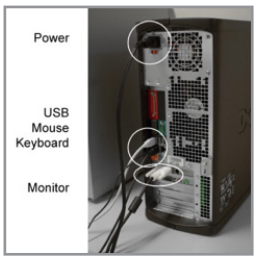
8. Insert a thin screwdriver or other thin, flat tool into the keyhole located on the edge of the access door.
9. Use the tool to carefully pry open the access door.

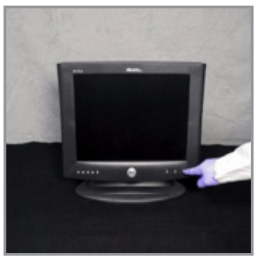
Connecting the System (Continued)

10. Make sure that the heating cover is pulled fully forward.
11. Close the access door of the instrument.

12. Connect the Universal Serial Bus (USB) cable to an available USB port on the computer.
13. Unpack and connect the mouse, the keyboard, and the monitor. Verify that all connections have been made correctly.



14. Power on the computer and monitor.



15. Connect the Universal Serial Bus (USB) cable from the computer to the back of the instrument.

Shutting Down the System

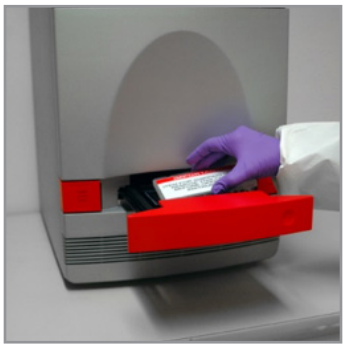
Short-term

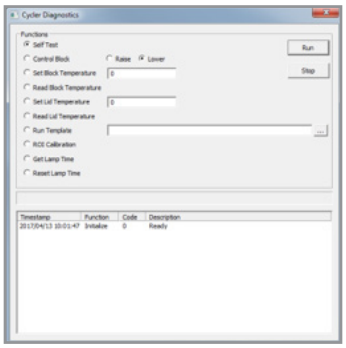
If you need to shut down the system for seven or fewer days, simply check that no rack is in the tray, then press the power button to power down the BAX® System instrument.

Long-term

If you are shutting down the system to move, ship or store it, follow these steps:

1. Load the original shipping plate that came with the system into the tray. If you no longer have the shipping plate, use an unused rack plate.
2. Select DIAGNOSTICS > CYCLER from the menu bar. The Instrument Diagnostics window opens.
3. Select **Control Block** and **Raise** from the list of options.
4. Click the **RUN** button. The bottom pane of the window displays the status.
5. Power down the instrument.





This procedure allows the instrument optics block to rest upon the plate, protecting it during storage or shipment.

Note: *Do not turn on power to the instrument after performing this long-term shutdown procedure until the instrument is in place for use. Powering on the instrument lowers the plate, exposing the optics block to potential damage during storage or shipment. If you power on the instrument by mistake, make sure to repeat steps 2-5 for long-term shutdown.*

Performing Maintenance

The BAX® System has been designed for ease of use and reliability. Careful design has created a system that does not require routine maintenance other than cleaning, as described below.

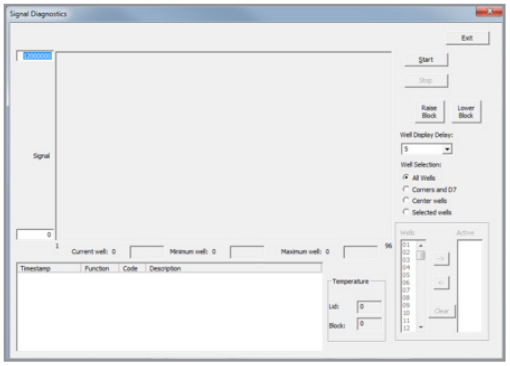
Routine maintenance

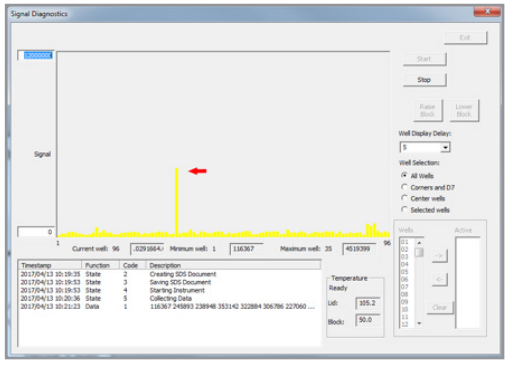
To prevent the accumulation of dust and debris wipe the instrument surfaces with a lint-free cloth. If needed, the casing can be wiped off with a damp cloth using mild soap or common laboratory disinfectant, such as 70% ethanol. Do not use cleansers containing iodine or acetone.

Cleaning the sample block

First, use the Well Signal Diagnostic to determine if the sample block should be cleaned. The Well Signal Diagnostic should be run if the background dye calibration plate fails, or according to the standard operating procedures of your laboratory. The results of this diagnostic allow you to see which wells are producing strong background signal – these are the wells that should be cleaned. Then, follow the steps below to clean the sample block with deionized water.

Note: Do not use bleach to clean the sample block.

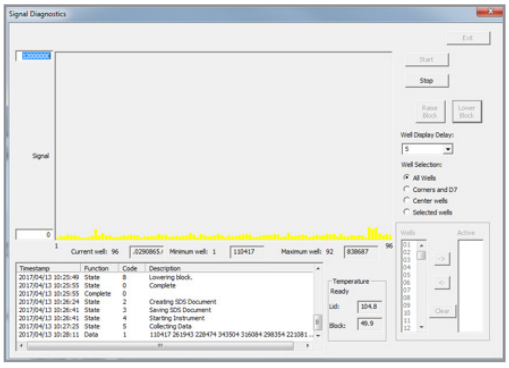




Part 1. Using the Well Signal Diagnostic

1. Make sure there are no samples or PCR tubes in the cycler/detector.
2. From the menu bar, select DIAGNOSTICS > WELL SIGNAL to open the Signal Diagnostics window.
3. Click the **START** button to run the Well Signal diagnostic. The results will display in the Signal Diagnostics window when the test is complete.
4. If one or more wells show a signal significantly higher than the other wells (see the example to the left), then the sample block should be cleaned. Click the **LOWER BLOCK** button to lower the sample block, then follow the procedure described below.

Performing Maintenance (Continued)



5. If the signal appears relatively low and uniform across the wells, the sample block does not need to be cleaned at this time.

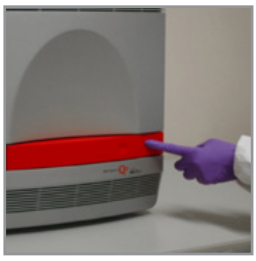
Note: *To save the results of the Well Signal Diagnostic, press the Print Screen ("PrtScn") button on the computer keyboard to create a screen capture of the results. Then, launch a program such as Microsoft® Word or Microsoft® Paint and select the Paste option to save the screen capture.*

Part 2. Cleaning the Sample Block

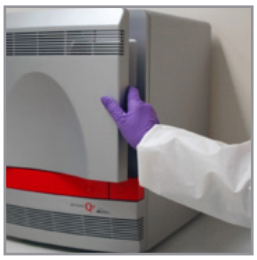
⚠ WARNING - PHYSICAL INJURY HAZARD

During instrument operation, the sample block can be heated as high as 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

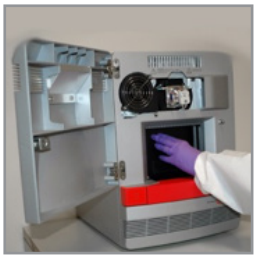
1. Power down and unplug the BAX® System instrument. Allow to cool for 15 minutes.

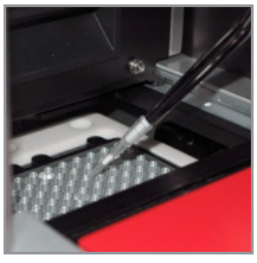


2. Insert a thin screwdriver or other thin, flat tool into the keyhole located on the edge of the access door.
3. Use the tool to carefully pry open the access door.



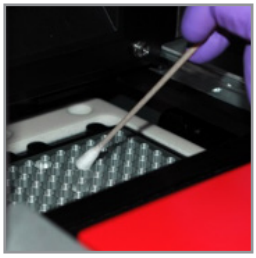
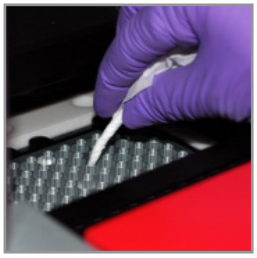
4. Remove the heated cover door to the back of the instrument.
5. Pipette a small volume of deionized water into each well (small hole). Let sit for 5 minutes.





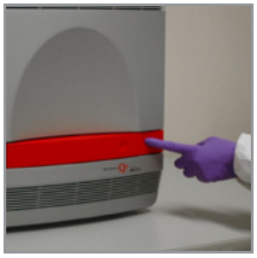
Part 2. Cleaning the Sample Block (Continued)

6. Scrub the inside of each well with a CleanFoam® swab (ITW Texwipe P/N TX751B)
7. Absorb the excess water with a lint-free cloth or a dry swab
8. Re-run the Well Signal Diagnostic described above to determine if the sample block has been cleaned sufficiently.
   a. If the block is clean, proceed to step #9.
   b. If the block is not sufficiently clean, repeat the "Cleaning the Sample Block" procedure using 95% ethanol instead of deionized water. Make sure to rinse the wells thoroughly with deionized water to remove all traces of ethanol before re-running the Well Signal Diagnostic.

9. Pull the heated cover door to the front of the instrument, and close the access door


10. Plug in and power on the BAX® System instrument

Changing the Fuse

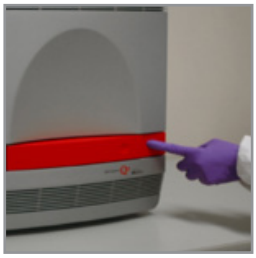
If the fan does not run, or the power indicator does not illuminate, or the halogen lamp does not work after replacing the bulb, a fuse may have to be replaced. You'll need a flat-head screwdriver to remove the fuse holders.

CAUTION - FIRE HAZARD

For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the same type and rating as those currently in the instrument.

Changing the Fuse (Continued)

1. Power down and unplug the BAX® System instrument. Allow to cool for 15 minutes.



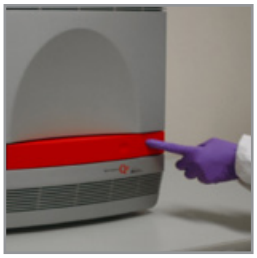

2. Using a flat-head screwdriver, unscrew and remove fuse holders from back of instrument.

3. Remove each fuse from its holder and inspect for damage. Carbon typically coats the inside of failed fuses.


4. Replace a failed fuse with a new one (12.5A, 250V, 5x20mm), then replace fuse holders in instrument.

5. Plug power cord into receptacle and power on the instrument. Installation is successful if unit powers on.

Note: *Fuse failure can be the result of fluctuations in the supplied power to the instrument. To prevent further failures, consider installing an electrical protective device*

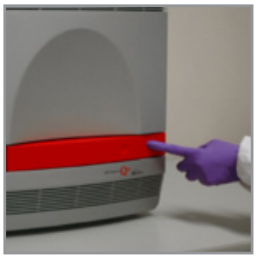



Changing the Halogen Bulb

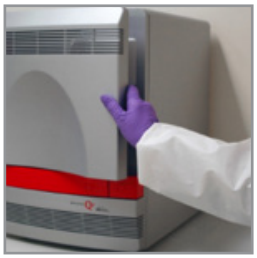
The bulb in the halogen lamp should be replaced after approximately 2,000 hours of life. After changing the halogen bulb, you must perform a dye calibration to re-calibrate the instrument and create a new calibration file. Follow the steps described in the next section, “Calibrating the Instrument”, to complete this process.

Note: *The quality of replacement bulbs can vary widely. For warranted results, use only bulbs that have been tested and approved for use with the BAX® System. You can order approved replacement bulbs from Hygiena (Part No. MIS2001 [D13758507]) or directly from AB (Part #4345287).*

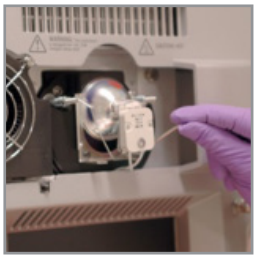
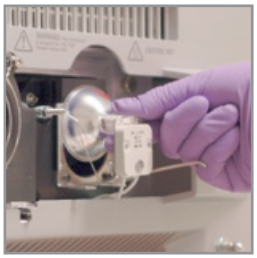
1. Power down and unplug the BAX® System instrument. Allow to cool for 15 minutes.



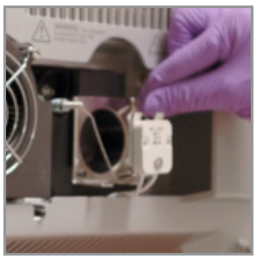
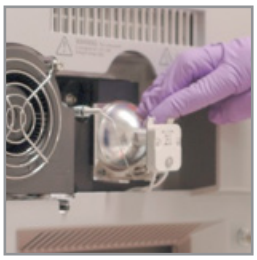

2. Insert a thin screwdriver or other thin, flat tool into the keyhole located on the edge of the access door.
3. Use the tool to carefully pry open the access door.

4. Pull the lamp release lever forward to release the halogen bulb from its mount.
5. Firmly grasp the failed bulb and lift it up and out of the slotted mount.

6. Push the lamp release lever back into the upright position.
7. Insert a replacement bulb firmly into the slotted mount and slide it down into place.

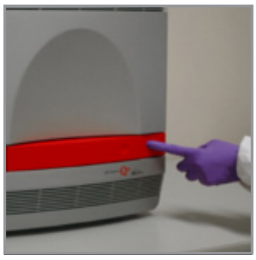



Changing the Halogen Bulb (Continued)

8. Close the access door, then plug the power cord back into the receptacle.
9. Power on the system.

10. Check that light is visible behind the grill on the front panel by selecting DIAGNOSTICS > CYCLER > SELF TEST from the menu bar, then click the **RUN** button.
11. Select DIAGNOSTICS > CYCLER > RESET LAMP TIME from the menu bar, then click the **RUN** button.

Note: *We recommend that you re-calibrate the instrument after changing the halogen bulb.*



Calibrating the Instrument

Note: *Calibration is important when running real-time PCR assays. For systems purchased prior to September 2006, contact your technical account manager for calibration before beginning to run real-time PCR assays.*

BAX® System Q7 instruments are calibrated prior to shipment. The calibration file is included on a CD that is attached to the back of the instrument for backup. Each time you reinstall the BAX® System application, **you must back up your calibration file** to safeguard against it being overwritten or damaged during the reinstallation process. See Chapter VIII: BAX® System Software for more details.

You must calibrate the BAX® System Q7 instrument under the following circumstances:

- The calibration report does not include the desired assay in the list of calibration files
- You have changed the halogen bulb
- Repairs were made to the instrument outside the U. S. and a new calibration file must be created

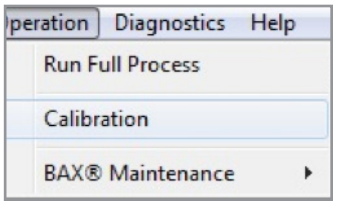
Note: *To ensure that calibration is performed successfully, clean the sample block and wells before performing a dye calibration. See "Cleaning the Sample Block" for instructions.*

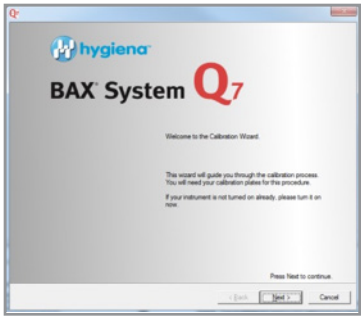
Calibration Kits

Calibration kits are available directly from Hygiena (Part No. KIT2026 [D12778652]).

Kits should be stored at -15°C to -25°C in the original foil envelope to protect it from exposure to light. Before use, bring the plates to room temperature and check that the plates are clean and the liquid levels are even across all the wells. Calibration kits can typically be used multiple times as long as they are kept frozen when not in use.

1. From the OPERATION menu, select CALIBRATION. The **Calibration Wizard** screen appears.
2. Make sure the Q7 instrument is powered on and click **NEXT** .





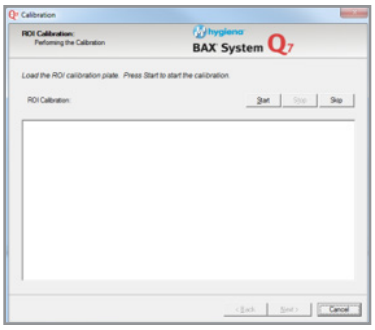
3. The next screen lists the 10 calibration plates needed to complete the 11 prompts of the calibration wizard.

   Click **NEXT** .

Note: *Plate order may vary during calibration.*

Calibration Kits (Continued)

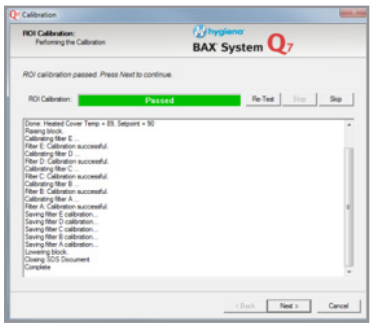
4. At the **ROI Calibration** screen, load the ROI plate and click the **START** button at the top of the window. The window displays a status bar and remarks on calibration progress.



Note: *Make sure calibration plates are at room temperature before use.*

Note: *Check plates before use. If there are droplets on the top film, flick the plate so drops return to the wells. If one or more wells have significantly less liquid, the calibration kit should be replaced.*

5. At the end of ROI calibration, the status bar displays a green "Passed" result. Click **NEXT** . At the prompt, load the **Background** plate and click **START** . Repeat this procedure at each prompt until all eleven calibration plates have passed.

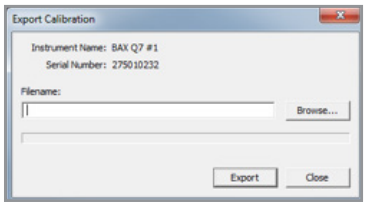


6. When you click **NEXT** on the last plate (Pure Dye VIC), the final screen appears. Click **FINISH** to exit the wizard.

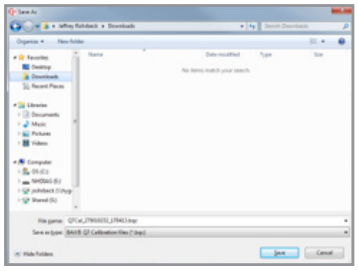
Note: *Make sure to remove the last calibration plate*

Calibration Kits (Continued)

7. Back up your calibration files to external media in case you need to restore them. From the OPERATION menu, select BAX® MAINTENANCE > EXPORT CALIBRATION.



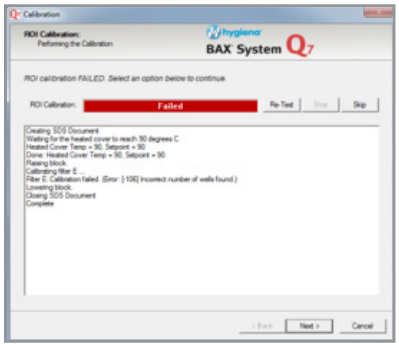
8. Click the **BROWSE** button to navigate to the folder where you want to store the file. The system suggests a name for the file based on the date.

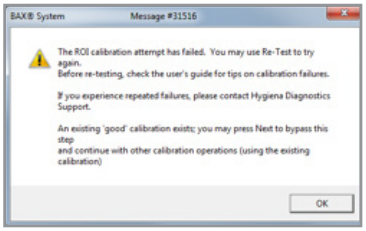


Calibration failure

If the plate does not pass calibration, the status bar displays a red “Failed” result.

A message box automatically appears that explains your options to re-test or continue with calibration using the last successful calibration file for that plate. Click **OK** to continue.





For tips on troubleshooting problems with calibration, see Appendix D: Troubleshooting.

Customized PPH Plate

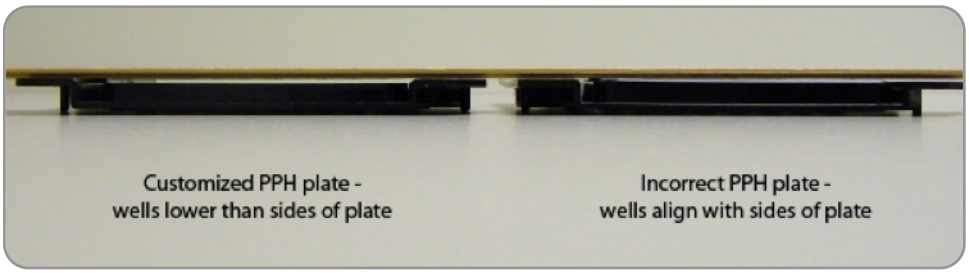
The BAX® System Q7 instrument is supplied with a customized PPH plate - the plastic rack inside the Q7 instrument drawer. The modifications made to this plate from the manufacturer's version provide for tighter seating of the PCR tubes included in BAX® System kits, which is needed for optimal performance.

If your lab also uses other thermal cyclers that are similar to the BAX® System Q7 instrument, there is a potential risk of confusing the PPH plates in each unit. Using a non-customized PPH plate in the Q7 instrument could interfere with performance. We recommend that whenever you clean the Q7 units or have them serviced, check that the correct customized PPH plate is in place before use.

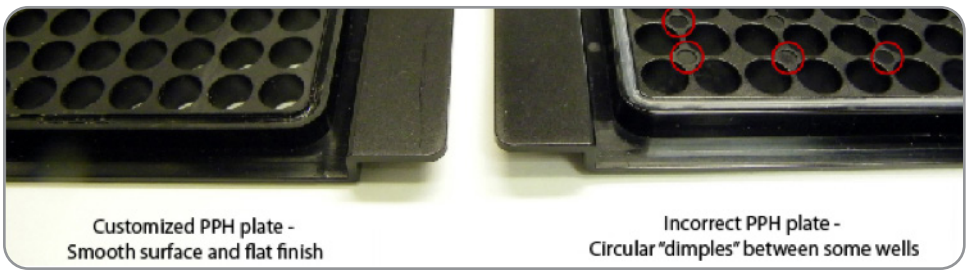
Modifications to the PPH Plate

To ensure that the correct customized PPH plate is used with your BAX® System Q7 instruments, check that the PPH plate contains the following features:

1. **The customized PPH plate does not have a rubber gasket around the perimeter of the wells.** The incorrect PPH plate may have a clear or white-tinted rubber gasket around the wells.



2. **The wells of the customized PPH plate have been milled down to be shorter than the sides of the plate.** The incorrect PPH plate has a well height that aligns with the sides of the plate.

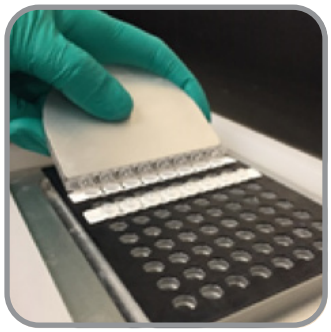


3. **The surface of the customized PPH plate is smooth and has a flat (not shiny) finish.** The incorrect PPH plate has a shinier finish and has circular "dimples" between some of the wells.

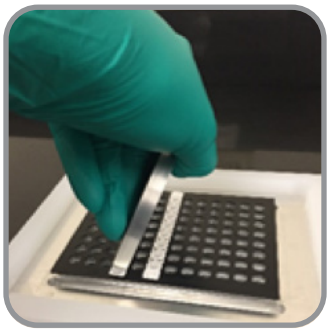
Capping/Decapping Tools

With your purchase of the BAX®System start-up package, you receive a tool* that facilitates decapping and resealing the PCR tubes. This tool allows you to remove the domed caps from a strip of up to eight PCR tubes at once, without jarring the contents of the tubes (A). The rounded end is used to reseal the tubes with flat optical caps, using a rolling motion (B).

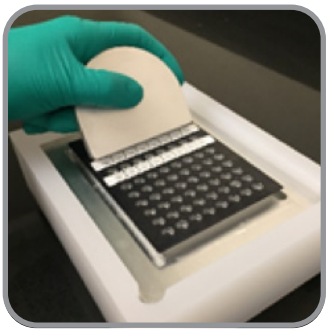
A. Removing Domed Caps from PCR Tubes



Place tool flange behind row of caps.

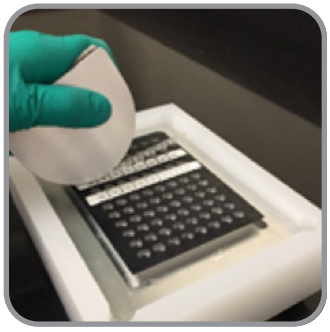


Tilt the tool forward and push down so that it locks over the caps.

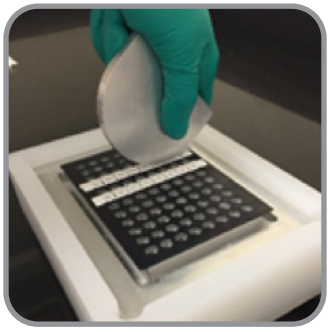


Tilt the tool backward so that the caps lift off.

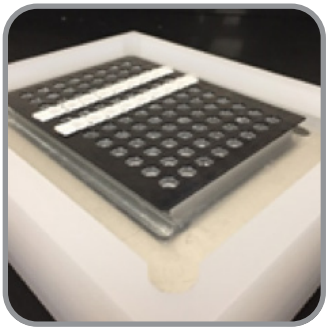
B. Sealing PCR Tubes with Flat Optical Caps



Place rounded edge of tool over first cap.



Roll the tool over the strip to lock the caps in place.



Visually inspect the caps to make sure they are seated.

*U.S. patent 5.967.001

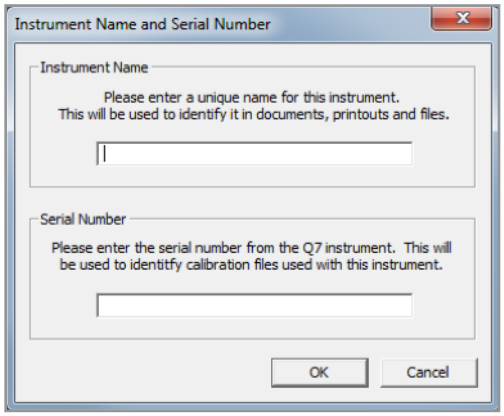
Shipping Instructions

In the event your BAX® System instrument needs service, please contact Hygiena Diagnostics Support to obtain a return authorization number and instructions for returning the unit:

- Thoroughly clean the unit of any biohazardous substances.
- Shut down the system for shipping, as described under long-term shut down procedures in this user guide.
- To avoid damage to the instrument, use ONLY the Hygiena-supplied loaner/replacement container for shipping the unit needing service.
- Ship the unit to the address on the return form.

VIII. BAX® System Software

The first time you open the application, you are prompted to enter a name for the BAX® System instrument to which the computer is connected. This name appears in the title bar of all windows, in files and on printed reports.



To change the instrument name later, select OPERATION > BAX® MAINTENANCE > UPDATE INSTRUMENT NAME AND SERIAL NUMBER.

You can run several copies of the BAX® System application concurrently. This is useful for reviewing data on other files while the instrument is performing PCR and analysis on a rack of samples.

The first instance of the BAX® System application normally opens in standard mode, which provides all the features necessary for operating the instrument. Any concurrent copies of the application are restricted to data analysis mode and do not allow for operation of the instrument.

Window Sections

The main window is divided into these sections:

Title bar – shows the currently open rack file and the instrument name. When you are creating a new file, the title bar displays “untitled” until you save it with a name.

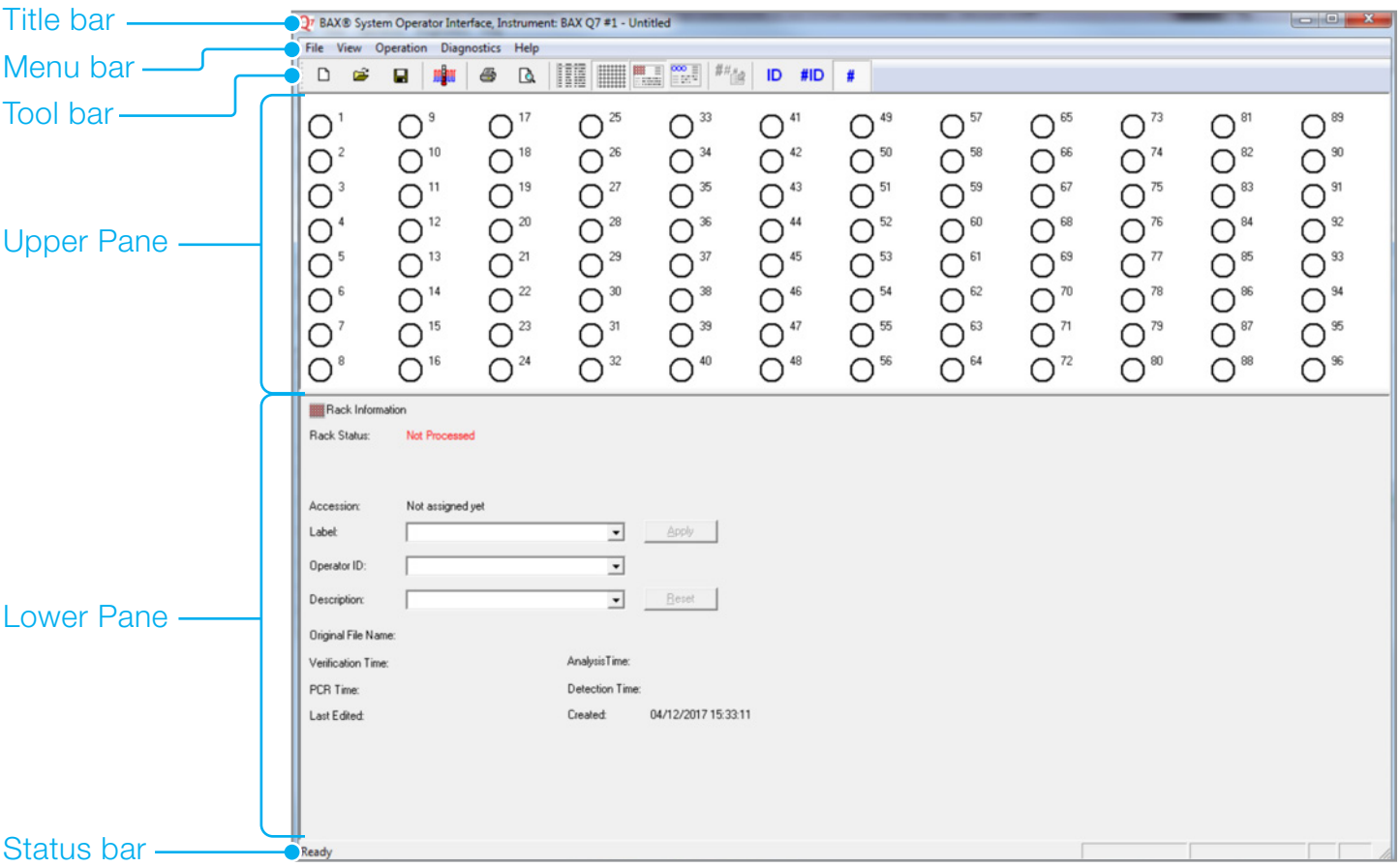
Menu bar – contains the main functions that you use to enter data, edit and otherwise manipulate the file.

Tool bar – displays icons of “shortcuts” for some of the common functions also found in the menu bar.

Upper pane – displays either a graphical layout that corresponds to the PCR tube rack when the grid icon in the tool bar is selected or a detailed report when the list icon is selected.

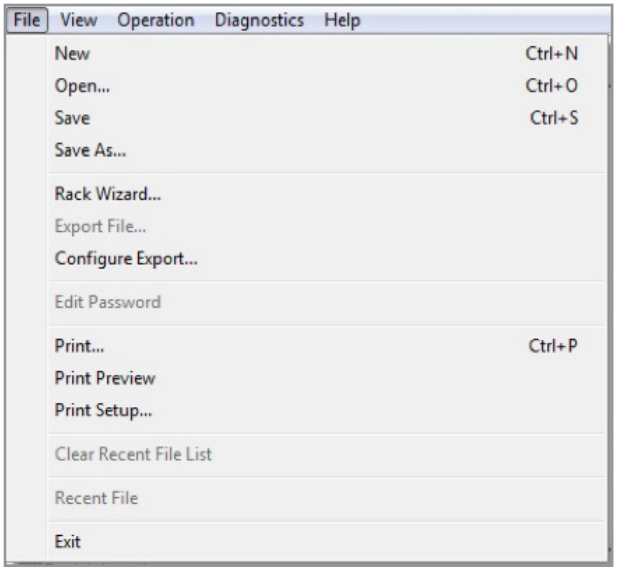
Lower pane – displays a form with fields for entering data. These fields change when you switch from “rack” view to “well” view.

Status bar – displays status messages, including explanations for the icons on the tool bar.



BAX® System application window elements

Menu Bar



FILE Menu

Creates, opens and saves your rack file. A rack file contains all the information you enter on a batch in rack view, on the individual samples in well view, and the test results.

NEW creates a blank file. If the current rack file has been edited, you are prompted to save it before the application creates a new file.

OPEN displays the standard Windows dialog box for navigating to the desired file. Files saved in this application use a ".bax" file extension.

SAVE writes the current file to disk.

SAVE AS allows you to save the current file under a different name.

RACK WIZARD guides you with screen prompts in creating a new rack file or importing an existing file.

EXPORT FILE allows you to save the file as delimited text to your folder of choice.

CONFIGURE EXPORT provides options for automatic export and for which data fields should be included in the text file.

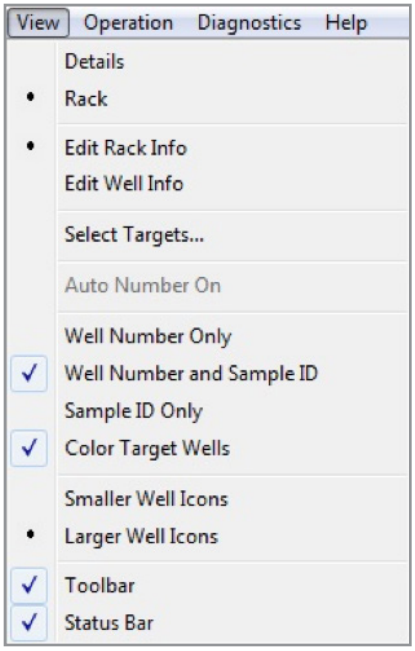
EDIT PASSWORD allows administrators to set passwords and users to change their passwords (available only if this security option was selected at installation – see "Security Options" in Chapter VIII: BAX® System Software).

PRINT, PRINT PREVIEW and **PRINT SETUP** perform according to the standard Windows methods.

CLEAR RECENT FILE LIST allows you to remove the list of recent files that appear in this section after use.

RECENT FILE is a placeholder for the list of recent files that appear in this section after use.

EXIT quits the entire application without asking for confirmation.



VIEW Menu

Allows you to switch among various views.

DETAILS displays all of your test results as a list in the upper pane.

RACK displays a graphical grid of the wells in the upper pane.

EDIT RACK INFO and **EDIT WELL INF**O display the data fields for rack or well information in the lower pane.

SELECT TARGETS opens a dialogue box that lets you define the targets in the drop-down menu list.

AUTO NUMBER ON toggles with **AUTO NUMBER OFF** to allow for customized numbering sequences.

WELL NUMBER ONLY displays the well numbers.

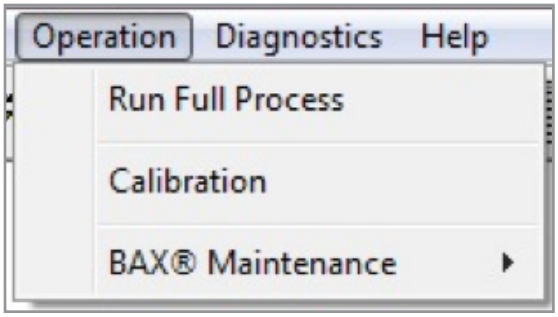
WELL NUMBER AND SAMPLE ID displays the well numbers and the ID you have assigned to each.

SAMPLE ID ONLY displays the wells with the ID you have assigned.

COLOR TARGET WELLS displays a target-specific colored box around each well.

SMALLER WELL ICONS and **LARGER WELL ICONS** toggle to adjust the size of your icons and font for longer label names.

TOOLBAR and **STATUS BAR** can be selected to display or deselected to remove from the window display.

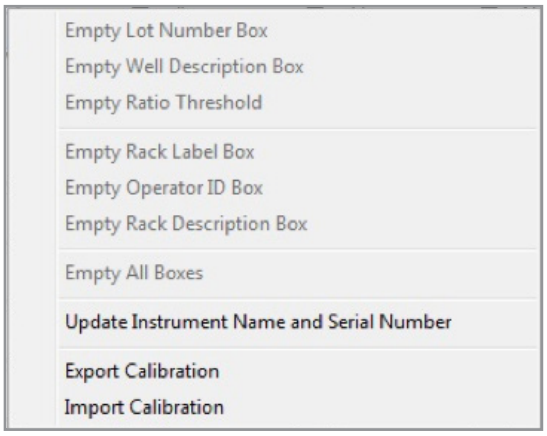


OPERATION Menu

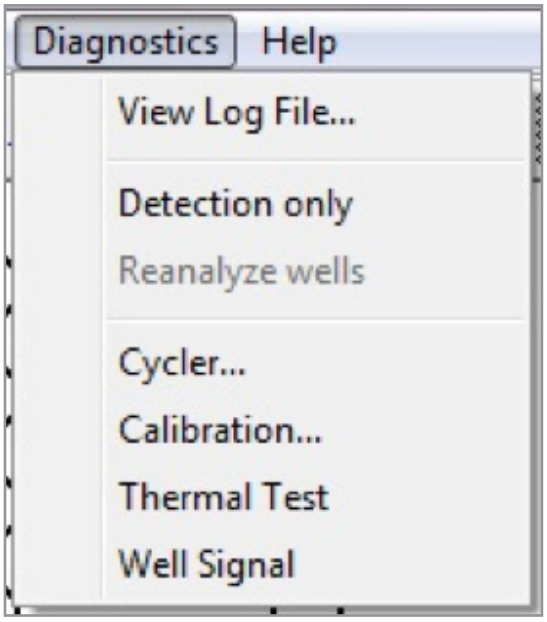
Allows you to process your samples and check the data.

RUN FULL PROCESS launches the PCR wizard for amplification and detection.

CALIBRATION launches the Calibration wizard that allows you to calibrate the instrument.



BAX® MAINTENANCE displays a submenu that allows you to clear the drop-down boxes on the lower pane of historical data, update the instrument name and serial number, and import/export calibration files.

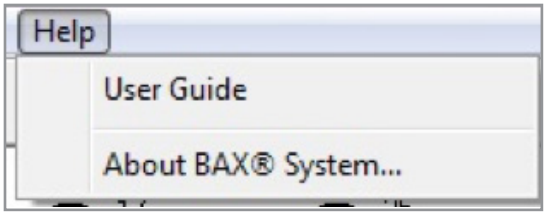


DIAGNOSTICS Menu

Provides for **VIEW LOG FILE, DETECTION ONLY** and **REANALYZE WELLS** features.

Allows access to **CYCLER, CALIBRATION, THERMAL** and **WELL SIGNAL** diagnostics, including self-test on the instrument.

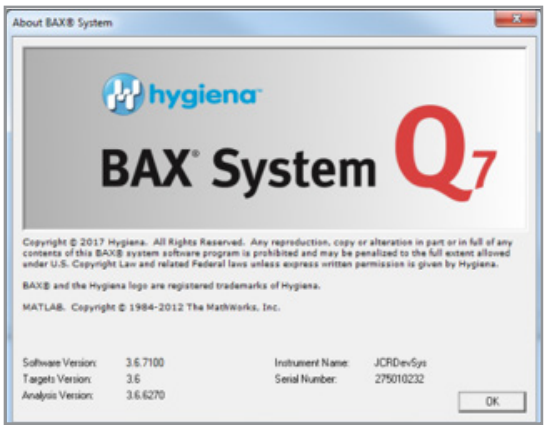
For Calibration Diagnostics, see “Troubleshooting Calibration” in Appendix D: Troubleshooting. For other tests, call Hygiena for instructions.



HELP Menu

USER GUIDE links to a PDF version of the user documentation that is installed with the application.

Note: *The first time you use this, you may need to install the Adobe Acrobat Reader from the Installation disk that was shipped with your system.*



ABOUT BAX® SYSTEM displays information about the current version of the application software, along with the instrument name, copyright and trademarks.

Tool Bar

The tool bar provides you with a series of quick-access buttons for common software features and commands.

	Create a new file
	Open an existing file
	Save the current file
	Run full process
	Print the current file
	Print preview
	Switch between the Details View and Rack view in the upper pane
	Switch between the Rack Information or Well Information view in the lower pane
	Auto number on/off
	Switch to display sample IDs only, well numbers with sample IDs, or well numbers only

Security Options

Your BAX® System startup package is shipped with the BAX® System application pre-installed on the computer. By default, the options for password protection and locked workstation are not enabled, thus allowing all users to launch the application and to use the workstation while samples are being processed.

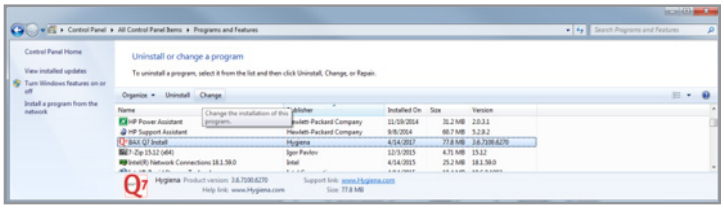
With password protection enabled, BAX® System passwords are required to launch the application. In addition, the workstation is automatically locked after a run has begun. This prevents canceling the run or otherwise interacting with the workstation while a run is in progress, unless unlocked via password.

Note: *BAX® System passwords are entirely separate from the passwords you use for the Windows® operating system. Before you can launch the BAX® System, you need to access the desktop by logging in with a Windows® password.*

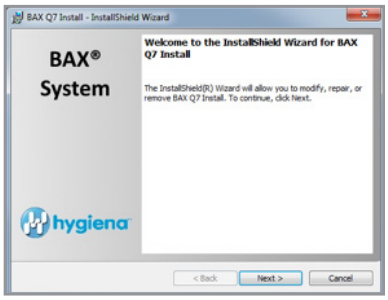
Modify the Installed Program

To enable the security features, you need to modify the BAX® System application, as described below.

1. After closing all windows and files, click the START button and select CONTROL PANEL > PROGRAMS AND FEATURES. A window appears that lists all currently installed programs.



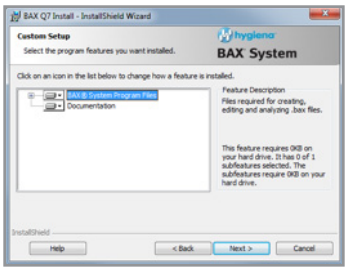
2. Scroll down the list to the BAX® System application and click the **CHANGE** button. This launches the Install Shield wizard. Click the **NEXT** button.



Modify the Installed Program (Continued)

3. Select **MODIFY** from the list of options in the Welcome window, then click the **NEXT** button. The Select Features window appears.

4. ClicK **NEXT** to modify the BAX® System program files.

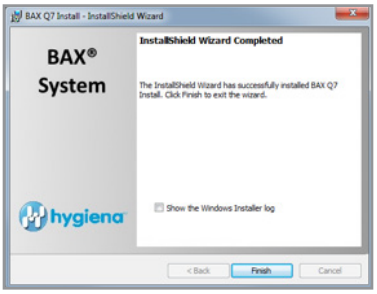


5. A window appears with the enable password option. Select “Enable Password Protection”.

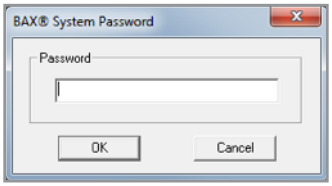
Modify the Installed Program (Continued)

6. Enter a password for the BAX® System application, and click **NEXT** .

7. Follow the wizard prompts to finish the maintenance. You do not have to restart your computer for the security features to take effect.



8. Each time you launch your BAX® System application, you will be prompted to enter a password.



Passwords

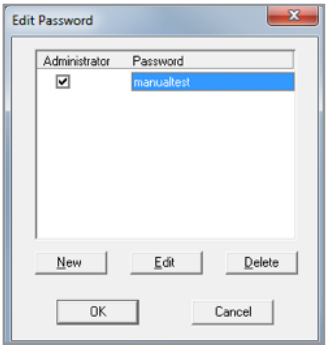
The person who sets the first BAX® System password is the administrator. If all the users in your lab share a single password, the administrator needs to tell them the BAX® System password so that they can launch the application and unlock the workstation during a run, if necessary.

Creating Additional Passwords

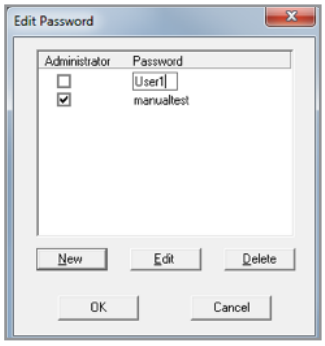
The administrator can create one or more additional BAX® System passwords for other users without administrator permissions, as follows:

1. After launching the BAX® System application with the established password, select FILE > EDIT PASSWORD from the menu bar. A window appears with the administrator box checked and its associated password visible

   This window is only available to administrators. It allows you to create BAX® System passwords for additional users, edit and delete them or give them administrator status.



2. Click the **NEW** button to create a second user. The Administrator box and the Password box are blank. Enter a password and click **OK**. Repeat this step for as many passwords as are needed.



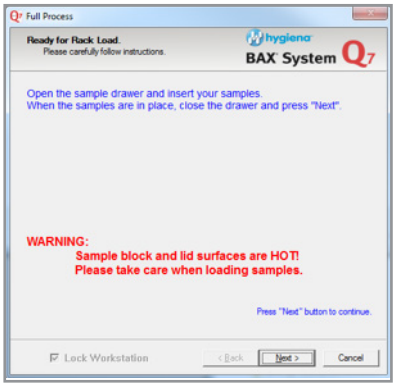
Changing Passwords

1. Users without administrator status can change their BAX® System password by launching the application under the password created for them by the administrator, then selecting FILE > EDIT PASSWORD from the menu bar. The Edit Password window appears.

2. Enter the BAX® System password assigned to you by the administrator in the old password field, then enter a new password, verify it and click **OK**. Each time you launch the BAX® System application, you can use your newly defined password.

Locked Workstation

With password protection enabled, the workstation automatically locks while amplification/detection is in progress on the instrument. The Ready for Rack Load window displays a checkbox with “Lock Workstation” message at the bottom.



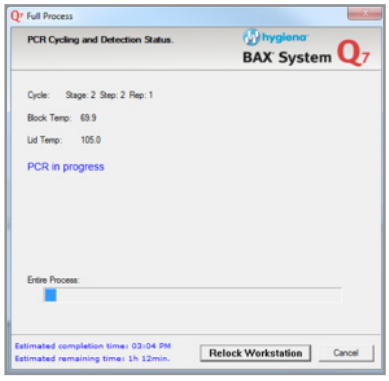
Click the **NEXT** button to begin amplification. A “Workstation locked” message travels around the window to remind you of this status.

If you move the mouse or touch any key after amplification has begun, a blank background covers the entire screen.

To unlock the workstation, enter the BAX® System password that was used to launch the application, and click **OK** .

The amplification/detection status windows reappear, but the workstation is now unlocked. At this point, you can cancel the run or open other applications.

To re-lock the workstation, click **RELOCK WORKSTATION** button.

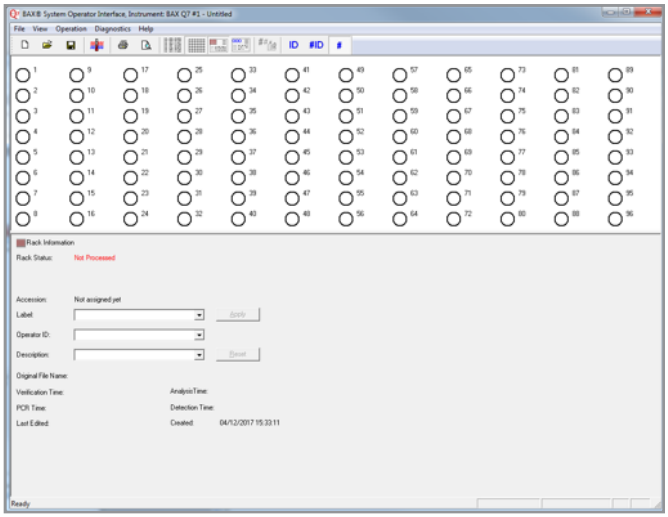


Creating a Rack File

1. Launch the BAX® System application from the shortcut on the desktop.

   Note: *If you are using security options, you are prompted to enter a password. See "Security Options" in Chapter VIII: BAX® System Software for more information*.

2. The screen displays a window with an empty rack view. This window is divided into an upper pane, which displays a grid of 96 blank wells and a lower pane with fields for data entry. The lower pane changes when you switch between "rack" and "well" view, as described in the following sections.



Empty rack view

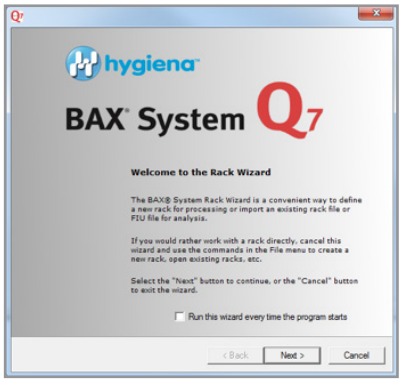
The rack file contains all the information on your samples that is required for analysis. You need to create this file and enter some information before loading your samples and running the program.

3. Select FILE > RACK WIZARD to create a rack file using screen prompts. You can also choose to enter the information manually.

Create a Rack File with the Rack Wizard

Select FILE > RACK WIZARD from the menu bar. You can choose to continue with the wizard by clicking **NEXT** or define a rack manually by clicking **CANCEL** .

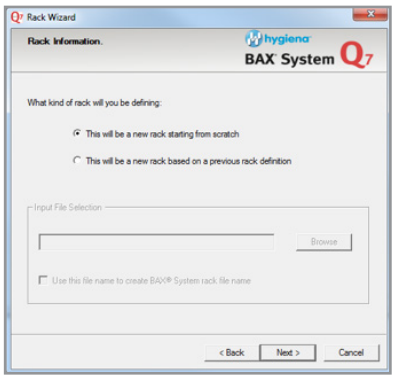
Note: *Use the checkbox at the bottom of the window to automatically run the rack wizard at startup.*



Choose the type of rack file you want to define:

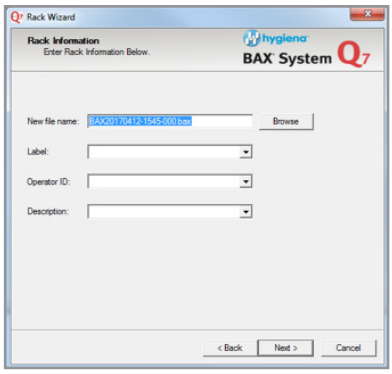
- New
- Based on a previous rack

Click **NEXT** to continue



Enter File Name, Label, Operator ID and Description. A file name based on the date is automatically generated, which you can change at this time.

Click **NEXT** to continue.



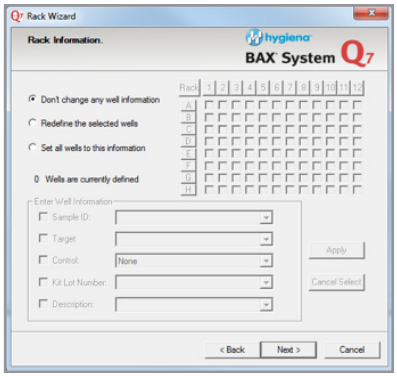
Create a Rack File with the Rack Wizard (Continued)

Choose how you want to define the information on your samples:

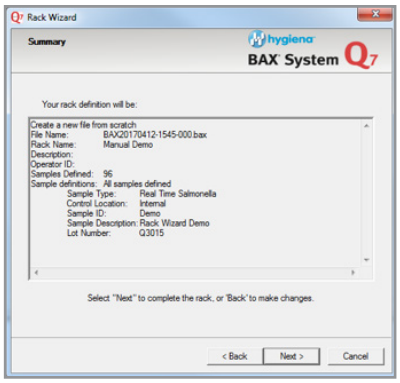
- Don't change any information
- Redefine selected wells
- Redefine all 96 wells

You can select wells by clicking on a row, a column or individual wells in the grid. Under "Well Information," you must select the Target from the drop-down menu. Other fields allow you to enter the Kit Lot Number, Sample ID and Description, if desired.

Complete the data fields, then click **APPLY**. When all sample information has been applied, click **NEXT** to continue.

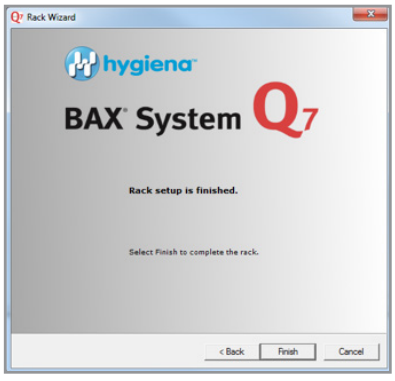


If you have created a new rack, a summary window appears that details the rack information. If you have imported a rack file, a shorter summary is displayed. After reviewing your definitions, click **NEXT** button to continue.



A final window appears that tells you the rack setup is complete.
Click **FINISH** to create a rack file with the name you assigned in the steps above and a ".bax" extension.

Note: *Once the rack file is created, you can change sample information by following the directions below for defining/editing wells.*



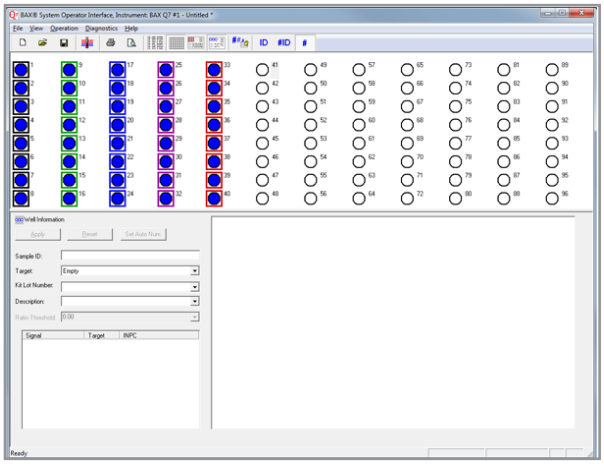
Manually Create a Rack File

1. At the empty rack view, select FILE > SAVE AS from the menu bar to create a new rack file with the ".bax" extension.

   Note: *If you don't save the file at this time, you are prompted to save it before the amplification begins.*

2. In the rack view lower pane, enter rack information:
   a. Enter a Label (optional) for the rack. Press the TAB key to move to the next field.
   b. Enter the Operator ID (optional).
   c. Enter a Description of this rack (optional).
3. Click on the **APPLY** button.

   Note: *After the rack file has been created, you can edit the information and click on the APPLY button to have those changes take effect on screen. However, the file itself is not modified until you select FILE > SAVE from the menu bar.*

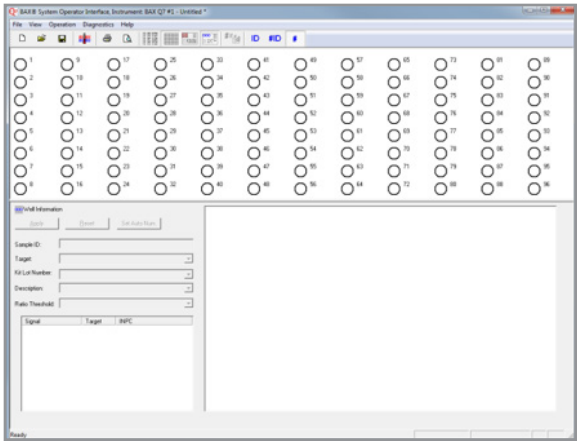


Defined rack

Define or Edit Wells

After you have defined your rack, you need to enter information on individual samples. This is done via the “well view” window, which appears when you click on a well in the upper pane. The upper pane of the window remains the same, but the lower pane changes to display information fields on each specific sample.

Switch to well view by clicking on a well in the upper pane. A different lower pane appears.



Empty well view

Note: *You can also get a well view by selecting VIEW > EDIT WELL INFO on the menu bar, or by clicking on the icon in the toolbar*

You can return to rack view any time by selecting VIEW > EDIT RACK INFO on the menu bar or by clicking on the icon in the toolbar

Enter Sample Information

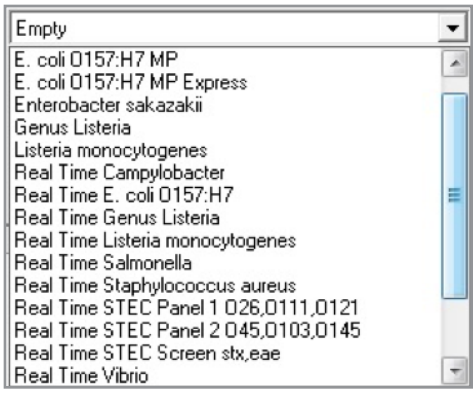
1. If you are not using the Auto Number feature (see next section), click on the first well, then enter the **Sample ID** (optional). Press the ENTER key to move to the next well and enter ID information.

   Note: *If you press the TAB key, you will move to the next data field for that well. If you press the ENTER key at any time, you will move to the same data field for the next well.*

2. Select and highlight all the wells with the same target organism.

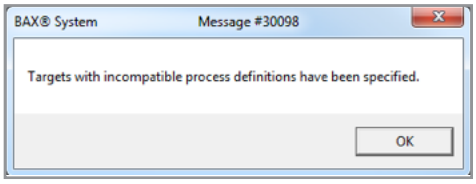
   Note: *You can enter data on a group of wells by clicking the mouse on the upper pane, and dragging it across a row or column to highlight the desired wells. You can also select a group by clicking on wells or columns, then pressing the CONTROL key and clicking on additional wells or columns. Any data you enter in the form is then applied to all the selected wells.*

3. Select the appropriate **Target** from the drop-down menu of possible targets. The **Control** type is automatically entered. These fields are required in order to run the process.



You have four target choices when testing for E. coli O157:H7:

- *E. coli O157:H7 MP –for single or mixed target batches using the MP assay (Part No. KIT2004 [D12404903])*
- *E. coli O157:H7 MP Express – for faster processing of single-target batches using the MP assay (Part No. KIT2004 [D12404903]). You cannot test for any other targets in the same rack when using MP Express.*
- *Real Time E. coli O157:H7 - for single-target batches using the real-time assay (Part No. KIT2000 [D14203648])*
- *Real Time E. coli O157:H7 EXACT - for single-target, faster batches using the real-time assay (Part No. KIT2039)*



You have two choices when testing for yeast and mold:

- *Yeast and Mold Enriched – use on samples that have been enriched. Cannot be mixed with other targets.*
- *Yeast and Mold Direct – use on samples that have not been enriched. Cannot be mixed with other targets.*

Note: *Except for MP Express, and Yeast & Mold protocols, the standard BAX® System PCR assays use the same set of cycling conditions, so you can test for mixed targets in a single rack. Some BAX® System real-time PCR assays also share a common set of cycling conditions, allowing you to mix these assays in a single rack. However, you cannot combine standard assays and real-time assays in the same rack. If you try to select incompatible targets, an error message appears.*

Enter Sample Information (Continued)

4. Enter the BAX® System Kit Lot Number from the box label (optional).
5. Use the Description field to enter any additional information (optional).
6. For Yeast and Mold Direct samples (see Chapter V: Protocols for Quality Assays), enter a customized Ratio Threshold (optional).
7. Click on the **APPLY** button. The grid displays blue filled circles to indicate defined samples. The list box in the lower half of the screen displays differently for standard PCR assays than for real-time PCR assays. Select FILE > SAVE from the menu bar.

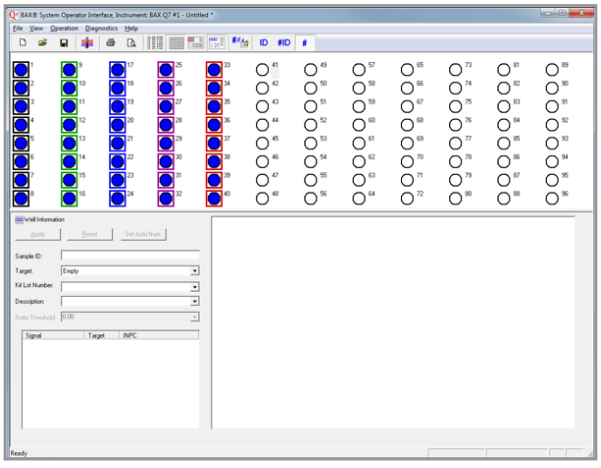
Note: *You can edit information in this file prior to loading your samples into the instrument. After the samples have been processed, you cannot edit the file. Any changes must be saved under a new file name.*

Setting Optional Preferences

Color Box Around Target Wells

Under the VIEW menu, you can select the COLOR TARGET WELLS toggle option. This will display a colored box around each well. Each color is specific to a target (also see kit label color), and thus provides a visual cue for tracking samples.

STANDARD ASSAYS		REAL-TIME ASSAYS	
Black:	*Salmonella*	Black:	*Salmonella*
Red:	*E. coli* O157:H7 MP *E. coli* O157:H7 MP Express	Orange:	*E. coli* O157:H7
		Red:	*E. coli* O157:H7 EXACT
Blue:	*Listeria monocytogenes* (including 24E)	Orange + Yellow:	STEC Screening (stx and eae)
		Orange + Brown:	STEC Panel 1 (O26, O111, O121)
Green:	Genus *Listeria* (including 24E)	Orange + Red:	STEC Panel 2 (O45, O103, O145)
		Green:	Genus *Listeria*
Mauve:	*Cronobacter*	Blue:	*L. monocytogenes*
Light Green:	Yeast and mold	Lilac:	*Staphylococcus aureus*
		Aqua:	*Vibrio cholerae/parahaemolyticus/vulnificus*
		Gold:	*Campylobacter jejuni/coli/lari*
		Brown:	*Shigella*



Defined wells in a mixed batch with Color Target Wells selected

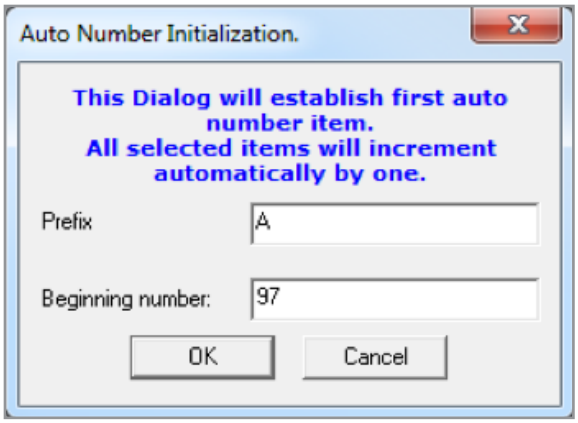
Auto-Number Wells

By default, the wells in the upper pane are numbered sequentially from 1 to 96. You can choose three different ways to display information in the upper pane by clicking on the appropriate icon in the tool bar:

#	Displays the well numbers only.
#ID	Displays the well numbers along with any Sample ID information you have entered in the lower pane.
ID	Displays only the Sample ID information you have entered for each well.

If desired, you can assign a different numbering scheme to your samples through the Auto Number feature. This option uses an alphanumeric sequence in place of Sample ID.

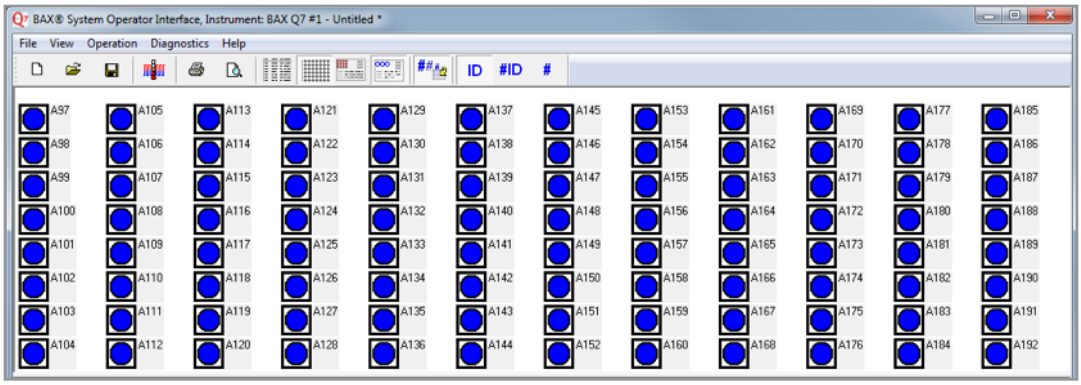
1. Make sure a Target (from the drop-down menu in the lower pane) has been selected for every well that contains a sample. When you click on **APPLY**, the selected wells change to blue-filled circles.
2. Select the group of wells you wish to auto-number.
3. Click on the ID icon in the tool bar, then click on the Auto Number icon. A window appears with two optional ID fields.



4. If desired, enter an alpha prefix (up to two characters) for all your samples.
5. If desired, enter a different initial number for this series of samples.
6. Click **OK**. The well view grid changes the labeling on blue-filled circles to reflect your auto-numbering scheme.

Note: *The auto-number scheme does not overwrite any Sample ID information that you have already entered manually.*

Auto-Number Wells (Continued)



Well view with Auto Number ON

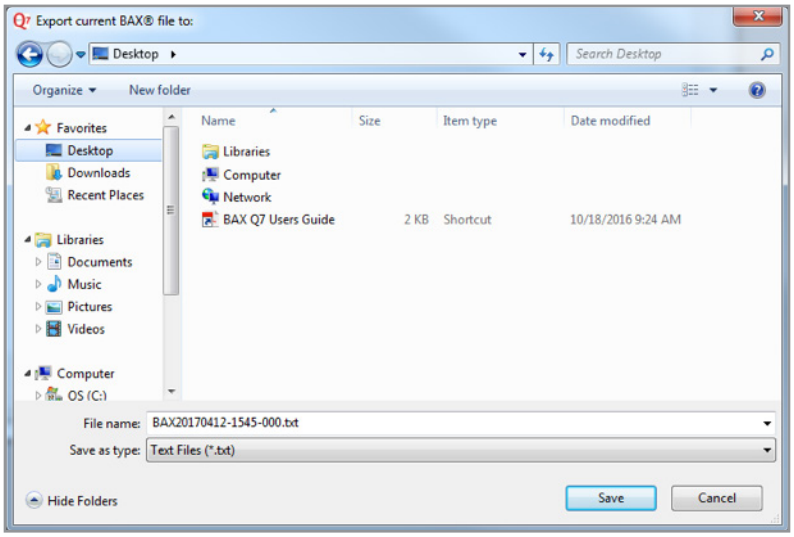
Note: *If desired, you can print a hard copy of these defined samples to use as a tracking sheet for the rest of the procedure. Click on either the* ***ID*** *or* ***#ID*** *icon, then choose FILE > PRINT > RACK REPORT from the menu bar.*

Smaller Well Icons

Under the VIEW menu, you can select the SMALLER WELL ICONS toggle option. This will re-size the display of the well icons to allow longer labels for each sample, both on-screen and in printed reports.

Configure Export Preferences

These options allow you to export information from your rack, including results, as tab-delimited text for integration with spreadsheets and other applications. You can export your rack file anytime by selecting FILE > EXPORT FILE from the menu bar. A “SAVE AS” export window appears that allows you to choose the folder in which to save this “.txt” file.

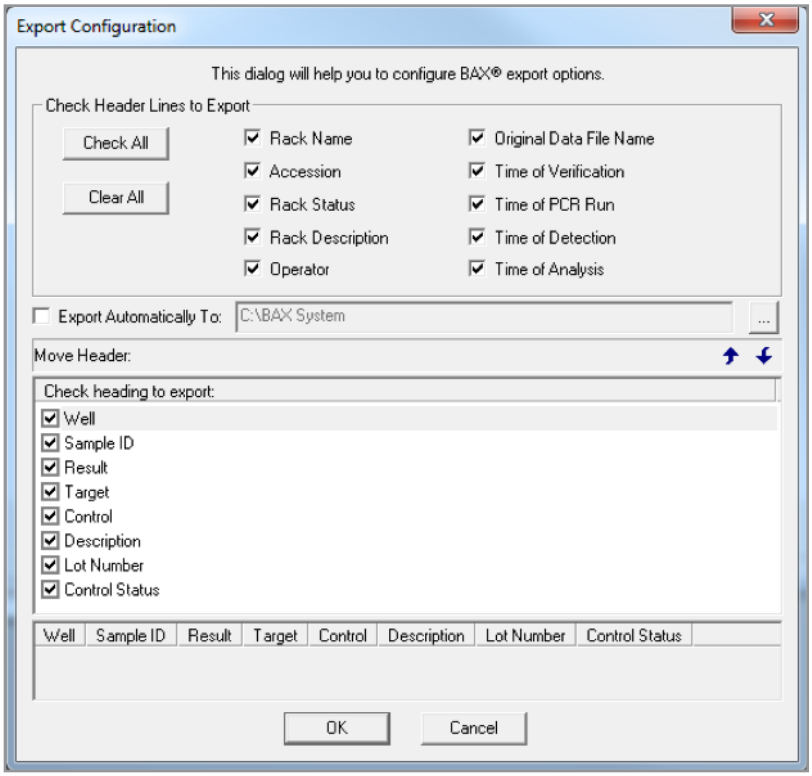


Export window

Configure Export Preferences (Continued)

You can configure your export preferences to include or exclude specific data fields, and you can re-order these fields to meet your import needs. You can also identify a folder to which this text file will automatically be exported at the end of the detection phase.

1. Under the FILE menu, select CONFIGURE EXPORT. A new window appears with three sections of options.
2. In the top section, check the data fields for rack and instrument information you wish to include in the header of the text file.
3. If you check the box in the center section, text files are automatically generated and exported at the end of the detection phase to the folder you select.
4. In the lower section, check the fields for sample information you wish to include in the text file. Click on one of the arrows in the “Move Header” box to move the highlighted field higher or lower in the order. A row of headings below these fields displays the order you have set for the columns.



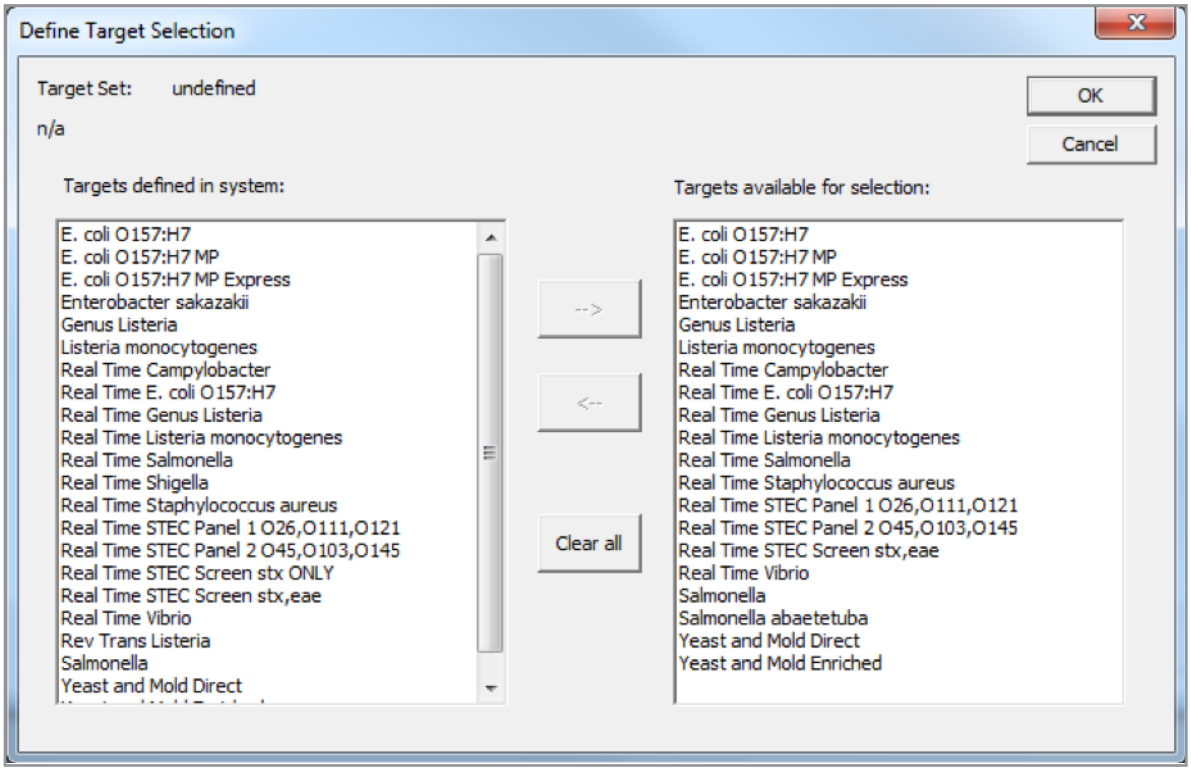
Export window

Select Targets

You can customize the list of targets in the drop-down Target menu (see “Window Sections” in Chapter VIII: BAX® System Software) to meet the needs of your lab.

Under the VIEW menu, choose the SELECT TARGETS option to display a dialogue box showing the complete system list of targets on the left and your defined list of targets on the right. To add to your defined list, highlight the desired targets in the system list, then click the right-arrow key. To remove targets from your defined list, highlight them, then click the left-arrow key. The CLEAR ALL button deletes all targets from the defined list.

Note: *If you are running the STEC Screening assay with the “stx only” option, you must manually add the target called “**Real Time STEC Screen stx ONLY**” to your drop-down menu of available target programs.*



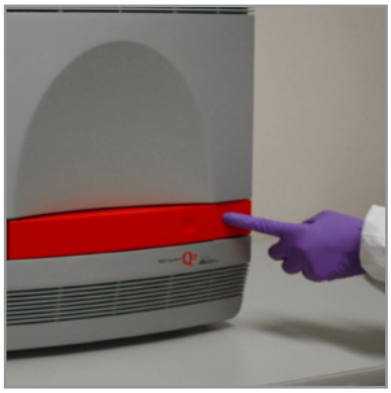
Define target selection dialogue box

Running a Full Process

Initialize the Instrument

Prior to loading samples for a full process run, the instrument requires about five minutes to heat to the appropriate temperature. We recommend that you initialize the instrument before starting lysis so that the program is ready to accept chilled samples immediately after you hydrate the PCR tablets.

1. Power on the BAX® System Q7 instrument.

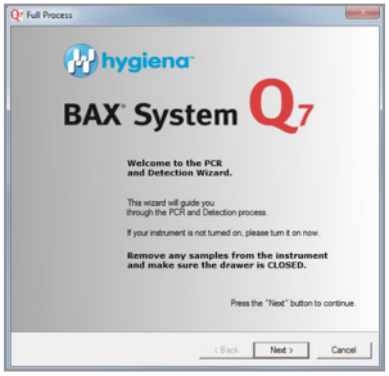


2. From the OPERATION menu, select RUN FULL PROCESS. This launches the PCR and Detection Wizard, which prompts you through the process.

   Note: *You can also launch a full process run by clicking the icon in the tool bar.*

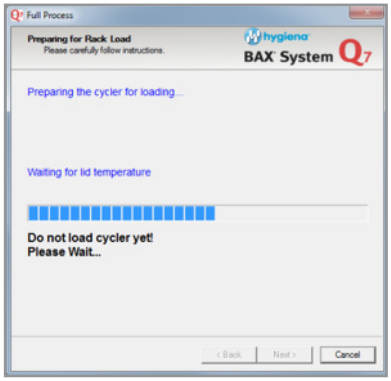
   

   If you have not saved your rack file, a navigation window appears for you to name and save the ".bax" file before processing.



A new screen automatically appears as the instrument begins to heat to its set temperatures. A message at the bottom of the screen warns you not to load samples yet.

While the instrument is heating, you can continue with lysis.

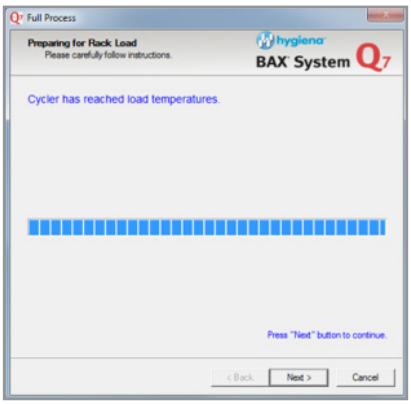


Amplify and Detect

PCR tablets must be loaded into the instrument immediately after hydrating in order to ensure accurate results. If you encounter a delay before loading, discard prepared samples and use additional sample lysate from cluster tubes to hydrate new PCR tablets as described above.

Follow the screen prompts in the PCR Wizard to load your samples, run the program and unload your samples:

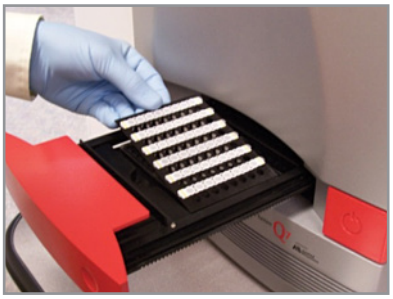
Preparing for Rack Load – displayed while the instrument warms to the correct temperature for loading the rack. Flashing text prompts you to wait. When the instrument reaches the appropriate load temperature, the wizard prompts you to click the NEXT button to continue.

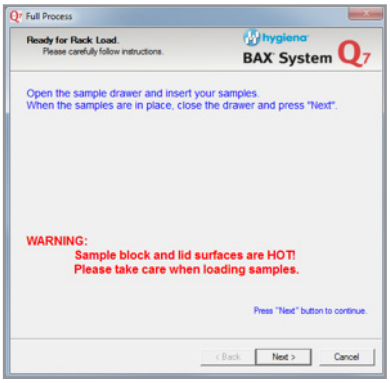


Ready for Rack Load – prompts you to load your samples.

1. Open the drawer by pressing the dimpled area on the right side.
2. Place the rack of PCR tubes into the instrument according to your rack file. Visually check to make sure the tubes are seated correctly and that the caps are sealed tightly.
3. Press the dimpled area to the right and push gently to close the drawer.
4. Click the NEXT button.

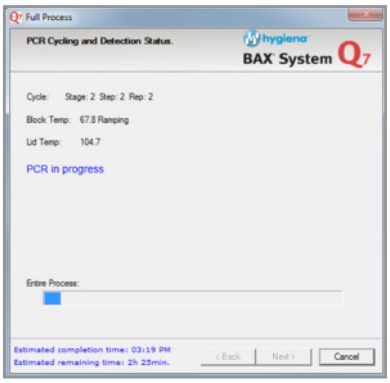
CAUTION: The heating block below the drawer is HOT! Take care to avoid touching this heated surface.





Amplify and Detect (Continued)

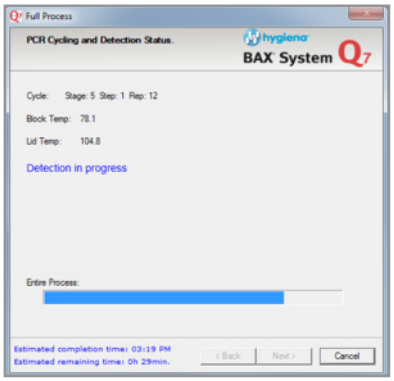
PCR Cycling Status –displays a status bar while the amplification portion of the program is running



Detection Status – displays a status bar while the detection portion of the program is running.

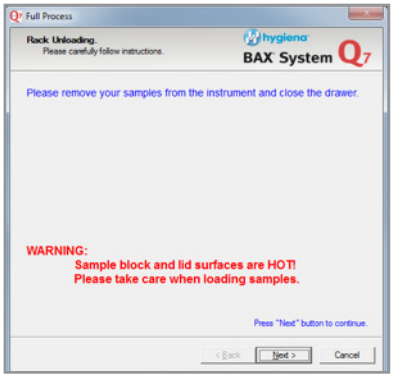
When this phase is finished, the instrument automatically shuts off heat to the block. If you have selected the automatic export option, a text file is sent immediately to the designated folder.

Note: *This screen does not appear when running real-time PCR assays because detection is integrated with amplification.*



Rack Unloading – prompts you to remove your samples. Open the drawer and carefully remove the rack of PCR tubes. Place the rack of samples in a bag and store in the refrigerator until you have finished reviewing the results, then dispose of waste according to your site practices.

Racks should be wiped with 10% bleach, rinsed with deionized water and blotted dry.



Process Completed Normally – Click the **FINISH** button to review the results on your processed samples.

Processing time

For standard PCR assays, automated amplification and detection in the BAX® System instrument takes approximately 3.5 hours to complete. The *E. coli* O157:H7 MP Express protocol reduces the processing time to approximately 2.5 hours.

For real-time PCR assays, processing is complete in 65 to 75 minutes, depending on the assay.

Some BAX® System assays can be run together in a single process in the BAX® System instrument. Refer to the table below to determine which assays are compatible.

<table>
<tr><th>Compatible Assays</th><th>Processing Time</th><th>Compatible Assays</th><th>Processing Time</th></tr>
<tr><td rowspan="3">Standard PCR assay for Salmonella
Standard PCR assay for E. coli O157:H7 MP
Standard PCR assay for Cronobacter
Standard PCR assay for Genus Listeria
Standard PCR assay for L. monocytogenes
PCR assay for Genus Listeria 24E
PCR assay for L. monocytogenes 24E</td><td rowspan="3">3 hours, 30 minutes</td><td>Real-time PCR assay for Salmonella
Real-time PCR assay for Genus Listeria
Real-time PCR assay for L. monocytogenes</td><td>75 minutes</td></tr>
<tr><td>Real-time PCR assay for Campylobacter
Real-time PCR assay for Staphylococcus aureus
Real-time PCR assay for Vibrio</td><td>70 minutes</td></tr>
<tr><td>Real-time PCR assay for E. coli O157:H7
Real-time PCR assay for E. coli O157:H7 EXACT
Real-time PCR assay for STEC Suite
Real-time PCR assay for Shigella</td><td>65 minutes</td></tr>
<tr><td>Standard PCR assay for E. coli O157:H7 MP Express</td><td>2 hours, 30 minutes</td><td>Standard PCR assay for Yeast and Mold</td><td>3 hours, 45 minutes</td></tr>
</table>

Reviewing Results

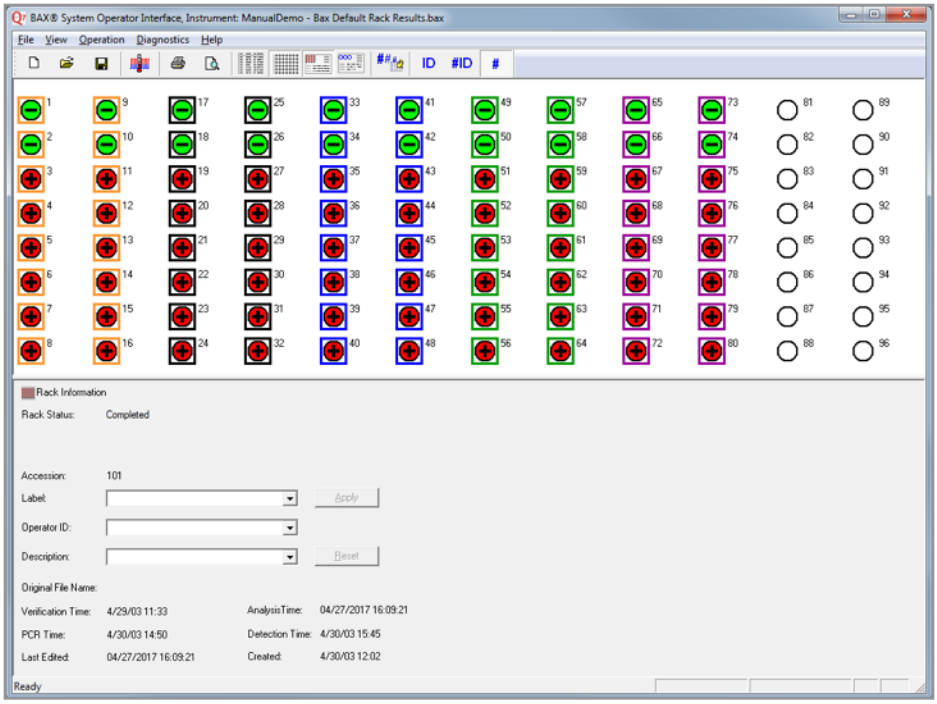
Standard PCR Assays

After you click on the **FINISH** button, a new window displays a modified rack view (see next page). The wells now appear in different colors with a symbol in the center to illustrate the results. You can view these results in rack view, rack details, well view and well details, as shown below.

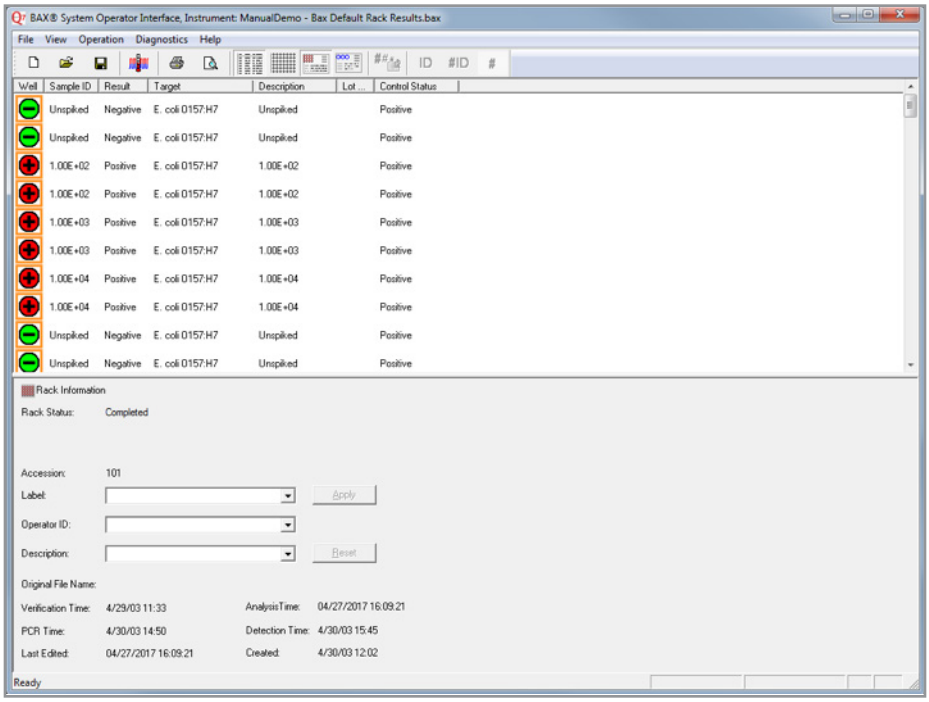
	Green (-)	=	Negative result
	Red (+)	=	Positive result
	Yellow	=	Indeterminate result (see Appendix D for troubleshooting)
	Yellow (?) with red slash	=	Signal error (see Appendix D for troubleshooting)
	Orange with check mark	=	Yeast and Mold Direct test has run successfully – see Detail View for ratio value

Rack View

The rack view shows all results along with information on the rack. You can display the rack results as a grid or as a detailed list.



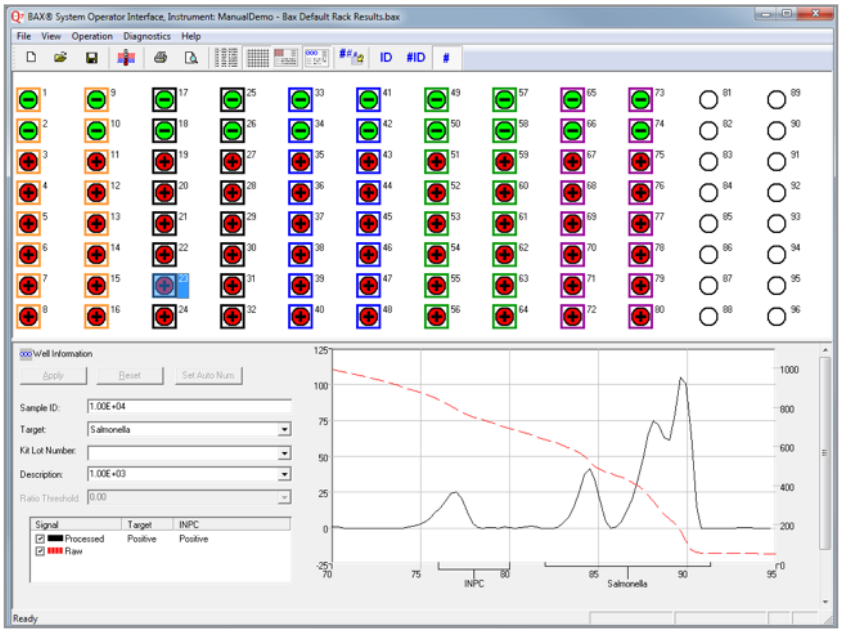
Results in rack view



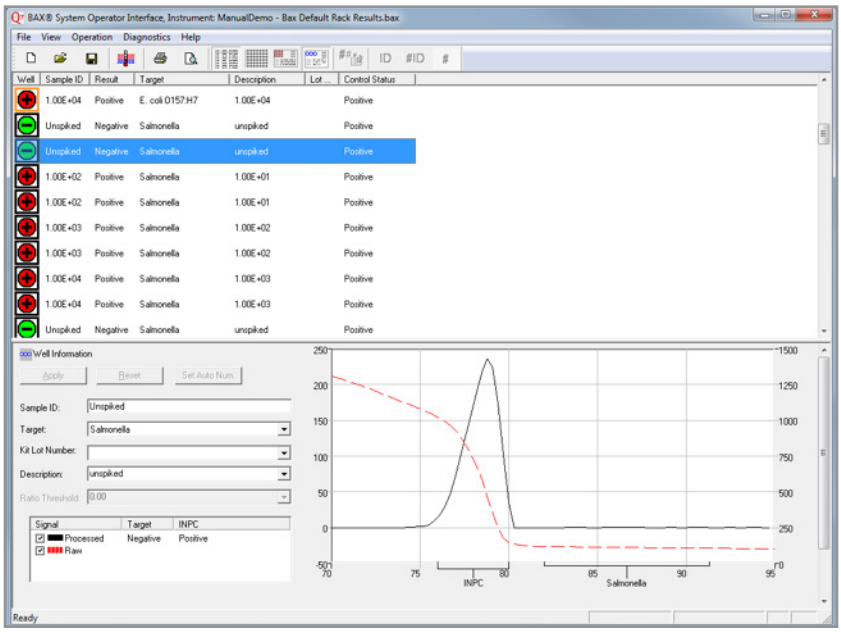
Results in rack detail view

Well View

You can examine the information on specific wells (or groups of wells) by selecting them. The list box in the lower pane then displays processed data with results for target and positive control in the list box. In standard PCR assays, the melting curve (solid black line) for processed data displays in the graph to the right. You can choose to overlay the raw data curve (red dashed line), if desired, by clicking on that option in the list box. You can also choose to display the upper pane as a detailed list.



Results in well view

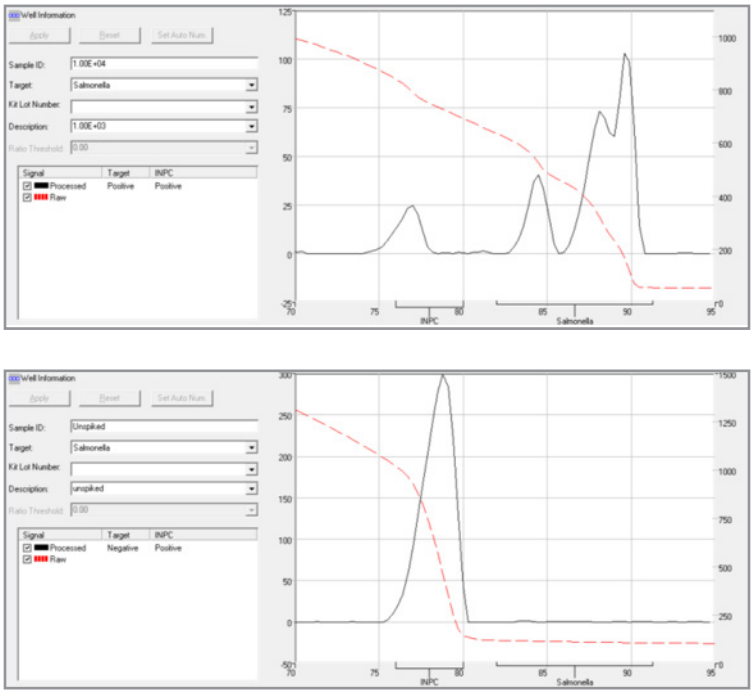


Results in well detail view

Melting Curves for Standard and 24E Assays

The melting curves produced by each target organism vary in the number and location of target peaks. See the melting curve profiles at the end of each BAX® System assay protocol for details.

Note that the lower pane of the well view contains a line graph of processed and/or raw data associated with the selected wells. The processed data graphs allow you to view the melting curve, which is unique to each target. The raw data displays the unprocessed fluorescent intensity against temperature.

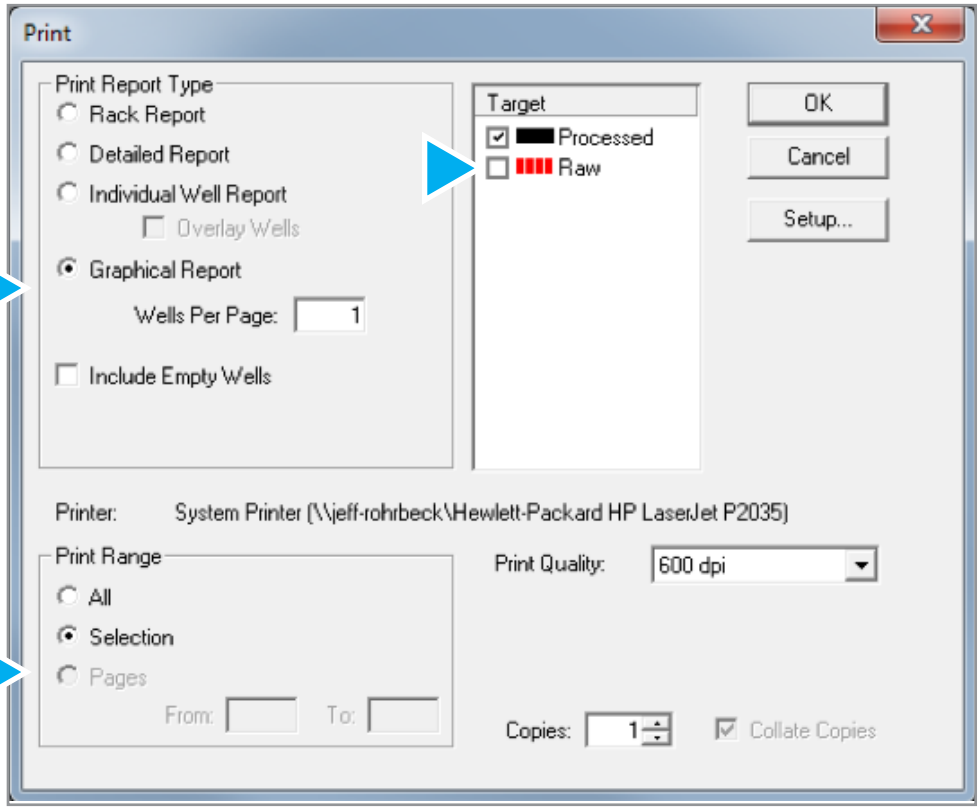


Typical positive (top) and negative (bottom) melting curve profiles

Typical Melting Curve Profiles

The BAX® System application provides several graphs of data for each reaction. You can examine a Processed view of the melting curve and interpret the graph as follows:

1. In the BAX® System results window, click on the well you want to review.
2. To enlarge the graph for easier viewing, select FILE > PRINT PREVIEW. A print options window appears.
3. Click on Graphical Report (1 Well Per Page), Processed View and Selection. If you have selected more than one well, the Selection option is automatically activated.



4. Zoom to enlarge the graph for viewing by clicking on the graph with the zoom tool. You can click the zoom tool two times.

For All Standard Targets

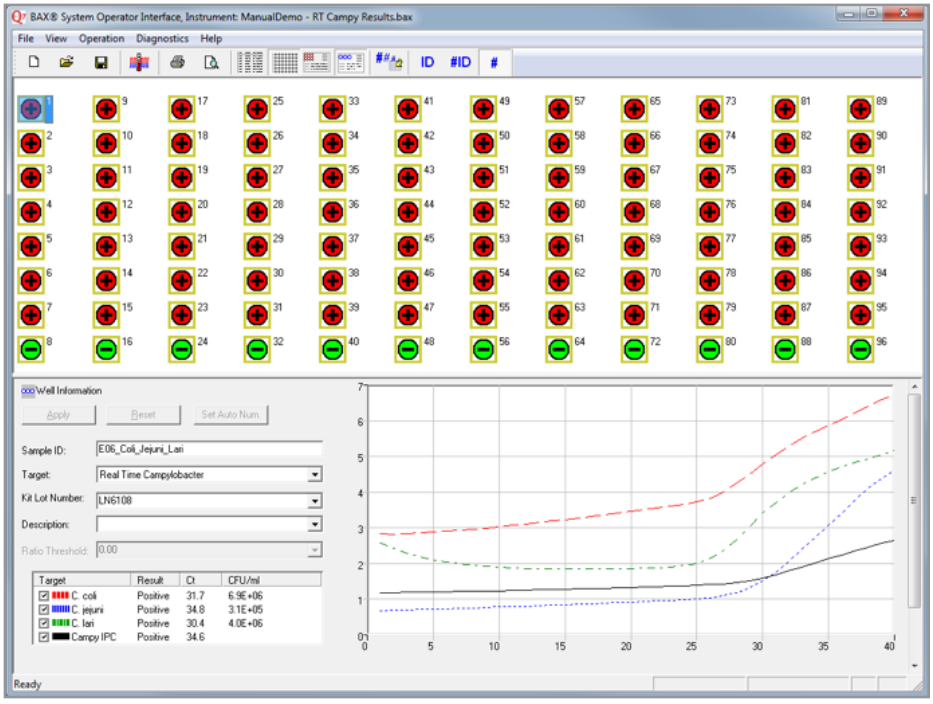
Target peaks - positive reactions show target peaks within a specified temperature range. Peaks can shift within their temperature range. In strong positive reactions, the peaks can shift to slightly lower temperatures, and in weaker reactions, the positives can shift to higher temperatures.

Control (INPC) peaks - occur in a specific temperature range for each target. Control peaks vary in height depending on the presence or absence of the target. **At high target levels, the control peak may be very small or absent**.

Strength of positive result - the strength of a positive result is determined by comparing the peak heights of the target to the control. Samples with high target levels will display a strong positive result as a tall target peak and shorter control peak. In reactions with lower target levels, the control peak grows taller as the target peak gets shorter. When the target peak displays at less than 1/10 the height of the control peak, the positive result is considered weak.

Amp Plots for Real-Time Assays

The results of real-time assays are displayed differently from the results of standard assays. Because real-time PCR assays use probe-based chemistry instead of fluorescent dye, the system does not generate melting curves. Instead, the graph on the right displays amplification plots for each target. For more details on typical amp plots for each real-time target, see the amplification plot profiles at the end of each BAX® System assay protocol in Chapter IV: Protocols for Real-Time Assays.



Results for real-time assays

Qualitative Results

As with standard PCR assays, the wells in the upper pane display color-coded icons:

	Green (-)	=	Negative result
	Red (+)	=	Positive result
	Yellow	=	Indeterminate result
	Yellow (?) with red slash	=	Signal error

*Refer to the troubleshooting section in the User Guide for assistance.

Quantitative Results

The lower pane displays a list box on the left with color-coded targets and results for each:

- Target – checked targets display the amplification plots in the graph on the right
- Result – displays a positive or negative qualitative result
- Ct – identifies the cycle number at which fluorescent signal reaches the detection threshold
- CFU/ml – if applicable, displays a quantitative value in scientific notation of the concentration level in the sample (going into lysis).

Target	Result	Ct	CFU/ml
C. coli	Positive	32.8	1.3E+06
C. jejuni	Positive	33.7	1.3E+06
C. lari	Positive	29.8	1.4E+06
IPC	Positive	34.4	

For some real-time assays, if target cell concentrations fall outside the detection range, the CFU/ml values display as follows:

< 1.0E+04 for concentrations below the range

> 1.0E+09 for concentrations above the range

For All Real-Time Targets

Fluorescence Intensity Units (FIU) – the Y-axis of the amp plot represents the level of fluorescence intensity units (FIU) measured by the BAX® System instrument. The X-axis represents the number of heating and cooling cycles the BAX® System performed.

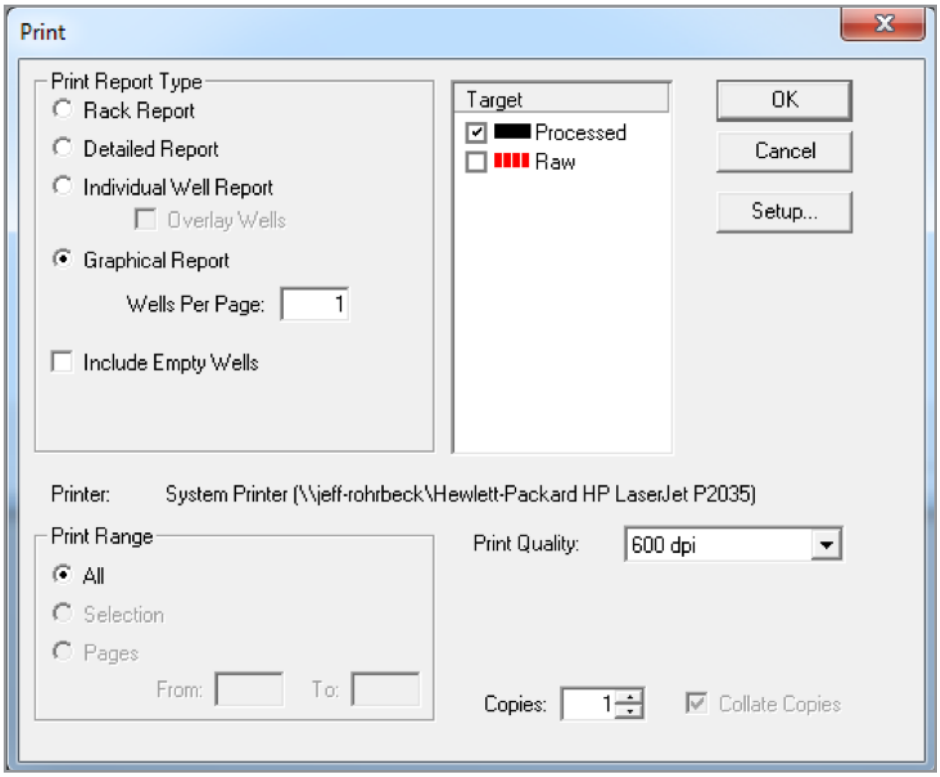
Sigmoid-shaped curves – positive reactions appear on the amp plot as a sigmoid-shaped curve. For a reaction to be considered positive, the Ct (or threshold crossing) value of this curve must be a positive value, typically this value will be between 20 and 50. A lower Ct value usually indicates a higher concentration of the target in a sample.

Internal Positive Control (IPC) – each amp plot displays an additional black curve that serves as in internal positive control. This INPC curve must have a positive Ct value. The Ct value of the IPC curve is generally between 30 and 40. If both the INPC reaction and the target reaction are negative, an indeterminate result will be displayed.

For more information on typical amp plots, see the amplification plot profiles at the end of each BAX® System assay protocol in Chapter IV: Protocols for Real-Time Assays.

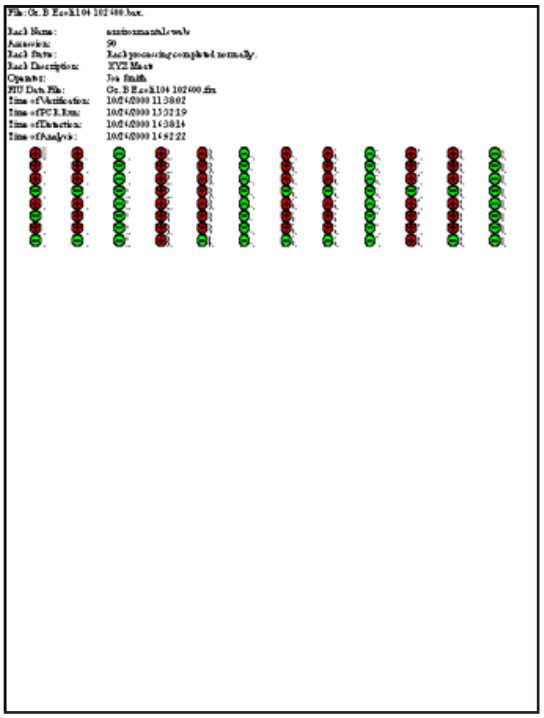
Printing Reports

You can print several types of reports by selecting FILE > PRINT PREVIEW from the menu bar and clicking on the appropriate option. You can designate the type of report for all or selected samples, along with the data type as individual or overlaid graphs.

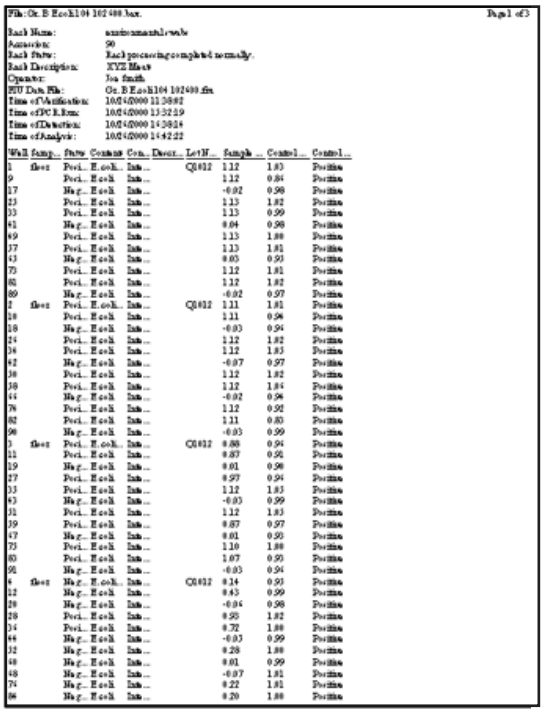


Print options

Every report includes the name of the file, the application version, and the instrument name on which the samples were processed.

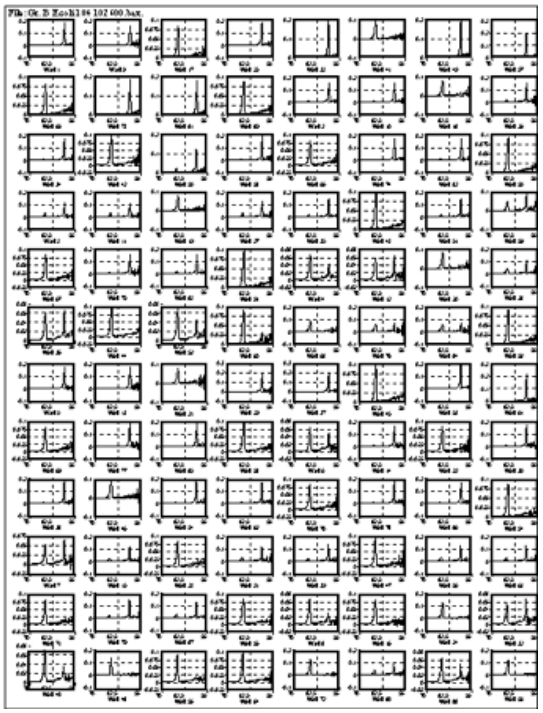


Rack Report: Provides a single-page print-out summarizing the results of all 96 wells as displayed in the Well Grid View.

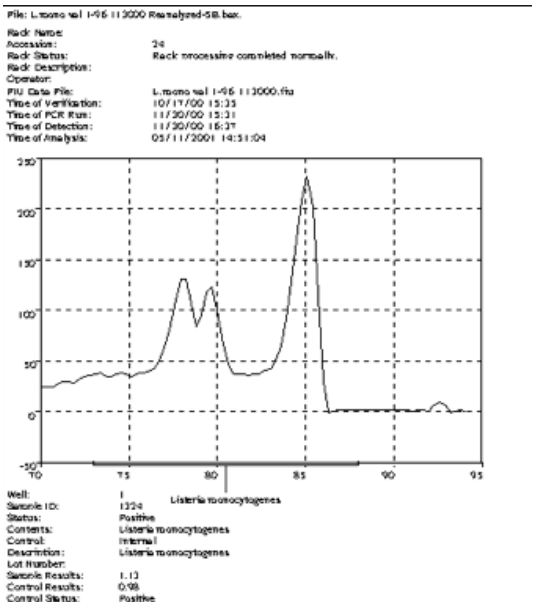


Detail Report: Provides a single-page print-out summarizing the details of each well result as displayed in the Well Detail View.

Note: *If the complete details for each column do not appear in the report, select the "Details View" on the main screen, then click and drag the edges of the column headers to expand each column before printing.*



Graphical Report: Provides a melt curve image for each sample selected (up to 96 samples) as displayed in the Well View.



Individual Well Report: Provides a detailed report for each sample selected (up to 96 samples), including the melt curve image as displayed in the Well Detail View.

Upgrading the Software

Upgraded versions of the BAX® System software are released regularly to include new assays, enhanced algorithms and other improvements. Using the most current software release is always recommended with the BAX® System. Follow these instructions to install an upgraded version of the BAX® System software onto the computer.

1. Back Up the Calibration File

Prior to upgrading or reinstalling the BAX® System application, you must back up the system's calibration file using the EXPORT CALIBRATION command. This safeguards against the file being lost or overwritten during reinstallation of the application.

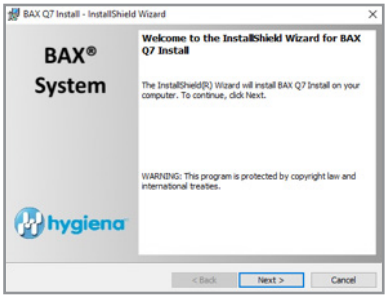
1. From the OPERATIONS menu, select BAX® MAINTENANCE > EXPORT CALIBRATION, then navigate to a new folder in which to store the backup file. Name the calibration file (example: "Q7Cal_date.bqc").
2. Click **SAVE** .

2. Install/Reinstall the Application

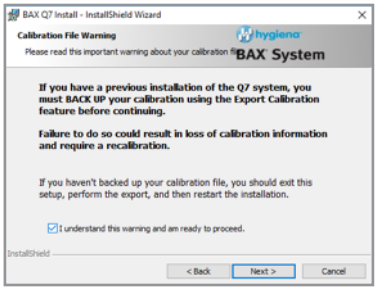
After backing up your calibration file, you are ready to install a new release or re-install another version. Although not required, it is recommended that you do not uninstall the existing BAX® System software before installing or reinstalling the application.

1. Close all programs and files. Place the BAX® System CD in your computer's CD drive. If the Install Wizard does not automatically launch, select **RUN** from the **START** menu, browse to the BAX SETUP.exe file on the CD, click **OK** .

2. When the install wizard appears, click **NEXT** .

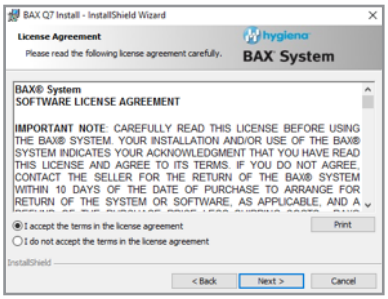


3. A screen appears with a reminder to update previous calibration files. If you have not backed up these files, click **CANCEL** and perform the export. If these files have been exported, click **NEXT** .

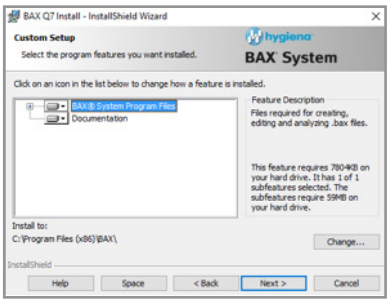


2. Install/Reinstall the Application (Continued)

4. The EULA screen appears. Select the “I accept…” option and click **NEXT** to accept the terms of the EULA.



5. A window appears in which the features to be installed are pre-selected. Click **NEXT** to continue.

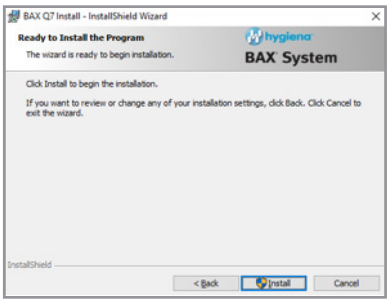


6. A message box displaying the password option appears. To skip password protection, select the “Do not use password protection” option, then click **NEXT** .

   To enable password protection, select the “Enable password protection” option. Enter a password for the BAX® System application, and click **NEXT** .

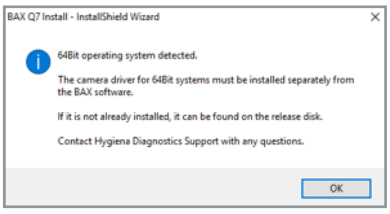
2. Install/Reinstall the Application (Continued)

7. The New Installation window appears. Click **INSTALL** to begin the installation.

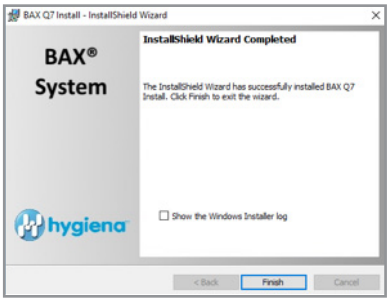


8. Follow the wizard prompts to finish installation. You do not have to restart your computer for the security features to take effect.

9. On a 64-Bit Windows system, you will get the information message shown about installing the camera driver. If you are already running a Q7 on this computer, then you have the camera driver installed and do not need to do anything.



10. Shut down the BAX® System instrument and computer, and then restart the system. Double-click the desktop icon to launch the updated software



3. Check the Calibration File

After installation, the calibration files should be installed automatically. To check that these files are installed successfully, select DIAGNOSTICS > CALIBRATION from the BAX® System menu bar to open the Q7 Calibration Diagnostics window and determine the status of your calibration files. If calibration coefficients are missing, import the backed-up calibration file by selecting OPERATIONS > BAX® MAINTENANCE > IMPORT CALIBRATION from the menu bar and selecting the backed-up calibration file.

Backing Up Data

We recommend that you back up your data frequently (at least once a week). For convenience, your BAX® System startup package includes a removable storage device. Please see the computer manufacturer's documentation for instructions on backing up data.

Appendix A. Enrichment Media Recipes

Appendix A. Enrichment Media Recipes

Enrichment recipes are included in this section for your convenience only and are subject to change without notice. Always check the following sources or your reference manual of choice for the most current update:

Reference method source	Link
USDA-FSIS Microbiology Laboratory Guidebook	http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
FDA Bacteriological Analytical Manual	http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
Health Canada Compendium of Analytical Methods	http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php

Important Tips on Enrichment Media

- Unless stated otherwise, all solutions should be pre-warmed to the specified enrichment temperature before use.
- Media should be warmed in a static incubator overnight. A hot water bath can also be used to warm media if a shorter pre-heating time is needed before preparing samples.
- Use a thermometer to ensure correct media temperature before preparing samples.

24 LEB Media (for 24E Assays)

Using 24 LEB Complete (blended media)

- Catalog MED2005 (D14654989) (Hygiena) or CM1154 (Oxoid) – 2.5 kg
- Completely dissolve 21.75 g 24 LEB Complete media in 500 mL distilled water. Autoclave at 121°C for 15 minutes, then cool to room temperature before use. Final pH 7.4±0.2 at 25°C.

24 LEB Media (for 24E Assays) (Continued)

Using 24 LEB Base and Selective Supplement

- 24 LEB Base – Catalog MED2004 (D13921126) (Hygiena) or CM1107 (Oxoid) – 500 g
- 24 LEB Selective Supplement – Catalog MED2015 (D13921133) (Hygiena) or SR0243E (Oxoid) – 10 vials
- Add 21.75 g 24 LEB base to 500 mL distilled water. Mix and autoclave at 121°C for 15 minutes. Cool mixture to ≤ 50°C. Hydrate 1 vial 24 LEB selective supplement with 5 mL sterile distilled water, mixing gently to dissolve. Add hydrated supplement to diluted base and mix thoroughly. Cool to 25-30°C before use. Final pH 7.4±0.2 at 25°C.

24 LEB Media with Buffer Supplement (for 24E Assays)

Required by NF VALIDATION for smoked fish with 24E assays and charcuteries with *L. monocytogenes* 24E assay

Note: *This buffer supplement may also be beneficial for other samples with a low pH or those that experience a drop-in pH during enrichment. Before testing any food types not certified by AOAC or AFNOR Certification, it is strongly recommended that you internally validate samples with this assay to determine if the buffer supplement is required.*

- 24 LEB media - see above
- 24 LEB Buffer Supplement – Catalog MED2000 (D15407304) (Hygiena) or BO1204 (Oxoid)
- Prepare 24 LEB media as described above. Add 10 mL pre-warmed (37°C) buffer supplement to each 225-mL volume of 24 LEB enrichment broth and mix. Cool to 25-30°C before use. Final pH 7.4±0.2 at 25°C.

Alkaline Peptone Water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Peptone	10 g	Dissolve peptone and NaCl into distilled water. Adjust pH so that value after sterilization is 8.5±0.2. Dispense into screw-cap tubes. Autoclave at 121°C for 10 minutes.
NaCl	10 g	
Distilled water	1 L	

Note: *Commercial preparations must be diluted to the concentration above before use with the Vibrio assay. If using Oxoid CM1117 dehydrated Alkaline Saline Peptone Water (www.oxoid.com), 20 g base powder should be rehydrated with 1 L of water instead of following the directions on the package in order to reach the correct concentration.*

BAX® System MP Media

(Previously BAX® System *E. coli* O157:H7 MP media broth)

StatMedia™ soluble packets

- BAX® System MP media – Catalog No. MED2016 (D12745725) – 33.75 g use 1.5 L sterile deionized water
- Add pre-warmed (42°C) sterile water to sterile container. Add soluble packet to container and mix until dissolved. Final pH, 7.2±0.2. Autoclaving is not required if used within 3-4 hours. For longer storage, autoclave at 121°C for 15 minutes, then store at 2-8°C for up to two weeks.

Bulk powder

- BAX® System MP media – Catalog No. MED2003 (D12404925) – 2.5 kg
- Dissolve 22.5 g in 1 L deionized water. Adjust pH to 7.2±2 at 25°C, then autoclave at 121°C for 15 minutes.

BAX® System *Listeria* Broth

- BAX® System media for *Listeria* – Catalog No. MED2002 (D12232794) – 2.5 kg
- Dissolve 60.4 g in 1 L deionized water. Adjust pH to 7.2±2 at 25°C, then autoclave at 121°C for 15 minutes.

  CAUTION: Do not boil the media or overheat the media in the autoclave. Do not extend autoclaving beyond 15 minutes.

Bolton Broth with Supplement (Single Strength)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Meat peptone	10 g	**THOROUGHLY MIX** 27.61 g in 1 L deionized water. Let soak 10 minutes. Swirl again and autoclave at 121°C for 15 minutes (in screw-capped bottles if possible). Before use, add 2 rehydrated vials of *Campylobacter* enrichment broth (Bolton formula) supplement (Oxoid SR0183 or Malthus Diagnostics X-131). If supplement is not available add 4 mL each of antibiotic concentrates (See http://www.cfsan.fda.gov/~ebam/m28a.html). Final pH, 7.4±0.2. Do not add blood to this mix. Store powdered media in a tightly fastened container in a cool, dry area to reduce oxygen infusion and peroxide formation, which can inhibit recovery of microaerophiles. Use prepared broth within 1 month of preparation (preferably less than 2 weeks).
Lactalbumin Hydrolysate	5 g	
Yeast extract	5 g	
NaCl	5 g	
Haemin	0.01 g	
Sodium pyruvate	0.5 g	
Alpha-Ketoglutamic acid	1 g	
Sodium metabisulphite	0.5 g	
Sodium Carbonate	0.6 g	
Deionized water	1 L	

Note: *Commercial preparations are available from Oxoid (www.oxoid.com) for Bolton broth base (CM0983) and supplement (SR0183).*

Bolton Broth with Supplement (Double Strength)

Prepare as above but use 55.22 g dry ingredients and add 4 vials of reconstituted supplement.

Brain Heart Infusion (BHI) Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Calf brain, infusion from 200 g	7.7 g	Dissolve ingredients in deionized water with gentle heat. Autoclave at 121°C for 15 minutes.
Beef heart, infusion from 250 g	9.8 g	
Proteose peptone (Difco) or polypeptone (Bioquest)	10 g	
NaCl	5 g	
Na_2HPO_4	2.5 g	
Dextrose	2.0 g	
Deionized water	1 L	

Note: *Commercial preparations of BHI broth are available from Oxoid (catalog #CM1032 for FDA-BAM formulation or #CM1135 for AFNOR Certification formulation) and other distributors.*

Brain Heart Infusion (BHI) Broth – Double Strength with NaCl

Dissolve 74 g of BHI base and 140 g of NaCl in 1 L of deionized water, and mix. Final pH, 7.4. Autoclave at 121°C for 15 minutes, and cool to 37°C before use.

Brilliant Green Dye Solution (1%)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Brilliant green dye	1 g	Dissolve dye in 10 mL deionized water, then dilute to 100 mL. Before use, test all batches of dye for toxicity with known positive and negative test microorganisms.
Deionized water	10 mL	

Buffered *Listeria* Enrichment Broth (BLEB)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
TSB	30 g	Dissolve ingredients without the 3 selective agents in deionized water. Autoclave at 121°C for 15 minutes. ***Note:*** *Optionally a filter-sterilized 10% (w/v) sodium pyruvate solution may be added after autoclaving.* Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C. Aseptically add 0.455 mL acriflavinee stock solution, 1.8 mL nalidixic acid acid stock solution and 1.15 mL cycloheximide stock solution to 225 mL enrichment after 4-hour incubation at 30°C.
Yeast extract	6 g	
Monopotassium phosphate	1.35 g	
Disodium phosphate (anhydrous)	9.6 g	
Sodium Pyruvate (sodium salt) (Sigma)	1.11 g	
Deionized water	1 L	
Cycloheximide	50 mg/L	
Acriflavine HCl	10 mg/L	
Nalidixic acid	40 mg/L	

Note: *Commercial preparations available from Oxoid (BLEB #CM0897 and supplement #SR0141).*

Buffered Peptone Water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Peptone	10 g	Dissolve ingredients in deionized water. Autoclave at 121°C for 15 minutes. Final pH 7.2±0.2.
Sodium chloride	5 g	
Disodium phosphate	3.5 g	
Mono-potassium phosphate	1.5 g	
Deionized water	1 L	

Note: *Commercial preparations are available from Hygiena (Part No. MED2010 [D15452608]) 2.5 Kg or (Part No. MED2011[D15452596]) 500 g or from Oxoid (#CM0509).*

Buffered Peptone Water with Novobiocin

Mix ingredients as above without antibiotic, then autoclave at 121°C for 15 minutes. Prepare novobiocin as a 20 mg/mL solution in deionized water. Filter sterilize through a 0.2 µm filter, then transfer 1 mL novobiocin solution to 1 L of the cooled (< 50°C) buffered peptone water broth. Final pH, 7.2±0.2. Use broth within 24 hours of adding the antibiotic.

Butterfield's Phosphate-Buffered Dilution Water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
KH_2PO_4	34 g	Dissolve in 500 mL deionized water. Adjust pH to 7.2 with 1 N NaOH. Bring volume to 1 L with additional deionized water. Sterilize at 121°C for 15 minutes. Store in refrigerator.
Deionized water	500 mL	

Complete Selective Enrichment Broth

Ingredients	Amount	Instructions
Tryptone soya broth powder	30 g	Dissolve ingredients without antibiotics in deionized water, adjust pH to 7.3, then autoclave at 121°C for 15 minutes. Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C. Add stock solutions: 0.45 mL acriflavine, 1.8 mL nalidixic acid and 1.15 mL cycloheximide to 225 mL enrichment broth.
Yeast extract	6 g	
Deionized water	1 L	
Cycloheximide	50 mg	
Acriflavine HCl	10 mg	
Nalidixic acid	40 mg	

Dey-Engley (D/E) Neutralizing Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptone	5 g	Dissolve all ingredients in deionized water. Autoclave at 121°C for 15 minutes. Final pH, 7.6±0.2.
Yeast extract	2.5 g	
Glucose	10 g	
Sodium thioglycollate	1 g	
Sodium thiosulfate	6 g	
Sodium bisulfite	2.5 g	
Polysorbate 80	5 g	
Lecithin (soy bean)	7 g	
Bromocresol purple	0.02 g	
Deionized water	1 L	

Note: *Commercial preparations of D/E neutralizing broth are available from Difco (#281910) and BBL (#298318).*

Demi-Fraser Broth

Ingredients	Amount	Instructions
Demi-Fraser broth base	55 g	Mix base in deionized water, adjust pH to 7.2±0.2, then autoclave at 121°C for 15 minutes.
(Difco #265320 or equivalent)	1 L	

Note: *Ferric ammonium citrate is not required in this formulation.*
Commercially available preparations are also available from Oxoid as Fraser broth base (#CM0895) and Half-Fraser Supplement (#SR0166).

EHEC Enrichment Broth (EEB)

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients without antibiotics in deionized water. Autoclave at 121°C for 15 minutes. Cool to room temperature before adding the filter-sterilized antibiotics. Final pH, 7.4 +/- 0.2. ***Note:*** *The level of cefixime has been reduced to ¼ strength from that described in BAM, Edition 8, Revision A /1998, because the original concentration of 0.05 mg/L was found to be inhibitory to the growth of O157:H7 positive control strains in the absence of competing microflora.*
Bile salts #3	1.5 g	
Dipostassium phosphate (K_2HPO_4)	1.5 g	
Deionized water	1 L	
Cefixime	0.0125 mg	
Cefsulodin	10 mg	
Vancomycin	8 mg	

Giolitti-Cantoni with Tween and Tellurite (GCTT) broth

Ingredients	Amount	Instructions
Giolitti-Cantoni broth (Oxoid CM0523 or equivalent)	54.2 g	Dissolve Giolitti-Cantoni broth in deionized water. Add 1 g Tween 80 to dissolved broth and mix. Final pH, 6.9±2. Autoclave at 121°C for 15 minutes, and cool to 37°C. Just before use, add 15.8 mL sterile solution of 3.5% Potassium tellurite per L.
0.1% Tween 80	1 g	
3.5% Potassium tellurite	15.8 mL	
Deionized water	1 L	

Lactose Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Beef extract	3 g	Dispense 225 ml portions into 500 ml Erlenmeyer flasks. After autoclaving at 121°C for 15 minutes and just before use, aseptically adjust volume to 225 mL. Final pH, 6.9±0.2.
Peptone	5 g	
Lactose	5 g	
Deionized water	1 L	

Modified EC broth with Novobiocin (mEC+n)

Ingredients	Amount	Instructions
Tryptone	20 g	Dissolve ingredients in deionized water. If necessary, adjust pH to 6.9+0.1 with 1 N HCl before autoclaving. Autoclave at 121°C for 15 minutes and cool. Add 5 mL of a filter sterilized, aqueous solution of 4mg/mL sodium novobiocin (adjusted for potency; Sigma N1628) for each L of medium (20 mg/L). Final pH, 6.9±0.1 at 25°C.
Bile Salts #3	1.12 g	
Lactose	5 g	
K_2HPO_4	4 g	
KH_2PO_4	1.5 g	
NaCl	5 g	
Deionized water	1 L	
Novobiocin	20 mg	

Modified LST Broth with Vancomycin

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptose or trypticase	20 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121°C for 15 minutes. Prepare vancomycin as a 10 mg/mL solution in deionized water. Filter sterilize through a 0.2 µm filter, then transfer 1 mL vancomycin solution to 1 L of the cooled (< 50°C) mLST broth. Final pH, 6.8±0.2. Use broth within 24 hours of adding the antibiotic. ***Note:*** *You can also use commercial brands of LST media, but you need to add extra NaCl (29.22 g) to the broth before autoclaving*
Lactose	5 g	
K_2HPO4	2.75 g	
KH_2PO_4	2.75 g	
NaCl	34.22 g	
Sodium lauryl sulfate	0.1 g	
Deionized water	1 L	
Vancomycin	10 mg	

Modified TSB with Novobiocin (mTSB+n) – FDA

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121°C for 15 minutes and let cool to room temperature. Final pH, 7.4 +/- 0.2. Add novobiocin just before use.
Bile salts #3	1.5 g	
Dipostassium phosphate	1.5 g	
Deionized water	1 L	
Novobiocin solution (R50)	0.2 mL	

Modified TSB with 8 mg/L Sodium Novobiocin (mTSB+n) – USDA

Source: USDA-FSIS Microbiology Laboratory Guidebook

Ingredients	Amount	Instructions
Modified Trypticase soy broth (mTSB) (Oxoid CM0989B or equivalent)	33 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121°C for 20 minutes. Let media cool to approximately 50°C. Add 2 mL filter-sterilized, aqueous solution of 4 mg/mL sodium novobiocin for each L of medium. Final pH, 7.4±0.2 at 25°C. If refrigerated, media must be pre-warmed to 18-35°C before use.
Casaminoacids (casein acid hydrolysate)	10 g	
Deionized water	1 L	
Sodium novobiocin solution (4 mg/mL concentration) (Sigma N1628 or equivalent)	2 mL	

MOPS-BLEB* Broth

** 3-(N-Morpholino) propanesulfonic acid - buffered Listeria enrichment broth*

Source: Silbernagel et al, *Journal of AOAC International* 87 (2) 395-410 (2004)

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Mix ingredients without antibiotics, adjust pH to 7.3±0.2, then autoclave at 121°C for 15 minutes. Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C. Add 3 mL acriflavine, 8 mL nalidixic acid and 5 mL cycloheximide to 1 L of *Listeria* enrichment broth.
MOPS free acid	6.7 g	
MOPS sodium salt	10.5 g	
Yeast	6 g	
Deionized sterile water	1 L	
Acriflavine	0.015 g	
Nalidixic acid	0.04 g	
Cyclohexamide	0.05 g	

Nonfat Dry Milk, Reconstituted

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Nonfat dry milk	100 g	Suspend 100 g dehydrated nonfat dry milk in 1 L deionized water. Swirl until dissolved. Autoclave at 121°C for 15 minutes.
Deionized water	1 L	

Shigella Broth with Novobiocin

Source: China GB 4789.5-2012

Ingredients	Amount	Instructions
Shigella broth (BD #214915 or equivalent)	500 g	Prepare the *Shigella* broth according to the manufacturer's instructions. Dissolve 50 mg novobiocin sodium salt into 1 L deionized water. Filter sterilize the solution through 0.45 µm membrane. Add 2.5 mL novobiocin solution to 225 mL prepared *Shigella* broth.
Novobiocin sodium salt (Sigma-Aldrich #N1628 or equivalent)	50 mg	
Deionized water	1 L	

Trypticase Soy Broth (TSB)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase peptone	17 g	Dissolve ingredients with heat in deionized water. Dispense 225 mL into 500 ml Erlenmeyer flasks. Autoclave at 121°C for 15 minutes. Final pH, 7.3±0.2. For trypticase soy broth without glucose, prepare as above, but omit 2.5 g glucose.
Phytone peptone	3 g	
NaCl	5 g	
K_2HPO_4	2.5 g	
Glucose	2.5 g	
Deionized water	1 L	

Trypticase Soy Broth (TSB) with Yeast

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients in deionized water. Autoclave at 121°C for 15 minutes. Final pH, 7.3±0.2
Yeast extract	6 g	
Deionized water	1 L	

Universal Pre-Enrichment Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptone	5 g	Dissolve ingredients with heat in deionized water. Autoclave at 121°C for 15 minutes. Final pH, 6.3±0.2.
Proteose peptone	5 g	
KH_2PO_4	15 g	
Na_2HPO_4	7 g	
NaCl	5 g	
Dextrose	0.5 g	
$MgSO_4$	0.25 g	
Ferric ammonium citrate	0.1 g	
Sodium pyruvate	0.2 g	
Deionized water	1 L	

UVM Broth, Modified

Source: USDA-FSIS Microbiology Laboratory Guidebook

Ingredients	Amount	Instructions
Proteose peptone	5 g	Dissolve ingredients in deionized water. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT; COOL AT ONCE AFTER REMOVAL FROM THE STERILIZER. IF THE MEDIUM BLACKENS OR DARKENS, IT HAS BEEN OVERHEATED AND MUST BE DISCARDED. Store in the refrigerator. Final pH, 7.2±0.2 at 25°C.
Tryptone	5 g	
Lab Lemco powder (Oxoid)	5 g	
Yeast extract	5 g	
NaCl	20 g	
KH_2PO_4	1.35 g	
Na_2HPO_4	12 g	
Esculin	1 g	
Naladixic acid (2% in 0.1 M NaOH)	1.0 mL	
Acriflavine	12 mg	
Deionized water	1 L	

Note: *Commercially available preparations are available from Oxoid as UVM Base (#CM0863) and Supplement (#SR0142).*

Appendix B. PCR Contamination Control

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When samples undergo PCR, the amplification process produces billions of copies of a specific DNA sequence from the targeted bacteria. If tubes are then unsealed, these copies can travel throughout the lab via aerosols, liquids and surface contact.

BAX® Systems with automated detection avoid this problem by processing only sealed tubes. However, as with any PCR technology, the risk of contamination cannot be completely eliminated. If the tubes are not properly sealed or are inadvertently opened after amplification, contamination of the lab should be assumed and care should be taken to minimize aerosols. See Decontamination procedures below.

Preventing PCR Contamination

In order to prevent contamination, always follow these best practices when working in the laboratory.

Work Area Preparation

- Before entering the work area, remove and dispose of any gloves you may already be wearing. Put on new gloves when entering and dispose of them before leaving the work area.
- Clean the work area and all materials and supplies before and/or after use with 10% hypochlorite bleach solution, rinse with deionized water or 70% ethanol solution and blot dry.
- A biohood in the work area is ideal. Make sure to use the UV light for 30 minutes before and/or after your use of the biohood. Lab tools and other equipment in the biohood should be rotated, if necessary, to ensure complete exposure to the UV light.

Sample Preparation

- Use barrier pipette tips for all pipetting events, if possible.
- Use new cluster tubes and caps for each sample.
- Change or clean gloves immediately before working with reagents.
- Review best practices procedures for loading samples, stressing the importance of ensuring the instrument has reached the load temperature before beginning to hydrate PCR tablets.

Materials Storage and Cleaning

- Store optical caps and cluster tube caps in a sealable container, such as a zip-lock bag or drawer. Never store optical caps and cluster tube caps in close proximity to the PCR tube rack or other items that are routinely placed in the PCR instrument.
- Clean the PCR tube rack and other items that are routinely placed in the PCR instrument with 10% hypochlorite bleach solution, rinse with deionized water or 70% ethanol solution and allow to dry after every use.
- Spray cooling blocks with 10% bleach, rinse with deionized water or 70% ethanol and blot dry before returning them to the refrigerator.
- Before performing calibration or if PCR product contamination is suspected, autoclave the pipettes according to the manufacturer's directions.

Materials Disposal

- DO NOT OPEN TUBES AFTER AMPLIFICATION. This will ensure that no amplified material is introduced into your work area.
- Dispose of used PCR tubes in a dedicated container that can be immediately autoclaved or in a designated container with bleach. Always treat tubes removed from the BAX® System instrument as positive enrichments or other potential sources of contamination.
- Discard barrier pipette tips in a plastic bag, autoclave the tips, and dispose of the bag after each use of the preparation area.

Identifying PCR Contamination

If you suspect that your lab is contaminated, prepare a set of negative controls (blank lysates) and run a full process in the BAX® System instrument. This "blank" acts as a negative control on the process. A positive result for the "blank" indicates contamination.

Collect environmental swab samples from a variety of areas around the lab (metal racks, decapping tools, cooling blocks, drawer handles, pipettor tips, etc.). Record the places from which environmental samples are collected; if contamination is observed at a location, these same areas should be sampled again once decontamination has been completed.

When collecting and processing samples, always use a new, unopened BAX® System kit (including protease, lysis buffer, PCR tubes and caps) and new, unopened boxes of supplies (including gloves, pipette tips and cluster tubes). The best aseptic technique should be used when processing environmental swab samples to ensure the highest integrity data.

Using the following protocol to collect and process the environmental swab samples:

1. Hydrate sterile swab in 500 µL diluent* (peptone water, DI water, lysis buffer, etc.).
2. Swab the sample area.
3. Place swab back into individual diluent* tubes and mix/vortex to agitate samples.
4. Transfer 5 µL of this solution into prepared BAX® System lysis reagent (lysis buffer + protease).

   Note: *If using the BAX® System real-time PCR assays for E. coli or STEC, transfer 20 µL solution into lysis reagent.*

5. Follow the BAX® System lysis procedure for the target organism to heat and cool samples and run a full process in the BAX® System instrument.

   ***** *If using the BAX® System real-time PCR assays for STEC, use sterile mTSB or BAX® System MP media as the diluent.*

Removing PCR Contamination

If contamination should occur, the work area must be thoroughly cleaned. During the cleaning process, it is important to change gloves, wipes and other decontamination materials often to prevent spreading PCR product to new areas via these materials.

While wearing gloves and a lab coat, perform the following procedures under sterile conditions:

- Wipe down the lab environment with 20% bleach solution*, followed by 70% ethanol solution. If possible, let the bleach solution sit for 10 minutes before wiping up with a dry wipe. If possible, irradiate the surfaces with a UV light source, being sure that all sides of the surface are exposed to UV light for at least 30 minutes.
  - Wipe the outside of all the equipment and the keyboard and mouse for each work station.
  - Wipe all cabinets, countertops, refrigerator handles, door handles, and anything else that is touched by hands in the lab environment.
  - Wipe heating blocks and timers.
  - Wipe all materials on sample prep areas, including pipettors, stands, table tops, etc.
- For electrical equipment, wipe down the surface with a cloth dampened with the bleach solution followed with a 70% ethanol rinse to prevent damage to the equipment.
- Soak smaller items in 20% bleach solution for at least 5 minutes, then rinse with water and allow to air dry.
  - Soak cluster tube racks and PCR racks.
  - Soak scissors, tweezers, cooling and heating block inserts, and two styles of decappers.
- Discard all disposable items (pipette tips, buffers and other solutions, etc.) that have been opened or partially used.
- Autoclave pipettes and all other ancillary equipment that can tolerate autoclaving, according to the manufacturer's directions.
- Mop or wipe all floors with bleach solution before leaving the lab after completing all other decontamination steps.

** A 10% bleach solution may also be used for decontamination in accordance with your laboratory SOP but may require repetition of the cleaning to assure removal of all amplicon.*

Monitoring PCR Contamination

To help prevent subsequent PCR contamination incidents, a laboratory may opt to incorporate a routine amplicon monitoring program that follows a procedure similar to established environmental monitoring programs. In the case of an amplicon monitoring program, the environmental target is DNA rather than viable bacteria.

- Routine amplicon monitoring programs may include a weekly rotation of sampling from representative areas throughout the lab, both before and after standard cleaning practices, as a means of monitoring the effectiveness of cleaning.
- If contamination is confirmed in any of these areas, a more thorough and rigorous monitoring procedure should be implemented until it is determined that contamination no longer exists.

Appendix C. Confirmation Utility

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Note: *This procedure is not part of the methods approved by AOAC.*

Good laboratory practice suggests that you not use the same method to both screen a sample and confirm its results. When using the BAX® System to screen a sample, any desired confirmation is usually performed though biochemical methods. Conversely, when screening with traditional biochemical methods, you can use the BAX® System to identify suspect target colonies on selective agar plates. The sample preparation steps vary slightly from those used with the screening protocol; otherwise, the method is the same. The only additional supplies you need are plastic disposable inoculating needles and sterile diluent, such as 0.1% peptone water.

To identify suspect colonies, follow these steps:

1. Examine selective agar plates for suspect colonies and identify those that require confirmation.
2. Prepare lysis tubes according to the screening protocol and fill out your sample tracking sheet.
3. Add 1 mL of sterile diluent to sterile tubes, and label them to correspond with your lysis tubes.

   Note: *If confirming samples with the BAX® System real-time STEC suite (Screening, Panel 1 or Panel 2 assays), use TSB or mTSB as the sterile diluent.*

   Note: *If your BAX® System results are positive, you can then use these tubes to streak plates for isolation and further identification of the target organism.*

4. Touch a plastic disposable needle to the agar plate to pick a suspect colony.

   Note: *Avoid picking larger colonies, as adding too much sample DNA to the dilution tube may affect results.*

5. Dip the needle into the corresponding dilution tube and swirl the needle to release the colony. Remove the needle, then cap the tube and mix.
6. Uncap the dilution tube, transfer 5 µL of the cell suspension into the corresponding lysis tube containing 200 µL lysis reagent, and mix.

   Note: *If confirming samples with the BAX® System real-time STEC suite (Screening, Panel 1 or Panel 2 assays), pipette 20 µL of the cell suspension into the corresponding lysis tube containing 200 µL lysis reagent.*

7. Repeat steps 4-6 for each of your suspect colonies, using a new needle for each pick.
8. Continue with the BAX® System testing protocol from “Perform Lysis.”

Atypical Colony Confirmation Results

When using the BAX® System to confirm a suspect colony, it is expected that positive results will be strong due to the purity of the culture (see the melting curve profiles at the end of each BAX® System assay protocol for details). However, if an excessive amount of non-target DNA is present in the lysis sample, weak positive results may occur for the target organism. If this occurs, the resulting graphs will display a large INPC peak relative to the target peaks.

If you do not find a strong positive confirmation, follow these steps to verify atypical positive results from a colony confirmation:

1. Prepare an overnight enrichment from the suspect colony according to your lab's standard procedure.

   Note: *You can also re-grow 10 µL of solution from the previous confirmation dilution tube (step 3, above) overnight to serve as the enrichment.*

2. Add 5 µL re-grown sample to 200 µL prepared lysis reagent.
3. Follow the appropriate BAX® System assay protocol (see Chapters II to V) to run a full process for the target organism.
4. View the displayed results. A positive result confirms the suspect colony for the target organism.

Appendix D. Troubleshooting

The troubleshooting tips provided in this chapter are intended to provide general guidance for the most common customer issues with the BAX® System instrument and procedures.

For additional technical assistance, contact your local distributor or call Hygiena Diagnostics Support at 800-863-6842.

Troubleshooting the Instrument

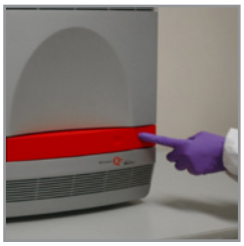
When the instrument is ready for use, the “Power” light on the front panel is displayed. If a red “Error” light displays instead, try these steps:

1. Press on the instrument door to ensure that it is closed.	
2. If the error light continues to display, open the instrument door and pull on the heated cover to verify that is fully forward. Close the instrument door.	

3. If the error light continues to display, check the monitor. If you don't see the desktop for the Windows operating system, turn off power to the instrument and wait for the monitor to display the desktop. Then power on the instrument.

4. If the error light continues to display, check the USB cable connections. Verify that one end is connected to the back of the instrument and the other to the computer.

5. If the error light continues to display, power off the instrument, wait 30 seconds, then power it back on.



6. If the error light continues to display, contact Hygiena Diagnostics Support or your distributor for technical assistance.

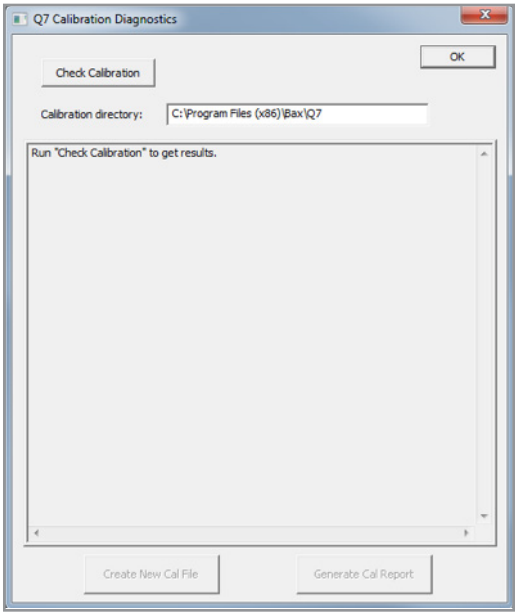
Troubleshooting Calibration

Due to the particular nature of the calibration process, any minor deviation from the protocol described under “Calibrating the Instrument” in Chapter VII: BAX® System Hardware may result in a calibration failure. If the calibration process fails, you can make one or more of the following adjustments and re-try performing the calibration.

- Check the failed calibration plate for droplets on the top film. If you see any, flick the plate so that the droplets return to the wells.
- Allow the calibration plates to reach room temperature.
- Remove any smudges or dust particles from the halogen bulb. Wear gloves when handling the bulb to avoid leaving fingerprints.
- Remove the halogen bulb from the socket, rotate it 180° and re-insert it into the socket.
- Replace the calibration plates if one or more wells have significantly less liquid than the other wells.

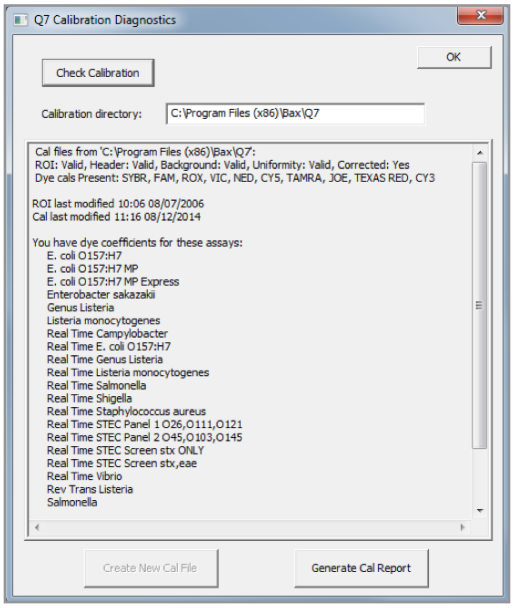
Calibration Diagnostics

After installing or re-installing BAX® System software, or if you suspect that there is a problem with the instrument, you can run calibration diagnostics to determine the status of your calibration files. This diagnostics feature also allows you to create some types of calibration files, if necessary.



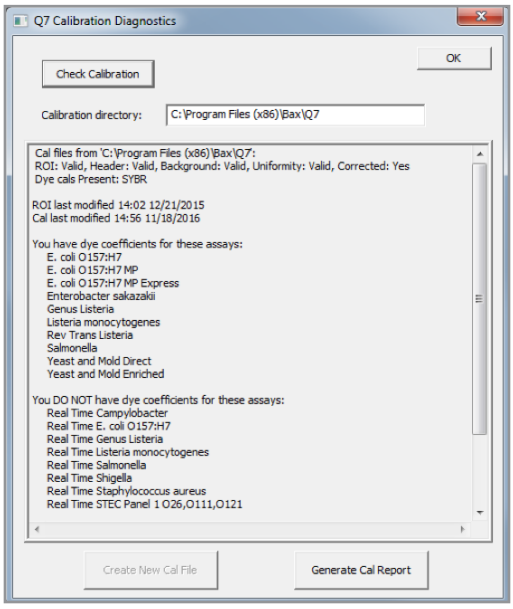
To run calibration diagnostics:

1. From the menu bar, select DIAGNOSTICS > CALIBRATION to open the Q7 Calibration Diagnostics window.
2. Click the **CHECK CALIBRATION** button to create a list of the valid calibration files.
3. After the calibration diagnostic has been run, the results will appear in the calibration window.



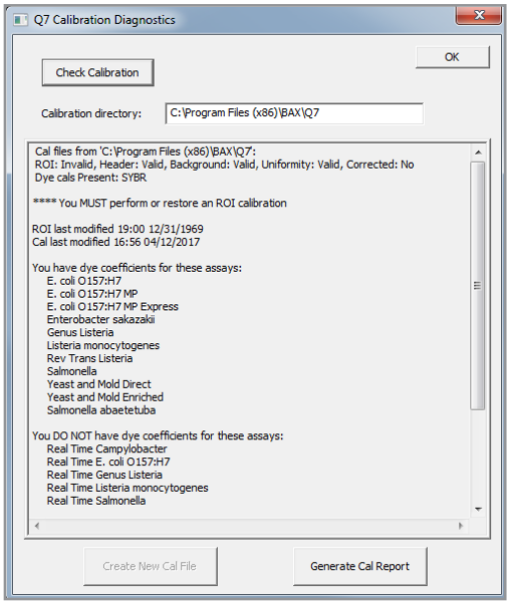
If the results state that you have coefficients for both standard and real-time assays (and does not list assays for which you do not have coefficients), then all calibration files are complete and properly installed to run standard, 24E and real-time assays. No further calibration is needed.

Calibration Diagnostics (Continued)



If the results state that you have coefficients for standard (melt) assays but do not have coefficients for real-time assays, then the instrument is not calibrated to run any real-time assay. The instrument is capable of running standard and 24E assays only.

To add coefficients to run real-time assays, follow the protocol described under “Calibrating the Instrument” in Chapter VII: BAX® System Hardware to complete calibration.



If the results state that you do not have an ROI calibration, then the instrument is not calibrated to run any standard, 24E or real-time assays.

To calibrate the instrument for ROI or real-time assays, follow the protocol described under “Calibrating the Instrument” in Chapter VII: BAX® System Hardware to complete calibration.

Troubleshooting Indeterminate or Error Results

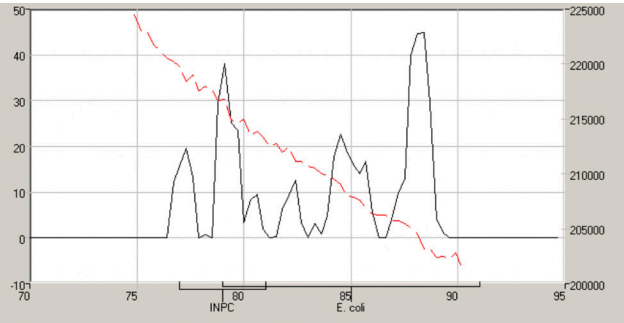
	Yellow	=	Indeterminate result
	Yellow (?) with red slash	=	Signal error

Indeterminate and error results are most commonly caused by

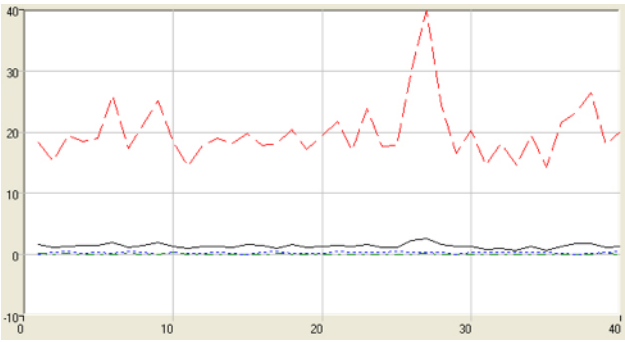
- Missing PCR tubes
- Non-specific reactions
- Straight line signal
- Exponential decay

In these situations, you can examine the shape of the melt curve and its Y-axis scale (standard assays) or the attributes of the amp plot (real-time assays) to help diagnose the problem.

Missing PCR Tubes



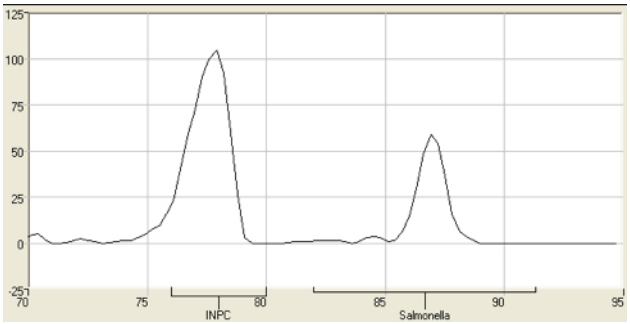
Melt Curve Diagnosis (standard and 24E assays):
The Raw view shows a jagged pattern of multiple steps. The Processed view shows system noise at low signal levels.



Amp Plot Diagnosis (real-time assays):
All target curves and the IPC curve appear as jagged lines (system noise). The IPC may also appear as a straight horizontal line, and no target curves appear.

Cause	Solution
PCR tubes were not placed in the rack according to your rack file layout. This happens most frequently when you are running a partial rack of samples and mistakenly place an entire strip of PCR tubes in the wrong column of the rack.	Although not evident in the mislabeled wells, the data has been captured and does exist in the file. Re-label the wells in your file according to the sample placement in the rack and assign the appropriate target. Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

Non-Specific Reactions

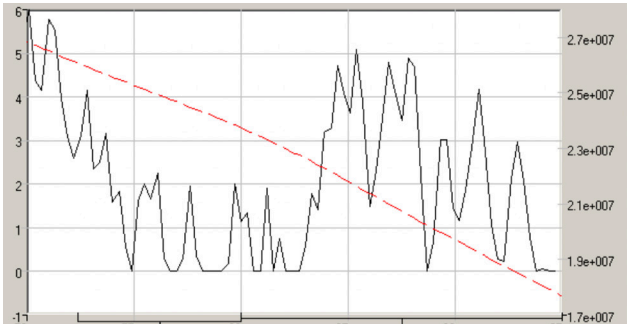


Melt Curve Diagnosis –

The Processed view shows small peaks in the 80-95°C range and broad peaks in the 88-95°C range. This view may also show subtle system noise.

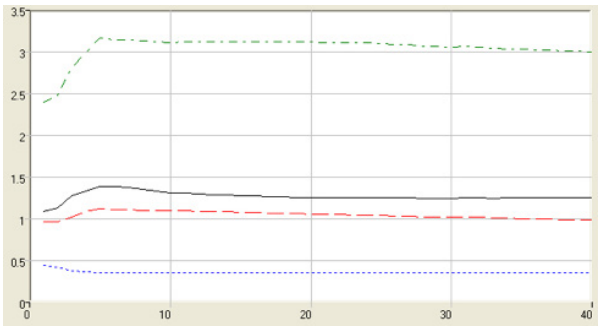
Cause	Solution
Samples were not loaded into the BAX® System cycler immediately after hydrating PCR tablets.	Repeat the assay from last enrichment. Do not begin to hydrate PCR tablets with lysate until the cycler has reached the correct load temperature and the "Ready for Rack Load" prompt appears. Load samples into the instrument immediately after hydrating tablets.
Samples were not adequately chilled to 2-8°C after heating for lysis.	Repeat the assay from last enrichment. Ensure that all cooling blocks are chilled to 2-8°C before use. Keep samples in the cooling block for a full 5 minutes before using to hydrate PCR tablets.
After lysis, samples were not maintained at 2-8°C before loading into the cycler.	Repeat the assay from last enrichment. Ensure that all cooling block inserts have been chilled to 2-8°C before use, and do not remove cooling blocks from the refrigerator until they are needed to keep them as chilled as possible. Finish using the cooling blocks within 30 minutes of removing inserts from the refrigerator. ***Note:*** *If you are using the E. coli O157:H7 MP assay, ensure that the cooling block for PCR tubes is maintained at -20°C.*

Straight Line Signal



Melt Curve Diagnosis –

The Raw view shows a negatively sloped line. The Processed view is blank or may show system noise.



Amp Plot Diagnosis (real-time assays):

The IPC and target curves appear flat. The IPC curve reports a Ct value of 0.0.

Cause	Solution
Second lysis step (95°C) was not performed properly.	Check that the heater is reaching 95°C by inserting a thermometer into one of the two threaded holes in the block. Repeat the assay from last enrichment, confirming that the lysis tubes heat for a full 10 minutes at 95°C.
Missing tablet – can happen when the tablet sticks to the pipette tip.	Repeat the assay from last enrichment. Check that the PCR tablet does not stick to the pipette tip and get discarded when the tip is ejected.
Dry tablet – can happen when pipettor is clogged or tips are loose.	Repeat the assay from last enrichment. Check that the pipettor is dispensing accurately, and that the tips are secure.
Powder from gloves is interfering with PCR	Use only powder-free gloves for handling samples. Repeat assay from last enrichment.

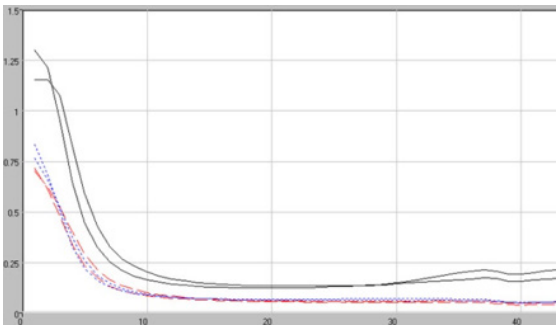
Straight Line Signal (Continued)

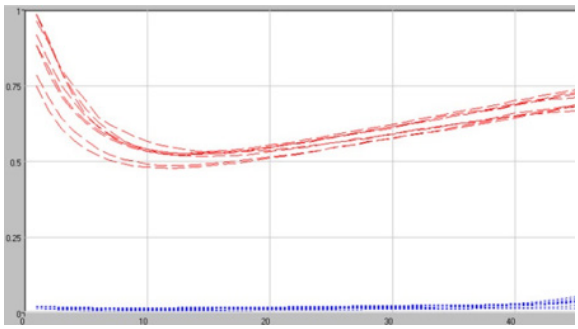
Cause	Solution
Samples were run in “Detection only” mode instead of “Run Full Process.” You can verify this by selecting VIEW > EDIT RACK INFO and checking the field for PCR Time in the lower pane. If the field is blank, no amplification occurred.	Repeat the assay from the last enrichment. Take care to select Run Full Process.
High concentration of detergent/disinfectant is interfering with PCR – can happen when sampling from recently sanitized areas without using a neutralizing buffer as wetting agent	Repeat the assay from the last enrichment to rule out sample prep errors. If problem persists, call Hygiena Diagnostics Support for assistance.
The sample type or enrichment protocol being tested has not been validated.	Repeat the enrichment according to the protocol described in the BAX® System User Guide. If you are testing a non-validated sample type, call Hygiena Diagnostics Support for assistance.

Exponential Decay

Amp Plot Diagnosis (real-time assays):

The IPC and target curves begin relatively high, then drop and remain flat or rise slightly for the rest of the reaction, which can cause an indeterminate result.

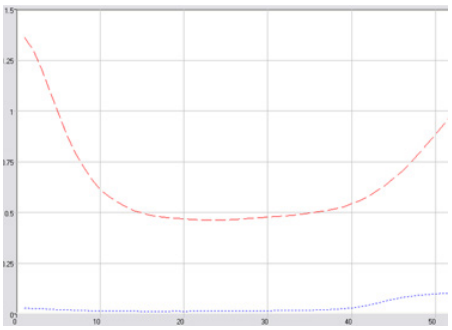




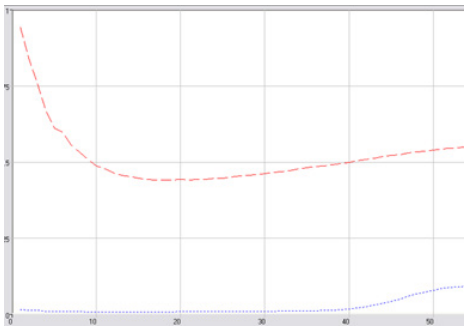
Exponential Decay (Continued)

Cause	Solution
PCR tablets were exposed to air for more than 15 minutes before being hydrated with lysate.	Repeat the assay from the last enrichment using new PCR tubes. Do not uncap PCR tubes until you are ready to hydrate the tablets. Only uncap one strip of PCR tubes at a time. OR Repeat the assay from the last enrichment using new PCR tubes. After hydrating PCR tablets with lysate, vortex (15-30 seconds) and centrifuge the plate to ensure all the liquid is at the bottom of the tube.
PCR tablets were not fully hydrated (insufficient volume of lysate transferred to PCR tubes).	Check that all pipettors are delivering the correct volume of lysate to the PCR tubes, then repeat the assay from the last enrichment using PCR tubes from a new BAX® System kit.
PCR tubes were exposed to humidity during storage or were stored improperly.	Discard any remaining PCR tubes from the kit, and repeat the assay from the last enrichment using PCR tubes from a new BAX® System kit. Before storing PCR tubes, re-seal the PCR tablet bag tightly. If storing PCR tubes in an open kit for more than 3 weeks, seal the bag of PCR tubes into a larger bag with desiccant or store at 4°C in a desiccation unit, if possible.

Note: *Some signs of exponential decay can also appear in samples that return positive or negative results (not indeterminate). These signs do not affect the validity of the BAX® System results, and action can be taken based on the plus/minus calls.*



Positive result with exponential decay



Negative result with exponential decay

Troubleshooting Process Interruptions

In some cases, a full process run in the BAX® System instrument may be interrupted before results are displayed for such reasons as

- unexpected power failure
- data communication error
- accidental cancellation of the process

In these cases, it may be possible to re-run the same PCR tubes in the instrument to generate results with the Detection Only operation. This option allows you to re-analyze samples that have already begun the detection phase of the standard full process. Results are available after about 1 hour.

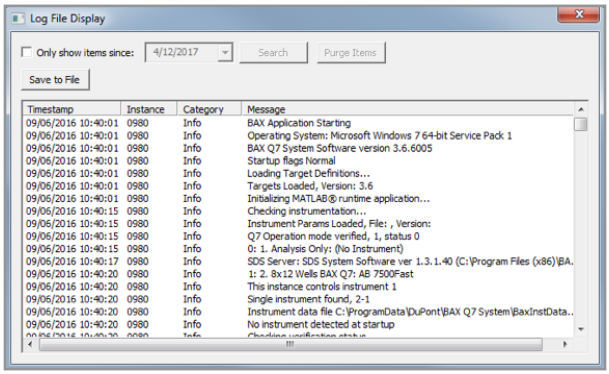
Note: *The Detection Only option SHOULD NOT be used to re-analyze samples for which results have already been displayed. To reanalyze results that have already been produced, select OPERATION > REANALYZE WELLS and follow the screen prompts.*

Note: *This option is not available for real-time assays.*

Review the Log File

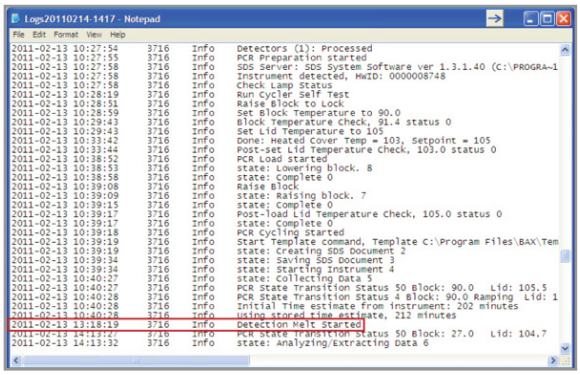
From the menu bar, select DIAGNOSTICS > VIEW LOG FILE to open log file display.

Locate the log file entry with the date and time of the interrupted process run.



Double-click on the log file to view details.

Note: *You can also click the SAVE TO FILE button to save a text version of the log file to your computer desktop.*



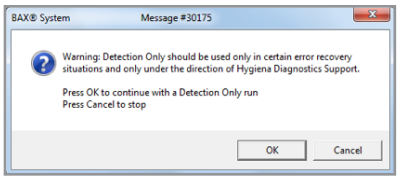
Locate the line in the log file that states “Detection Melt Started”. If the “Detection Melt Started” line appears in the text file, follow the instructions below to re-run samples using the Detection Only operation.

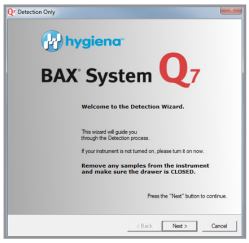
If this line does not appear in the text file, new PCR tables must be hydrated from the saved lysate to re-run the full process in the BAX® System instrument.

Perform Detection Only

Note: *This operation should be run* ***as soon as possible*** *after the process interruption occurs. Contact Hygiena Diagnostics Support for guidance before performing Detection Only.*

1. Power on the BAX® System Q7 instrument. From the menu bar, select DIAGNOSTICS > DETECTION ONLY to launch the Detection Wizard. You will get a warning dialog to confirm that you want to do a Detection Only run. Select **OK** to get to the run wizard.
2. Remove any samples from the instrument and close the sample drawer. Click **NEXT** .

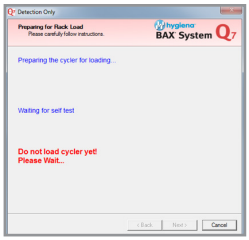




3. A new screen automatically appears as the instrument heats to its set temperatures. The red color in the banner indicates that it is a Detection Only run.

Note: *DO NOT LOAD SAMPLES into the instrument until prompted to do so.*

4. Follow the screen prompts to perform detection. The process completes in approximately 1 hour.



BAX® SYSTEM LIMITATION OF WARRANTY AND LIABILITY

BAX® SYSTEM LIMITATION OF WARRANTY AND LIABILITY

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BAX® System

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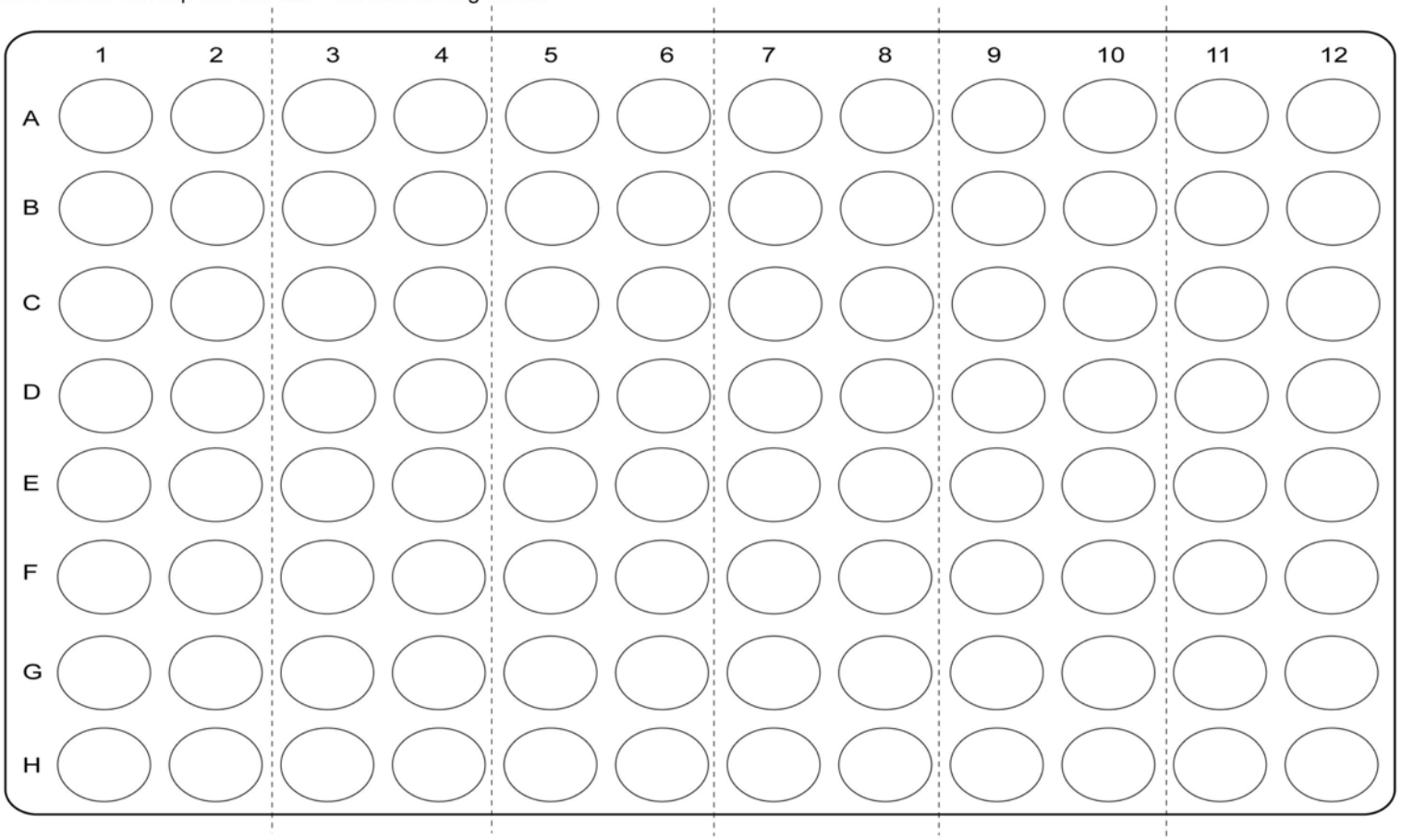
BAX® System Sample Tracking Sheet

Organism: _____

Date: _____

Enter one sample ID number into each circle.
These sheets correspond with the PCR rack arrangement.



Date lysis: _____ Kit lot # _____ Media lot # _____

Date PCR: _____ Exp. date: _____ Exp. date: _____

Technologist: _____

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