Study Report: Evaluating the Effects of Pre-Warming Media Before Enrichment

Introduction

The BAX® System User Guide states that, unless otherwise specified, media should be pre-warmed to a specified temperature before samples are added for enrichment. In order to demonstrate the purpose of this recommendation, an internal study was performed to evaluate the effect of pre-warming enrichment media before sample incubation.

The results of this study support the recommendation of Hygiena to pre-warm all media before enrichment, unless otherwise specified.

Methodology

Equipment, reagents and supplies

BAX® System PCR assay for *Salmonella*
BAX® System instrument and peripherals
Standard BAX® System equipment and supplies
Brain Heart Infusion (BHI) broth
Buffered Peptone Water (BPW)
Brain Heart Infusion (BHI) plates

Sample preparation

Salmonella Typhimurium (DD 586) was selected from the Qualicon Culture Collection for use in this study. This strain was selected due to its relatively frequent presence in a wide variety of naturally contaminated matrices. The *Salmonella* strain was incubated at 37°C in BHI broth overnight to an assumed concentration of 10^9 CFU/mL, then serially diluted in BPW to a concentration of 10^3 CFU/mL, or one log below the claimed sensitivity of the BAX® System assays. Before testing began, 250 mL BPW was added to two stomacher bags. One stomacher bag was placed into an incubator set to 37°C to pre-warm the media overnight. The second bag of media was left at room temperature and not prewarmed. To spike the prepared enrichment media, 1 mL of the serially diluted *Salmonella* strain was added to each stomacher bag of BPW and agitated slightly to mix. Immediately after mixing, a 1-mL aliquot of spiked media was removed from each bag for testing both by culture and with the BAX® System method. The stomacher bags were then incubated at 37°C for 11 hours, with additional 1-mL aliquots removed for testing every 30 minutes.

Method

Throughout the 11-hour enrichment period used in this study, a total of 23 aliquots were removed from each stomacher bag of spiked media for testing. Each media aliquot was tested by both a standard plating method and a non-commercial BAX® System assay for *Salmonella*.

Plating method

For each media aliquot, 100 µL of the enrichment was streaked in triplicate onto BHI agar plates and incubated at 37°C overnight. Plates were then examined for typical *Salmonella* colonies, and total colonies were counted to determine target cell growth.

Internal BAX® System method

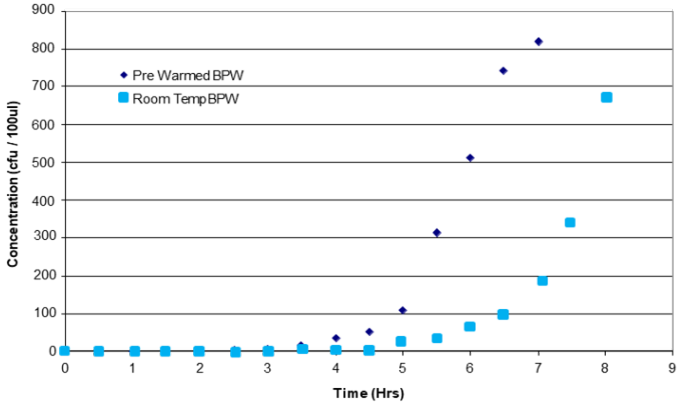
For each media aliquot, 5 µL of the enrichment was added to lysis tubes containing 200 µL prepared BAX® System lysis reagent, and lysis was performed according to the BAX® System real-time protocol for preparing Gram-negative bacteria. This lysate was then used to hydrate PCR tablets in tubes, and all samples were processed in the BAX® System Q7 instrument.

Results

Plating method

The results of the standard plating method are summarized in Figure 1. Although both growth curves illustrate typical bacterial growth for *Salmonella*, samples taken from the pre-warmed BPW media provided countable growth on the BHI plates approximately 1.5-2 hours earlier than samples taken from the room-temperature BPW.

Figure 1. *Salmonella* growth in pre-warmed and room-temperature media



Furthermore, samples taken from pre-warmed BPW reached target *Salmonella* concentrations approximately 1.5 hours sooner than the samples taken from room-temperature media. For example, while the enrichment in pre-warmed BPW reached a target cell count of 100 CFU/mL after about 5 hours of incubation, the room temperature BPW required at least 6.5 hours to reach the same cell concentration. This data suggests that the lag phase for *Salmonella* growth can be decreased by approximately 1.5 hours when pre-warmed BPW is used as the primary enrichment.

BAX® System Method

The BAX® System results for each time point tested are summarized in Table 1. While samples enriched in pre-warmed BPW media grew to detectable levels of *Salmonella* in about 6 hours, samples enriched in room-temperature media required at least 8 hours before positive results were obtained.

Conclusions

The results of this study support the recommendation of Hygiena to pre-warm all media before enrichment, unless otherwise specified in the protocol, in order to sooner reach detectable levels of the target organism. This data suggests that, when pre-warmed BPW is used during primary enrichment, the total time to result for the BAX® System method can be achieved approximately 1.5 to 2 hours* earlier than if room-temperature media is used.

**This time difference is based on research with pure culture only; times may vary due to variables such as the sample matrix tested, sample size and sample temperature.*

Table 1. BAX® System Results for *Salmonella* Testing

Enrichment time (hours)	Pre-warmed media result	Room-temperature media result
0.0	Negative	Negative
0.5	Negative	Negative
1.0	Negative	Negative
1.5	Negative	Negative
2.0	Negative	Negative
2.5	Negative	Negative
3.0	Negative	Negative
3.5	Negative	Negative
4.0	Negative	Negative
4.5	Negative	Negative
5.0	Negative	Negative
5.5	Negative	Negative
6.0	**Positive**	Negative
6.5	**Positive**	Negative
7.0	**Positive**	Negative
7.5	**Positive**	Negative
8.0	**Positive**	**Positive**
8.5	**Positive**	**Positive**
9.0	**Positive**	**Positive**
9.5	**Positive**	**Positive**
10.0	**Positive**	**Positive**
10.5	**Positive**	**Positive**
11.0	**Positive**	**Positive**