The Innovate System

Implementation Guide

The Easy-to-Use Rapid Microbial Screening System

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1 Introduction

This Implementation Guide has been developed to provide an understanding of the methods and experimentation required to implement the use of the RapiScreen™ Kit for releasing end products on the Innovate system. It is intended to be used as a reference to aid you in your implementation process.

If the RapiScreen method is new to you, it should be reassuring to know that many of the world's largest beverage product companies are using the method routinely to positively release their end products. The method for microbial testing in finished products has been accepted by the manufacturing industry as a valid alternative screening method, which has been in widespread use for over two decades.

We acknowledge that each customer has a unique range of products, which differentiates you from competitors. However, when you look at microbiological testing procedures, very similar methods are applied throughout the industry. This guide provides an overview of those methods.

- Section 2 introduces and describes in detail the technology, reagents, methodology and instrumentation necessary to perform the assays.
- Section 3 guides you through product implementation in a step-by-step manner.
- Section 4 provides worksheets to help your company record, calculate and interpret the results.

The methods described assume that the user is performing the tests on the Innovate Luminometer in conjunction with Innovate RapiScreen reagents. The methods described are applicable to other instrumentation and reagents; however, the protocols will vary and are specific to each instrument. Throughout this implementation guide incubation times are listed as typical based on a majority of user methodologies. All users should determine the most appropriate incubation times and temperatures based on their product types and microorganisms of interest.

A customer may decide to perform product sample inoculations to show that the method is capable of detecting contamination. The use of Quanti-cult®cell suspensions is referenced throughout this document. Quanti-cult microorganisms were chosen for their availability and ease of use. Alternatively, the inoculation studies may be completed using contaminating organisms isolated from the plant environment or Reference strains can be purchased from an alternate source (examples include ATCC (USA), CBS (The Netherlands), DSMZ (Germany), and JCM (Japan).

2 Innovate System

2.1 Introduction to ATP Bioluminescence

Adenosine triphosphate (ATP) is the universal currency of free energy in biological systems. All living organisms contain ATP as a vital part of their energy metabolism. It is this ATP that is released and detected by a standard bioluminescence test. ATP rapidly reacts, in the presence of the luciferase enzyme, with luciferin and oxygen to give a photon of yellow-green light:

$$\text{ATP} + \text{D-Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{AMP} + \text{Oxyluciferin} + CO_2 + \text{PPi} + \text{Light}$$

ATP bioluminescence is a commonly used method for the microbial screening of beverage products and is applicable to a very wide range of sample types. A luminometer automatically adds the required reagents to a sample and detects the emitted light.

The reaction is very efficient; every molecule of ATP can cause emission of a photon of green light. Due to the fact that all organisms rely on ATP as the main carrier of metabolic energy, ATP bioluminescence can detect living microorganisms with great sensitivity. This provides a much more rapid detection system than waiting for visible colonies to grow on agar plates.

2.2 Outline of the RapiScreen Testing Methodology

RapiScreen kits can be used to detect microbial contamination in finished products.

- An incubation/enrichment step is required before the ATP assay is performed. During incubation any microbes present in the product are allowed to multiply.
- Following incubation, an aliquot of the product is added to a microtiter plate which is placed in the luminometer.
- The Innovate and Innovate.im Software automatically inject the reagents and measure the light output. Results indicate the presence of microbial ATP if the product sample gives a signal significantly above that of a sterile sample.
  - ATP depleting reagent (ATX) is first injected automatically and a timed period is provided for the reagent to deplete free ATP. Most beverage products naturally contain sources of free ATP. Sources of free ATP include somatic and/or microbial cells killed during the manufacturing process.
  - Microbial extractant (CellSolver) is injected automatically, and a timed period allowed for this detergent based solution to permeabilize the microbial membranes.
  - ATP bioluminescence reagent (Sensilux) is then injected and any light produced is measured by the instrument in Relative Light Units (RLU).

2.3 Equipment and Accessories

Innovate products

Innovate Luminometer MCH4000 (Cat. no. 220050):
Automated luminometer providing high throughput screening in a microtiter plate format, including Windows-based Innovate.im software designed for the operation and control of the Innovate luminometer.

Application Specific Reagent Kit
Pipettes and sterile tips (to dispense 50 µl of sample) General laboratory materials

Incubator
Quanti-cult® organisms (optional) Agar plates for confirmatory purposes

2.4 Method

Step 1: Enrichment – Incubation of product in final package for a user determined time.
Note: typical incubation time for UHT dairy products is 48-72 hours. All users should determine the most appropriate sample preparation, incubation times and temperatures based on their product types, microorganisms of interest, and regulatory requirements.

Step 2: Measurement – Measurement of sample using ATP bioluminescence method. See the kit insert for detailed reagent reconstitution and handling guidelines.

- Pipette 50 µl of incubated product into sample well for standard protocol*
- Place microtiter plate into luminometer
- Injection of reagents and measurement is automatically completed by instrument
- Results are expressed in Relative Light Units (RLU)

*Alternative protocols are available for product matrices other than UHT dairy products. Instrument specific parameters will be entered into the Innovate.im Software and measurement protocols will be verified by technical support during equipment installation.

2.5 Daily Controls

The following controls should be performed to ensure proper system operation. All controls should yield a result of OK prior to performing sample analysis.

Negative Control

- Instrument Blank – Measure 2 empty wells with the InsBlank protocol.

Negative Control

- Reagent Blank – Measure 2 wells with the ReaBlank protocol.

Positive Control

- ATP Positive Control – Pipette 50 µl of Innovate ATP Pos Control into two sample wells. Measure wells using ATP protocol.

2.6 Interpretation of Results

Result interpretation is automatically performed by the Innovate.im software based on specific cut-off thresholds determined during product evaluation. Typically a sample with RLU greater than 3x the average product baseline is considered positive; however implementation data will be collected to confirm the appropriate cut-off value.

The following guidelines are used for interpreting the results and will be displayed by the Innovate.im software:

PASS Sample Result = Negative → Sample is sterile

FAIL Sample Result = Positive → Sample is non-sterile

3 Customer Implementation

3.1 Introduction

Customer implementation is used to demonstrate that the new method is equivalent to, or better than, the conventional method. The implementation process encompasses the function of the whole system of instrument and reagents and can be divided into three sections:

Determining RLU baselines	Detection of microorganisms (optional)	Parallel testing
The RLU baseline is the average RLU reading obtained from sterile product. This value is used to calculate the cut-off limits for the interpretation of the sample results.	To determine the ability of the method to detect contaminants in beverage products.	To ensure that contaminated samples are detected and uncontaminated samples will give negative results.

3.2 Implementation by Product Grouping

Prior to beginning the implementation, it is common practice to divide products into product groups. There are a number of reasons for product grouping:

- Testing of every single product variant is expensive and time consuming to do.
- Most manufacturers consider it acceptable to divide the range of products into formulation groups (% of fat, pulp, pH, etc.) and select one product from each group as representative of the group.
- As new products are developed, a product grouping approach can replace the need to test each new product separately.

3.3 Determining RLU Baselines

Most beverage products naturally contain sources of free ATP. Sources of free ATP may include somatic cells and/or microbial cells killed during the manufacturing process. Reagent kits contain an ATP depleting enzyme that will deplete these sources of free ATP. Subsequent measurement of product with no viable microbial cells will give what is referred to as the product's "RLU baseline". This baseline value is the average RLU reading obtained from sterile product. This value is used to calculate cut-off limits that establish if a given sample is negative or positive for microbial contamination. The RLU baseline should be determined for each product (or product group) to be tested. In addition, baseline values are not fixed and can change over time for a number of reasons such as seasonal or raw material changes. It is good practice to continually monitor a products baseline and make changes to the threshold if needed.

Samples

A suggested sampling would be 30 samples of each product from a minimum of two production runs.

Method

Standard Assay

- Incubation of product in final package for specified incubation time and temperature
- Shake samples well to homogenize
- Pipette 50 µl of each sample into a well on a microtiter plate
- Place microtiter plate into luminometer
- Setup plate prep and Start measurement
- Injection of reagents and measurement is automatically completed by instrument
- Results are expressed in Relative Light Units (RLU)

Additional assay protocols are available for products other than UHT Dairy. Technical Support can help determine if use of alternate protocols are advised.

Interpretation of RLU values

Use Worksheet 1 to record results, calculating the average RLU from the 30 samples. The RLU values should be stable. Calculate 3x the average baseline RLU value for the cut-off limits. Interpretation of values is:

PASS $RLU_{sample} < 3x\ RLU_{baseline}$ → Sample is negative

FAIL $RLU_{sample} > 3x\ RLU_{baseline}$ → Sample is positive

3.4 Detection of Inocula Using a Range of Microorganisms (optional)

For each product to be tested, the ability of different microorganisms to grow to a detectable level is established using both the Innovate method and the traditional microbiology method. The first step is to select the range of microorganisms to be evaluated. It is suggested to use common spoilage organisms of your specific beverage products. To facilitate inoculation studies, the use of Quanti-cult cell suspensions is recommended. Alternatively, organisms isolated from previous spoilage incidents may be used.

Samples

The number of samples required is one product sample per organism and one product sample as negative control to be tested for each product.

Materials

Quanti-cult organisms may be ordered. Each quanti-cult vial delivers 10-100 CFU's per 100 µl.

Method

- Remove quanti-cult cultures from refrigerator and rehydrate each vial per enclosed kit instructions, paying special attention to the following:
  - Pre-warm the rehydrating fluid vial(s) in a 35-37 °C incubator
  - Allow culture vials to warm to room temperature
  - Replace blue cap on rehydrating fluid vial with red cap containing the microorganisms
  - Invert vial and incubate at 35-37 °C for 15 minutes
  - Invert vial several times by hand to mix
  - Visually ensure that organisms are dissolved and in suspension
- Prior to adding inoculum to product, plate 100 µl of microbial suspension on conventional media and count the colonies after incubation to confirm the CFU level of the inoculum.
- Inoculate sample package with 10-100 colony forming units (CFU) or other specified number of cells
- Incubate inoculated sample(s) and one non-inoculated package for specified time and temperature
- Shake sample well prior to testing to homogenize
- Pipette 50 µl of each sample in duplicate onto a microtiter plate. The non-inoculated sample will serve as a control to verify that sample was not contaminated prior to spiking.
- Place microtiter plate in the instrument and start measurement
- Confirm results by streaking 10-100 µl of spiked sample on conventional media and checking for growth after incubation at 30 ± 2 °C for 24-72 hours

Interpretation of Results

Use worksheet 2 to record results

3.5 Parallel Testing

An implementation program will involve running the method in parallel with a traditional method (Agar Plates or pH) for a significant number of routine samples to ensure that contaminated samples are detected and that uncontaminated samples will give a negative result and will, therefore pass the QC check.

Samples

The number of samples tested in a parallel study will vary according to the individual user requirements. Typically, the number of samples for parallel testing is 50 samples per product.

Method

ATP method

- Incubation of product in final package for specified time and temperature
- Shake samples well to homogenize
- Run samples using the appropriate protocol
- Place microtiter plate into luminometer and measure

Traditional method

- Test same sample using current microbiological method

Interpretation of results

Use worksheet 3 to record results

- Compare the results of the Method with the traditional method. Criteria for acceptance of the new method will vary with the individual user requirements. Typically, the following criteria are considered:
  - Overall agreement of the two methods ≥ 95%
  - No false negative results
- If parallel testing data is satisfactory, finished products may be released with the Innovate method. It is important that any discrepancies between methods be investigated and that the cause is determined.

4 Worksheets

4.1 Worksheet 1 – Determining Product Baselines

Date: _____ Product: _____

	Production run 1 RLU	Production run 2 RLU
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		
Sample 8		
Sample 9		
Sample 10		
Sample 11		
Sample 12		
Sample 13		
Sample 14		
Sample 15		
Sample 16		
Sample 17		
Sample 18		
Sample 19		
Sample 20		
Sample 21		
Sample 22		
Sample 23		
Sample 24		
Sample 25		
Sample 26		
Sample 27		
Sample 28		
Sample 29		
Sample 30		

Calculation of the Cut-off limits:

Average RLU - value _____

_____

3 x Baseline RLU _____

4.2 Worksheet 2 – Detection of Inocula Using a Range of Microorganisms (optional)

Date: _____

Product	Organism	Target inoculum	Actual inoculum Mean CFU	Mean RLU	Detection by Innovate (+ or -)	Plate Growth (+ or -)	Comment

4.3 Worksheet 3 – Parallel Testing

Date: _____

Sample ID	Sample Type	Innovate Results RLU	Innovate Confirmation Results	Reference Method	Comment