# Validation of BacT/ALERT® Media for Sterility Testing in a Skan PSI Isolator after Three $H_2O_2$ Decontamination Cycles

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## INTRODUCTION

Pharmaceutical and biotechnology manufacturers using the BacT/ALERT® Dual-T (BTA) system for sterility testing of aseptically produced products conduct the test under aseptic conditions to mitigate the risk of false positive results from environmental contamination. The Skan PSI Isolator, using $H_2O_2$ for decontamination, provides an aseptic environment where samples can be introduced into the BTA media. Typically, enough media are decontaminated to perform a scheduled test. Unused media are removed from the isolator and discarded unless performance is validated after multiple decontamination cycles. Additionally, interruptions to the decontamination cycle such as an electrical event can result in additional $H_2O_2$ exposure to the media and potentially to laboratory personnel.

**A study was performed to validate BTA media for sterility testing after exposure to three isolator decontamination cycles.**

## MATERIALS AND METHODS

**Decontamination Cycle:**

BTA media (iAST, iNST, iFA, iPF, iFN, iLYM) and water-filled bottles (1 ml purified) were exposed to three consecutive decontamination cycles in a Skan Pharmaceutical Isolator (PSI), type SIS-700, equipped with a vaporizer.

**Set Values of the cycle parameters:**

| Parameter | | Set Value |
|---|---|---|
| q 1 | Quantity of H2O2 vaporized during main dosage (g) | 70 |
| q 2 | Quantity of H2O2 vaporized during redosage (g) | 7 |
| x | Number of redosages | 2 |
| t 1 | Holding time between redosage (sec) | 180 |
| t 2 | Aeration time (sec) | 10800 |
| Relative humidity of start conditioning phase (%) | | < 20 |
| Temperature of start conditioning phase (°C) | | < 40 |
| Concentration of the H2O2 solution (%) | | 49.5 |

Top caps on the iNST, iFA, iPF and iFN bottles were removed prior to decontamination. Two load configurations were tested: 209 bottles and 409 bottles over six shelves of two racks. Three biological indicators (*Geobacillus stearothermophilus*, NAS-152, Apex Laboratories, Sanford, NC) were placed during the third cycle to validate that decontamination was achieved for each load pattern tested.

**Chemical-Physical Testing:**

Residual $H_2O_2$ was measured using the Amplex® UltraRed Assay (Life Technologies™). Nine decontaminated bottles from each load configuration and an equal number of controls were rinsed with 10 ml of purified water and tested for residual $H_2O_2$ (average $H_2O_2$ (mg) per bottle surface). Penetration of $H_2O_2$ was determined by direct testing of the water recovered from each of nine bottles per load configuration and an equal number of controls (average $H_2O_2$ ng / ml). The closures on 100 decontaminated and 100 control bottles were tested using a 1600 Type M durometer (Rex Gauge Co., Buffalo Grove, IL) to determine if exposure to $H_2O_2$ caused a change in the hardness of the stopper. All bottles were inspected for evidence that exposure to $H_2O_2$ had no impact on container integrity.

**Growth Promotion Testing:**

The growth promoting properties of control and decontaminated media were tested using BioBall® (BTF, Sydney, Australia), 25 cfu / bottle, five replicates of each microorganism / medium / incubation temperature combination listed in Table 1:
*Staphylococcus aureus* NCTC10788 (SA), *Escherichia coli* NCTC12923 (EC), *Clostridium sporogenes* NCTC12935 (CS), *Bacillus subtilis* NCTC10400 (BS), *Pseudomonas aeruginosa* NCTC12924 (PA), *Candida albicans* NCPF3179 (CA), and *Aspergillus brasiliensis* NCPF2275 (AB).

## RESULTS & DISCUSSION

**GROWTH PROMOTION TESTING (GPT):**

The chemical and physical test results were correlated to the GPT results. If analysis of Time to Detection (TTD) confirmed the media remained unaffected after decontamination, any differences in the chemical and physical tests were considered insignificant. A difference in mean TTD between the test and control bottles was considered statistically significant if p-value = <0.05. A difference between means with a positive value is interpreted as a decrease in TTD for the test bottles and a negative value is interpreted as an increase in TTD for the test bottles.

**Table 1: Growth Promotion Test Results**

| | | | Mean TTD, Hours | | | | Difference, | |
|---|---|---|---|---|---|---|---|---|
| | Medium | T°C | Cntrl | STD | Test | STD | Hours | P-Value |
| SA | i AST | 32.5°C | 23.4 | 0.6 | 24.9 | 1.1 | -1.5 | 0.025* |
| | i FA | | 21.8 | 1 | 21.9 | 1.2 | -0.1 | 0.869 |
| | i PF | | 20.8 | 0.7 | 21.4 | 0.4 | -0.6 | 0.154 |
| | i FN | | 23 | 0.8 | 23.5 | 0.6 | -0.5 | 0.320 |
| | i NST | | 19.5 | 0.8 | 19.9 | 0.9 | -0.3 | 0.541 |
| EC | i AST | 32.5°C | 16.7 | 0.4 | 16.5 | 0.5 | 0.2 | 0.514 |
| | i FA | | 17.7 | 0.3 | 16.6 | 0.6 | 1.1 | 0.008* |
| | i PF | | 23.7 | 0.6 | 22.3 | 0.8 | 1.4 | 0.014* |
| | i FN | | 15.4 | 0.3 | 15 | 0.4 | 0.4 | 0.086 |
| | i NST | | 14.6 | 0.1 | 14.4 | 0.3 | 0.2 | 0.252 |
| CS | i FN | 32.5°C | 23 | 1.2 | 23.8 | 0.7 | -0.8 | 0.232 |
| | i NST | | 22.1 | 1.2 | 21.7 | 0.7 | 0.4 | 0.565 |
| BS | i AST | 32.5°C | 18.4 | 0.3 | 18.1 | 0.7 | 0.2 | 0.538 |
| | i FA | | 19.4 | 0.4 | 19.3 | 0.3 | 0.1 | 0.613 |
| | i PF | | 18.6 | 0.4 | 18.9 | 0.7 | -0.3 | 0.408 |
| | i AST | 22.5°C | 41.5 | 2.5 | 43.2 | 2.4 | -1.7 | 0.317 |
| | i FA | | 44.9 | 2.8 | 44.9 | 0.8 | 0 | 0.977 |
| | i PF | | 45.1 | 2.2 | 45.8 | 2.8 | -0.8 | 0.656 |
| PA | i AST | 32.5°C | 22.8 | 0.7 | 22.5 | 0.5 | 0.3 | 0.482 |
| | i FA | | 22.2 | 0.5 | 22.5 | 0.4 | -0.3 | 0.357 |
| | i PF | | 22.1 | 0.4 | 22.2 | 0.6 | -0.1 | 0.665 |
| | i AST | 22.5°C | 45 | 1.1 | 43.6 | 0.4 | 1.4 | 0.028* |
| | i FA | | 44.9 | 3.1 | 43.5 | 0.8 | 1.3 | 0.404 |
| | i PF | | 43.3 | 1 | 43.8 | 1 | -0.5 | 0.471 |
| CA | i AST | 32.5°C | 28.6 | 0.7 | 28.5 | 0.8 | 0 | 0.933 |
| | i FA | | 44.2 | 1.6 | 42.3 | 2.5 | 2 | 0.182 |
| | i PF | | 56.2 | 6 | 58.6 | 6.9 | -2.4 | 0.575 |
| | i LYM | | 25.2 | 0.5 | 25.5 | 1 | -0.3 | 0.555 |
| | i AST | 22.5°C | 52.8 | 0 | 49 | 2.1 | 3.8 | 0.016* |
| | i FA | | 53.8 | 2.1 | 50.9 | 1.1 | 2.9 | 0.028* |
| | i LYM | | 46.5 | 1 | 45.1 | 0.8 | 1.4 | 0.004* |
| | i PF | | 56.6 | 1.3 | 52.3 | 2 | 4.3 | 0.034* |
| AB | i AST | 32.5°C | 58.6 | 2.7 | 58.1 | 3.1 | 0.5 | 0.803 |
| | i FA | | 58.6 | 10.1 | 55.2 | 5.1 | 3.4 | 0.526 |
| | i PF | | 51.8 | 2.1 | 51.8 | 1.3 | 0 | 1.000 |
| | i LYM | | 32.5 | 2.1 | 33.4 | 2.1 | -0.9 | 0.540 |
| | i AST | 22.5°C | 106.6 | 3.1 | 104.6 | 2.5 | 1.9 | 0.641 |
| | i FA | | 102.2 | 9.1 | 102.7 | 7.3 | -0.5 | 0.929 |
| | i PF | | 96.5 | 4.3 | 105.1 | 10 | -8.6 | 0.113 |
| | i LYM | | 59.5 | 2 | 60.5 | 3.6 | -1 | 0.614 |

*Indicates statistically significant difference in TTD.

Eight microorganism / medium / incubation temperature combinations had p-values <0.05.

Although statistically significant, the results were considered practically insignificant since seven of eight results for the decontaminated bottles showed improvements in TTD. The GPT results indicate that the decontaminated bottle $H_2O_2$ penetration levels were also insignificant.

**CHEMICAL-PHYSICAL TESTING:**

**Negative Control:** Biological indicators from each load were negative for growth after 7 d incubation at 55-60 C. This validated that the cycle parameters were effective for Decontamination of the load configurations tested.

**Container – Stopper:** No visible effects to the container were observed and label and barcode readability were maintained. Residual amounts of $H_2O_2$ found on the surface of the decontaminated bottles were measured to provide information on potential user exposure (Table 2). Durometer hardness measurements show that three $H_2O_2$ decontamination cycles do not cause the bottle stopper to harden and impact its ability to seal (Table 4).

**Residues:** Mean levels of $H_2O_2$ inside the decontaminated bottles were higher than the controls, however, the levels were still very low – 17.15 and 6.16 ng / ml (ppb) (Table 3).

**Table 2: Surface Residual levels of $H_2O_2$**

| Surface | Load 1 | | Load 2 | |
|---|---|---|---|---|
| Residual | Mean, mg / bottle | Std Dev | Mean, mg / bottle | Std Dev |
| Test | 0.9152 | 0.2379 | 1.3402 | 0.2547 |
| Control | 0.0001 | 0.0001* | 0.0002 | 0.0002 |
| Total | 0.9151 | | 1.34 | |

*4 of 9 control bottles were below the detection limit of the test (3.9 ng $H_2O_2$/ml)

**Table 3: Bottle Penetration levels of $H_2O_2$**

| Penetration | Load 1 | | Load 2 | |
|---|---|---|---|---|
| | Mean, ng / mL | Std Dev | Mean, ng / mL | Std Dev |
| Test | 47.8 | 12.07 | 17.08 | 11.17* |
| Control | 30.65 | 3.97 | 10.92 | 5.62* |
| Total | 17.15 | | 6.16 | |

*1 of 9 bottles was below the detection limit of the test (3.9 ng $H_2O_2$/ml)

**Table 4: Durometer Hardness**

| Stopper | Durometer Hardness Measurement, Shore M | | | |
|---|---|---|---|---|
| | Mean | Std Dev | Minimum | Maximum |
| Test | 53.08 | 2.74 | 48 | 58 |
| Control | 53.87 | 2.34 | 49 | 58 |

## CONCLUSION

**This study verified the integrity of the BTA media container and validated that the BTA media remained unaffected after exposure to three typical $H_2O_2$ decontamination cycles. BacT/ALERT bottles (iAST, iNST, iFA, iFN, iPF and iLYM) are suitable for use in an Isolator.**

## REFERENCES

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