Biologics Safety Testing

Meeting USP/EP/CFR sterility testing requirements with a two media assay

BioReliance® by SAFC

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About BioReliance

BioReliance Corporation is a leading provider of cost-effective contract services to the pharmaceutical and biopharmaceutical industries, offering more than 1,000 tests or services related to biologics safety testing, specialized toxicology and animal health diagnostics. Founded in 1947, BioReliance is headquartered in Rockville, Maryland, with laboratory operations in Rockville and Scotland and offices in Tokyo, Japan, and Mumbai, India. The Company employs more than 650 people globally. For more information, visit www.bioreliance.com.

Key Services:

- **Custom Assay Development** to fulfill your exact requirements
- **Biosafety Testing** of biologicals for viruses, bacteria, mycoplasma, fungi
- **Cell Line Characterization** including identity testing, genetic stability, EM, sequencing
- **Final Product Testing** including biopotency testing, residual DNA, host cell proteins, cross-reactivity
- **Virus/TSE Validation Studies** for all biological products
- **Contract GMP Production and Testing** of viral vectors and cell banks
- **Veterinary Vaccine Services** including characterization/identity, extraneous agent testing
- **Regulatory and Consulting Services**

All work undertaken by BioReliance is in compliance with appropriate GLP or GMP standards.

Further information is available by visiting our website at **www.bioreliance.com**

Abstract

Historical data from BioReliance presented herein supports that only 2 media, Fluid Thioglycollate (FTM(THIO)) and Soybean-Casein Digest (SCDM(TSB)) when used in a standard USP/EP/CFR sterility test is sufficient to detect microbial contaminants.

Introduction

BioReliance currently offers a panel of standard sterility assays that comply with US and EU requirements (**Table 1**). The two media outlined in the USP, FDA, EP and JP requirements are Fluid Thioglycollate (FTM(THIO)) and Soybean-Casein Digest (SCDM(TSB)) (USP<71>, 21CFR 610.12, EP 2.6.1, and JP 4.06 respectively). These media detect anaerobic and aerobic bacteria as well as fungal contaminants. An additional series of assays that employ the use of four media as opposed to the standard two defined in the compendial methods have also been offered by BioReliance (**Table 1**). Sabouraud Dextrose Agar (SDA or SAB), designed to support fungal growth, and Pre-reduced Peptone Yeast Glucose broth (PYG), designed for anaerobic bacteria have been used in select sterility assays. These media were added to the compendial media with the intent of offering broader coverage of microbial contaminants in sterility tests.

Historical data from the past half decade (2005–2009) of BioReliance sterility testing were analyzed and assessed in the context of the two versus four media assay formulations and are presented in this report. The data demonstrate that microorganisms detected in the five year data set are capable of growth on the two media FTM(THIO) and SCDM(TSB). From this we conclude that the addition of SDA(SAB) and PYG to the testing scheme does not offer any competitive advantage or additional information to the compendial Sterility Tests currently available at BioReliance.

Table 1. BioReliance Sterility Tests currently available.

GLP Assay Number	GMP Assay Number	Assay Description	Media
510011	510811	Direct Inoculation Sterility for Final Bulk, 2 Media	SCDM(TSB)/FTM(THIO)
510036	510836	Direct Inoculation Sterility for Final Vials, 2 Media	SCDM(TSB)/FTM(THIO)
510006	510806	Membrane Filtration Sterility	SCDM(TSB)/FTM(THIO)
510000[1]	510888[1]	Direct Inoculation Sterility for Prebanking or Cells at Limit, 4 Media	SCDM(TSB)/FTM(THIO)/SDA(SAB)/PYG
510D00[1]	510D88[1]	Direct Inoculation Sterility for Unprocessed Bulk, 4 Media	SCDM(TSB)/FTM(THIO)/SDA(SAB)/PYG
510R00[2]	510R88[2]	Direct Inoculation Sterility for Cell and Virus Banks, 4 Media	SCDM(TSB)/FTM(THIO)/SDA(SAB)/PYG

[1] Replaced by 510011 and 510811.

[2] Replaced by 510036 and 510836.

Fluid Thioglycollate Medium (FTM(THIO))

The intended use of THIO medium is the cultivation of anaerobic, microaerophillic and aerobic microorganisms and for detecting the presence of bacteria in normally sterile materials (Difco manual, 10th edition). FTM(THIO) conforms to the requirements of USP and AOAC for testing the sterility of antibiotics, biologics, and food. The formulation of THIO supports the growth of a wide variety of fastidious microorganisms having a range of growth requirements, including anaerobic bacteria (**Table 2**).

Soybean-Casein Digest Media or Tryptic Soy Broth (SCDM(TSB))

Tryptic Soy Broth is used for cultivating a wide variety of microorganisms and conforms to the Harmonized USP/EP/JP requirements for microbiological testing, including sterility. This medium is also recommended by the Clinical and Laboratory Standards Institute (CLSI) for inoculum preparation in disk diffusion sensitivity tests for testing bacterial contaminants in non-sterile pharmaceutical and cosmetics. TSB complies with established standards in the food industry and is the media of choice by the USDA APHIS for detecting viable bacteria in live vaccines (9CFR:113.26).

Commonly referred to as Soybean-Casein Digest Medium, Tryptic Soy Broth (TSB) is a general purpose medium with a rich nutritional base used for isolating fastidious and non-fastidious microorganisms, including fungi (**Table 3**).

Table 2. Growth characteristics of control organisms for growth promotion in Fluid Thioglycollate medium after 18-48 h incubation at 35ºC incubated under ambient (aerobic) conditions

(Difco 10th ed.; Difco and BBL Manual online).

Organism	Growth
Staphylococcus aureus ATCC® 6538™	Good to excellent
Pseudomonas aeruginosa ATCC® 9027™	Good to excellent
Bacteroides vulgatus ATCC® 8482™	Poor to fair*
Bacillus subtilis ATCC® 6633™	Good to excellent
Bacteroides vulgatus ATCC® 8482™	Poor to fair*
Candida albicans ATCC® 10231™	Good to excellent
Clostridium sporogenes ATCC® 11437™	Good to excellent
Kocuria. rhizophila ATCC® 9341™ (formerly Micrococcus luteus)	Good to excellent
Neisseria meningitidis ATCC® 13090™	Good to excellent
Streptococcus pyogenes ATCC® 19615™	Good to excellent

* Growth good to excellent after 3 days incubation.

Table 3. Growth characteristics of control organisms in TSB (Difco and BBL Manual online).

Organism	Incubation temperature	Recovery
Escherichia coli ATCC® 25922™	30–35ºC	Growth by 48 h
Streptococcus pneumoniae ATCC® 6305™	30–35ºC	Growth by 24 h
Staphylococcus aureus ATCC 25923	30–35ºC	Growth by 48 h
Bacillus subtilis ATCC® 6633™	20–25ºC	Growth by 3 days
Candida albicans ATCC® 10231™	20–25ºC	Growth by 5 days
Aspergillus brasiliensis ATCC® 16404™	20–25ºC	Growth by 5 days

Sabouraud Dextrose Agar (SDA or SAB)

SAB is a general-purpose medium devised by Sabouraud for culturing of yeasts, molds, and aciduric microorganisms and is used for the growth of fungi, particularly those associated with skin infections (dermatophytes) (Sabouraud, 1892). The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and slightly inhibitory to contaminating bacteria in clinical specimens. This medium is recommended in the *USP* for use in performing total combined mold and yeast counts (Microbial Limit Tests).

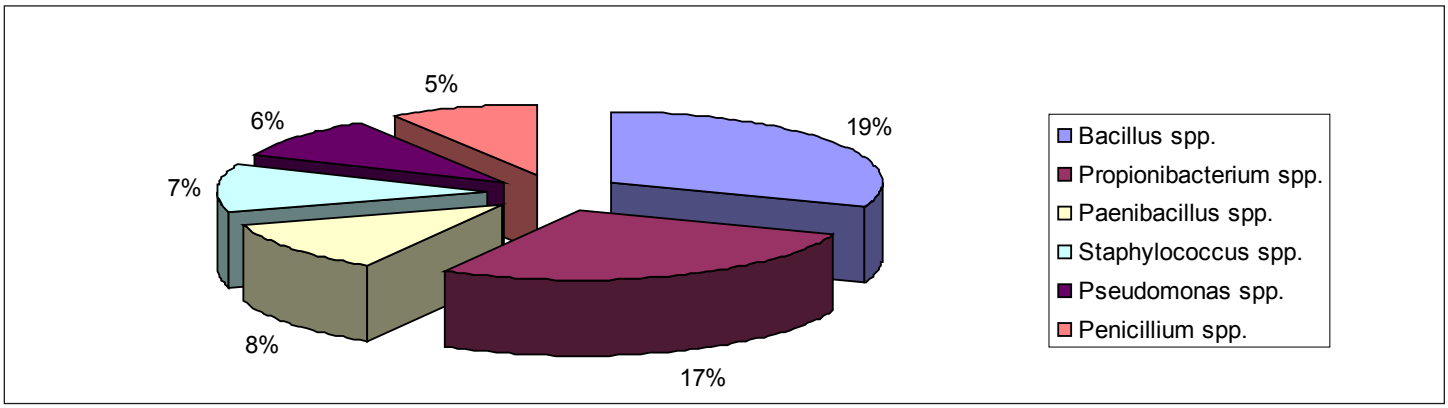
Pre-reduced Peptone Yeast Glucose Broth (PYG)

PYG was developed by the Virginia Polytechnic Institute (VPI) anaerobe laboratory for the cultivation of anaerobes for downstream identification tests (Holdeman, et al., 1977). The anaerobic bacteria cultivated in the PYG broth are also found in the anaerobic phase of the FTM(THIO) media employed in the standard two media sterility tests. Sodium thioglycollate lowers the oxidation–reduction potential of the FTM(THIO) media creating anaerobic conditions in this medium and the small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium (McFaddin, 1985) rendering the inclusion of PYG redundant in the sterility assays.

Historical Data Analysis

The data from Sterility Assays performed by BioReliance over the past 5 years (**Figure 1**) indicate that *Bacillus* species (19%), especially *Bacillus circulans*, was the most commonly identified contaminant in non-sterile biologics tested. The second most common cause of failure was *Proprionibacterium spp.* (17%), followed distantly by P*aenibacillus spp.* (8%) and *Staphylococcus spp.* (7%). The remaining contaminants identified in test articles were found at a frequency of 1-3%. The fungal contaminant, *Penicillium* (5%) was found as infrequently as *Staphylcoccus spp.*, even when SDA(SAB) was included in the tests. In comparison, an analysis of FDA product recall data for 134 non-sterile pharmaceutical products from 1998 to September 2006 demonstrated that 48% of recalls were due to contamination by *Burkholderia cepacia*, *Pseudomonas spp.*, or *Ralstonia picketti*, while yeast and mold contamination were found in 23% of recalls. Gram-negative bacteria accounted for 60% of recalls, but only 4% were associated with Gram-positive bacteria (Jimenez, 2007).

Figure 1. Distribution of the most frequent contaminants found in samples tested from 2005-2009.



Taken together, these data indicate that aerobic and microaerophilic bacteria constitute the primary concern in sterility testing among pharmaceutical and biologics manufacturers. These organisms isolated from contaminants of biologics introduced during the manufacturing process are capable of growth on FTM(THIO) and SCDM(TSB). These two (2) media, independently or together detect representatives from all genera picked up on PYG and/or SDA(SAB), including aerobic and anaerobic bacteria and most fungal species (**Table 4**). Therefore, the addition of PYG or SDA(SAB) does not offer any additional detection advantage over the two media assay. Furthermore, testing using two media as recommended in the USP, EP, and CFR guidelines offers greater sensitivity and accuracy than use of four media for testing. Spreading the test article across fewer media increases the likelihood that contaminants will be detected and that they will be detected in replicate media. Performing sterility testing using the two media to compliance as outlined per USP<71>, 21CFR 610.12 and EP 2.6.1 is scientifically suitable and will meet regulatory standards worldwide for the development and production of safe products intended for human use.

Notification

Effective October 1, 2010, BioReliance will be discontinuing the availability of all four media sterility assays. We encourage that all clients contact their Account Manager or Program Manager to discuss two media assay alternatives.

Table 4. Genera and species identified in sterility testing at BioReliance in the past five (5) years. The designation spp. indicates more than one species of a given genus has been detected in the sterility assays.

Organism	
Acholeplasma laidlawii	*Mesorhizobium huakuii*
Acinetobacter johnsonii	*Methylobacterium extorquens*
Acinetobacter radioresistens	*Micrococcus luteus*
Actinomyces meyeri	*Paecilomyces variotii*
Afipia genomospecies 4	*Paenibacillus spp.*
Arthrobacter spp.	*Patulibacter minatonensis*
Bacillus spp.	*Penicillium spp.*
Bifidobacterium bifidum	*Phyllobacteriaceace*
Bradyrhizobiaceae	*Propionibacterium acnes*
Candida parapsilosis	*Pseudomonas spp.*
Cauolobacter vibrioides	*Ralstonia pickettii*
Cellulosimicrobium funkei	*Serratia liquefaciens*
Cryptococcus spp.	*Staphylococcus spp.*
Cupriavidus metallidurans	*Stenotrophomonas maltophilia*
Enterococcus	*Streptococcus spp.*
Herminiimonas saxobsidens	*Tsukamurella* (formerly *Corynebacterium)*
Leuconostoc gelidum	

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