510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number: K190405

B. Purpose for Submission:

To obtain a substantial equivalence determination for the BACT/ALERT MP Reagent System which includes an adjusted formulation of the BACT/ALERT MP Culture Bottle, the BACT/ALERT MP Antimicrobial Supplement (AS) and the BACT/ALERT MP Nutrient Supplement (NS).

C. Measurand:

Mycobacterial species.

D. Type of Test:

Liquid culture medium for recovery of mycobacteria from sterile body specimens other than blood, and from digested-decontaminated clinical specimens, using a colorimetric sensor to detect CO_2 dissolved in the culture media.

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

BACT/ALERT MP Reagent System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2560, Microbial Growth Monitor

2. Classification:

   Class I

3. Product code:

   MDB

4. Panel:

   83 - Microbiology

H. Intended Use:

1. Intended use(s):

   The BACT/ALERT MP Reagent System consists of the BACT/ALERT MP Culture Bottle with a removable closure used in conjunction with the BACT/ALERT MP Antimicrobial Supplement and the BACT/ALERT MP Nutrient Supplement. The BACT/ALERT MP Reagent System is designed for use with the BACT/ALERT 3D Mycobacteria Detection Systems for recovery and detection of mycobacteria from sterile body specimens other than blood, and from digested-decontaminated clinical specimens.

2. Indication(s) for use:

   Same as the Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   BACT/ALERT (BTA) 3D Microbial Detection Systems

I. Device Description:

The BACT/ALERT MP Reagent System consists of three reagents: BACT/ALERT MP Culture Bottle, the BACT/ALERT MP Antimicrobial Supplement (AS) and the BACT/ALERT MP Nutrient Supplement (NS).

The BACT/ALERT Mycobacteria Detection System, used in conjunction with the BACT/ALERT MP Reagent System, provide both a microbial detection system and a culture media with suitable nutritional and environmental conditions to recover mycobacterial species commonly isolated from patient specimens other than blood. The BACT/ALERT MP Antimicrobial Supplement kit is intended to reduce the incidence of break-through contamination due to bacteria which may survive the digestion-decontamination process. Reconstituted BACT/ALERT MP Antimicrobial Supplement must be added to BACT/ALERT MP bottles prior to inoculation of all non-sterile specimens. For recovery of mycobacteria present in sterile specimens, only the BACT/ALERT MP Nutrient Supplement should be added to the BACT/ALERT MP Culture Bottle. BACT/ALERT MP Nutrient Supplement contains components which are necessary to ensure optimal growth of mycobacteria present in the patient sample and is used for reconstitution of BACT/ALERT

MP Antimicrobial Supplement. Inoculated bottles are loaded into the instrument where they are incubated and continuously monitored for the presence of mycobacteria that will grow in the BACT/ALERT MP Culture Bottle. The BACT/ALERT MP Culture Bottle, the BACT/ALERT MP Antimicrobial Supplement and the BACT/ALERT MP Nutrient Supplement formulations were adjusted to decrease Time-To-Detection (TTD) of select mycobacterial species.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BACT/ALERT MP Culture Bottle

2. Predicate 510(k) number(s):

   K031737

3. Comparison with predicate:

Similarities		
Item	**Device: BACT/ALERT MP Reagent System (K190405)**	**Predicate: BACT/ALERT MP Culture Bottle (K031737)**
Classification	Regulation Section: 21 CFR 866.2560, Microbial Growth Monitor	Same
Product Code	MDB	Same
Intended Use	The BACT/ALERT MP Reagent System consists of the BACT/ALERT MP Culture Bottle with a removable closure used in conjunction with the BACT/ALERT MP Antimicrobial Supplement and the BACT/ALERT MP Nutrient Supplement. The BACT/ALERT MP Reagent System is designed for use with the BACT/ALERT 3D Mycobacteria Detection Systems for recovery and detection of mycobacteria from sterile body specimens other than blood, and from digested-decontaminated clinical specimens.	The BACT/ALERT MP Culture Bottle consists of the BACT/ALERT MP Culture Bottle with a removable closure used in conjunction with the MB/BacT Antimicrobial Supplement (and /or the MB/BacT Reconstitution Fluid). The BACT/ALERT MP System is designed for use with the MB/BACT or the BACT/ALERT 3D Mycobacteria Detection Systems for recovery and detection of mycobacteria from sterile body specimens other than blood, and from digested-decontaminated clinical specimens.
Sample Type	Sterile body specimens other than blood and digested-decontaminated clinical specimens	Same
Technology	Reflectance	Same
Color change based on CO_2 production	Yes	Same

Differences		
Item	**Device: BACT/ALERT MP Reagent System (K190405)**	**Predicate: BACT/ALERT MP Culture Bottle (K031737)**
MP Culture Bottle	Storage temperature: 15-30ºC	Storage temperature: 2-8ºC
	Formulation changes	
	Not present	Pancreatic digest of casein
	Not present	Bovine Serum Albumin (BSA)
	Not present	Catalase in Purified Water
	Magnesium	Not present
MP Antimicrobial Supplement (AS)	**Formulation changes**	
	Not present	Azlocillin
	Fosfomycin	Not present
	Polymixin B increased approximately 20% as compared to the predicate	Polymixin B (in trace amounts)
	Trimethoprim increased approximately 350% as compared to the predicate	Trimethoprim (in trace amounts)
	Not present	Vancomycin
MP Antimicrobial Supplement Vial	Bromobutyl Stopper Top	Chlorobutyl Stopper Top
MP Nutrient Supplement (NS) [1]	**Formulation changes**	
	Fatty acids (in trace amounts)	Not present
	Pancreatic digest of casein (in trace amounts)	Not present
	Fatty acid free BSA	BSA
	α-ketoglutarate	Not Present

[1] The MP Nutrient Supplement is called "Reconstitution Fluid" for the predicate BACT/ALERT MP Culture Bottle

K. Standard/Guidance Document Referenced (if applicable):

- CLSI M22-A3. Quality Control For Commercially Prepared Microbiological Culture Media – Third Edition.
- CLSI M100. Performance Standards for Antimicrobial Susceptibility Testing – 28th Edition.
- CLSI M48-A. Laboratory Detection and Identification of Mycobacteria – Approved Guideline. Vol. 28, No 17.
- CLSI M07. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Tenth Edition.
- CLSI MM09-A2. Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine – Second Edition.

- CLSI EP15-A2. User Verification of Performance for Precision and Trueness – Approved Guideline. Vol. 25, No 17.
- ANSI/ASQ. Sampling Procedures and Tables for Inspection by Attributes. Version Z1.4-2008.

L. Test Principle:

BACT/ALERT Mycobacterial Detection Systems utilize the same principle to detect microorganisms in cultures taken from processed patient samples as is described for the BACT/ALERT Microbial Detection System with respect to blood cultures. BACT/ALERT Mycobacteria Detection Systems utilize a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the organisms metabolize substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes to lighter green or yellow. The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes. At the time of detection, approximate colony forming units (CFUs) per mL are 10^6 - 10^7.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision:

Within-laboratory Precision

Within-laboratory precision was evaluated by conducting an in-house seed study over 12 non-consecutive days using multiple BTA 3D Systems, lots of each adjusted reagent (MP Culture Bottle, Nutrient Supplement (NS), and Antimicrobial Supplement (AS)), and operators. All species shown in **Table 1a** were tested at two target concentrations (300 CFU/bottle and 1000 CFU/bottle) representing a low concentration sample (~3x LoD) and the concentration found in a patient sample with a positive Acid-Fast Bacilli (AFB) smear. Actual inoculums for the 300 and 1000 CFU/bottle targets ranged from six to-388 CFU/bottle and 20 to 1,292 CFU/bottle, respectively. Each species was tested with two different sample matrices, representing a non-sterile patient sample (simulated sputum) and sterile sample (pleural fluid). A matrix equivalency study was conducted to support use of simulated sputum in all seeded studies. For more details, see the section titled, "***Simulated Sputum Matrix Equivalency Study***". For non-sterile samples, organisms were spiked into simulated sputum and digested-decontaminated by the NaOH method before inoculation into adjusted MP Culture Bottles with reconstituted Nutrient Supplement and Antimicrobial Supplement added. For sterile samples, each organism was spiked into pleural fluid and inoculated into adjusted MP Culture Bottles with NS only. Percent recovery data was analyzed for each organism and for each test concentration and matrix. Because no difference was noted, the data was combined and used to evaluate the percent recovery for the species listed in **Table 1a**."

Table 1a. Precision Study – Percent Recovery Results

Species (ATCC number)	Adjusted MP Bottle		% Recovery
	# Bottles Tested	# Bottles Positive	
M. fortuitum (6841)	384	384	100%
M. intracellulare (13950)	383	383	100%
M. kansasii (12478)	384	384	100%
M. scrofulaceum (19981)	384	384	100%
M. tuberculosis (25177)	383	383	100%

Performance was assessed by determining percent recovery (detection) and mean Time-To-Detection (TTD). TTD is the amount of time from a bottle being placed on the BTA 3D instrument until it is flagged positive. Percent recovery reflects positive flag by the instrument and subculture consistent with the appropriate seeded organism.

The study demonstrated that percent recovery was 100% for all mycobacterial species regardless of sample matrix or inoculum level (**Table 1a**). Percent recovery was unaffected by adjusted MP Culture Bottle lot.

Mean TTD was calculated independently for the following organism groups and species: (i) non-tuberculosis mycobacteria (NTM) rapid growers (i.e., *M. fortuitum*-ATCC 6841), (ii) NTM slow growers (i.e., *M. intracellulare*-ATCC 13950, *M. kansasii*-ATCC 12478) and *M. scrofulaceum-ATCC* 19981), and (iii) *Mycobacterium tuberculosis*-ATCC 25177. Mean TTD for these organism groups/species did not vary by MP Culture Bottle lot. Mean TTDs were faster for mycobacteria spiked into pleural fluid compared to simulated sputum (**Table 1b**). Although it was observed that mean TTD was delayed for bottles from lot four with pleural fluid, this was attributed to only testing lot 4 with a subset of slower growing mycobacteria (i.e., *M. intracellulare* and *M. tuberculosis*). Mean TTD results for the adjusted MP Culture Bottle are illustrated in **Table 1b**, stratified by reagent lot and sample type.

Table 1b. Time-To-Detection – Lot-to-Lot Difference by Sample Type

Adjusted MP Culture Bottle Lot	Sample Type	Species/Group	Target Inoculum			
			300 CFU/bottle		1000 CFU/bottle	
			# of Bottles	Mean TTD (in Days) (95% CI)	# of Bottles	Mean TTD (in Days) (95% CI)
1	Simulated sputum (processed)	NTM – rapid grower[1]	32	3.6 (3.5, 3.7)	32	3.2 (3.1, 3.3)
		M. tuberculosis (ATCC 25177)	24	16.3 (15.9, 16.7)	24	14.5 (14.3, 14.8)
		NTM – slow growers[2]	71	10.5 (10.3, 10.7)	72	9.4 (9.2, 9.6)
	Pleural fluid	NTM – rapid grower	32	2.7 (2.6, 2.8)	32	2.4 (2.4, 2.5)
		M. tuberculosis (ATCC 25177)	32	15.7 (15.4, 16.1)	32	14.1 (13.8, 14.4)
		NTM – slow growers	104	10.5 (10.2, 10.8)	104	9.8 (9.5, 10.1)
2	Simulated sputum (processed)	NTM – rapid grower	32	3.4 (3.3, 3.5)	32	2.9 (2.8, 3.1)
		M. tuberculosis (ATCC 25177)	40	15.6 (15.1, 16.0)	40	13.6 (13.2, 14.0)
		NTM – slow growers	96	10.6 (10.4, 10.8)	96	9.7 (9.5, 9.9)
	Pleural fluid	NTM – rapid grower	32	2.5 (2.5, 2.5)	32	2.4 (2.3, 2.5)
		M. tuberculosis (ATCC 25177)	8	15.1 (14.7, 15.6)	8	14.0 (13.7, 14.2)
		NTM – slow growers	80	10.9 (10.5, 11.3)	80	9.9 (9.6, 10.3)
3	Simulated sputum (processed)	NTM – rapid grower	32	3.7 (3.4, 3.9)	32	3.0 (2.8, 3.2)
		M. tuberculosis (ATCC 25177)	32	15.0 (14.8, 15.2)	32	13.1 (12.9, 13.3)
		NTM – slow growers	120	10.5 (10.3, 10.6)	120	9.5 (9.5, 9.6)
	Pleural fluid	NTM – rapid grower	32	2.8 (2.5, 3.2)	32	2.4 (2.3, 2.5)
		M. tuberculosis (ATCC 25177)	16	15.1 (14.9, 15.4)	16	13.8 (13.5, 14.1)
		NTM – slow growers	80	10.8 (10,4, 11.2)	80	10.2 (9.8, 10.5)
4	Pleural fluid	*M. tuberculosis* (ATCC 25177)	39	17.0 (16.8, 17.2)	40	16.1 (16.0, 16.3)
		M. intracellulare (ATCC 13950) - NTM slow grower	24	9.8 (9.6, 9.9)	24	9.1 (9.0, 9,2)

1. *M. fortuitum* (ATCC 6841)
2. *M. intracellulare* (ATCC 13950), *M. kansasii* (ATCC 12478), and *M. scrofulaceum* (ATCC 19981)

Reproducibility

Reproducibility was evaluated by conducting a multi-center study at two external sites and internally at bioMérieux, over five days, using multiple BTA 3D instruments, lots of adjusted reagent (MP Culture Bottle, NS, and AS), and operators. The combined data from a total of 1,800 bottles (5 organisms x 2 matrices x 2 inoculum levels x 2 operators x 5 days x 3 reps per run x 3 sites) was used to evaluate the percent recovery for the species listed in **Table 2a**. All species were tested at two target concentrations (300 CFU/bottle and 1000 CFU/bottle) representing a low concentration sample (~3x LoD) and the concentration of a patient sample with a positive AFB smear. Each species was tested with two different sample matrices, representing a non-sterile patient sample (simulated sputum) and sterile sample (pleural fluid). For the non-sterile samples, organisms were spiked into simulated sputum and digested-decontaminated before inoculation into adjusted MP Culture Bottles supplemented with reconstituted AS. For sterile samples, each organism was spiked into pleural fluid and inoculated into adjusted MP bottles supplemented with NS.

Performance was assessed by determining percent recovery and mean TTD. The study demonstrated that overall percent recovery in pleural fluid was 99.1% (446/450) for mycobacteria spiked at a target inoculum of 300 CFU/bottle and 100% (450/450) for mycobacteria spiked at a target inoculum of 1000 CFU/bottle (**Table 2a**). Overall percent recovery in simulated sputum was 99.8% (449/450) at a target inoculum of 300 CFU/bottle and 100% (450/450) for mycobacteria spiked at a target inoculum of 1000 CFU/bottle (**Table 2b**). Percent recovery was not affected by adjusted MP Culture Bottle lot (**Table 2c**).

Mean TTD was calculated independently for the following organism groups and species: (i) non-tuberculosis mycobacteria (NTM) rapid growers (i.e., *M. fortuitum*-ATCC 6841), (ii) NTM slow growers (i.e., *M. intracellulare*-ATCC 13950, *M. kansasii*-ATCC 12478) and *M. scrofulaceum-ATCC* 19981), and (iii) Mycobacterium tuberculosis-ATCC 25177. Mean TTD for these organism groups/species did not vary by MP Culture Bottle lot. Mean TTD for mycobacteria was faster in pleural fluid than simulated sputum (**Table 2D**). Mean TTD results for the adjusted MP Culture Bottle with pleural fluid and simulated sputum are illustrated in **Table 2d**.

Table 2a. Reproducibility Study Percent Recovery Results for Pleural Fluid

Species	Inoculum Level (CFU/bottle)		% Recovery			
	Target	**Actual**	**Site 1**	**Site 2**	**Site 3**	**Overall [95% CI]**
M. fortuitum	300	18-382	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	105-1267.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. intracellulare	300	1-323	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) [94.0, 99.9%]
	1000	5-967.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. kansasii	300	141-389	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	480-1290	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. scrofulaceum	300	7-387	100% (30/30)	96.7% (29/30)	100% (30/30)	98.9% (89/90) [94.0, 99.9%]
	1000	15-1207.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. tuberculosis	300	1-162	100% (30/30)	93.3% (28/30)	100% (30/30)	97.8% (88/90) [92.2, 99.7%]
	1000	5-712.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
Overall	300	NA	100% (150/150)	98.0% (147/150)	99.3% (149/150)	99.1% (446/450) [97.7, 99.8%]
	1000	NA	100% (150/150	100% (150/150)	100% (150/150)	100% (450/450) [99.2, 100%]

Table 2b. Reproducibility Study Percent Recovery Results for Simulated Sputum

Species	Inoculum Level (CFU/bottle)		% Recovery			
	Target	Actual	Site 1	Site 2	Site 3	Overall [95% CI]
M. fortuitum	300	10-382	100% (30/30)	100% (30/30)	96.7% (29/30)	98.9% (89/90) [94.0, 99.9%]
	1000	90-1287.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. intracellulare	300	1-323	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	7.5-1117.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. kansasii	300	9-337	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	205-1280	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. scrofulaceum	300	9-304	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	22.5-1225	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
M. tuberculosis	300	0-275	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
	1000	0-1262.5	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [96.0, 100%]
All organisms	300	NA	100% (150/150)	100% (150/150)	99.3% (149/150)	99.8% (449/450) [98.8, 99.9%]
	1000	NA	100% (150/150)	100% (150/150)	100% (150/150)	100% (450/450) [99.2, 100%]

Table 2c. Reproducibility Study Results – Percent Recovery for Pleural Fluid and Simulated Sputum, Stratified by Lot

Adjusted MP Culture Bottle Lot	% Recovery [95% CI]	
	Pleural Fluid	Simulated Sputum
1	99.4% (358/360) [98.0, 99.9%]	100% (360/360) [99.0, 100%]
2	99.6% (269/270) [98.0, 99.9%]	99.6% (269/270) [98.0, 99.9%]
3	99.6% (269/270) [98.0, 99.9%]	100% (270/270) [98.6, 100%]

Table 2d. Reproducibility Study Results – Time-To-Detection (TTD) Stratified by Lot and Sample Type

Adjusted MP Culture Bottle Lot	Sample Type	Species/Group	Target Inoculum			
			300 CFU/bottle		1000 CFU/bottle	
			# of Bottles	Mean TTD (in Days) (95% CI)	# of Bottles	Mean TTD (in Days) (95% CI)
1	Simulated sputum	NTM – rapid grower[1]	27	3.9 (3.6, 4.2)	27	3.1 (2.8, 3.3)
		M. tuberculosis (ATCC 25177)	27	20.5 (19.5, 21.6)	27	16.6 (15.7, 17.5)
		NTM – slow growers[2]	81	10.2 (9.9, 10.6)	81	9.0 (8.7, 9.2)
	Pleural fluid	NTM – rapid grower	27	2.6 (2.5, 2.7)	27	2.3 (2.2, 2.4)
		M. tuberculosis (ATCC 25177)	26	16.6 (16.0, 17.2)	27	15.1 (14.9, 15.3)
		NTM – slow growers	81	9.9 (9.3, 10.5)	81	9.4 (8.9, 9.8)
2	Simulated sputum	NTM – rapid grower	26	3.9 (3.6, 4.2)	27	3.1 (2.9, 3.3)
		M. tuberculosis (ATCC 25177)	27	19.3 (18.5, 20.2)	27	16.4 (15.8, 17.0)
		NTM – slow growers	81	10.5 (10.1, 10.8)	81	9.3 (9.0, 9.6)
	Pleural fluid	NTM – rapid grower	27	2.7 (2.5, 2.8)	27	2.3 (2.2, 2.4)
		M. tuberculosis (ATCC 25177)	27	16.8 (16.3, 17.2)	27	15.2 (14.8, 15.6)
		NTM – slow growers	80	10.1 (9.6, 10.6)	81	9.5 (9.1, 9.9)

3	Simulated sputum	NTM – rapid grower	36	3.6 (3.4, 3.7)	36	2.9 (2.8, 3.0)
		M. tuberculosis (ATCC 25177)	36	20.4 (19.5, 21.3)	36	16.9 (16.1, 17.7)
		NTM – slow growers	108	10.4 (10.1, 10.7)	108	9.3 (9.0, 9.5)
	Pleural fluid	NTM – rapid grower	36	2.5 (2.5, 2.6)	36	2.4 (2.3, 2.4)
		M. tuberculosis (ATCC 25177)	35	16.9 (16.4, 17.4)	36	15.5 (15.3, 15.8)
		NTM – slow growers	107	10.1 (9.6, 10.5)	108	9.2 (8.9, 9.6)

1. *M. fortuitum* (ATCC 6841)
2. *M. intracellulare* (ATCC 13950), *M. kansasii* (ATCC 12478), and *M. scrofulaceum* (ATCC 19981)

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

External QC Testing

During the Clinical Study, external quality control (QC) was conducted on the BTA 3D System using negative and positive control bottles. Positive control organisms were seeded into adjusted MP Culture Bottles at target inoculum levels of 1000 CFU/bottle. Organism concentrations for bottle seeding were confirmed by plate counting procedures. A positive control inoculated with *M. fortuitum* 6841 and a negative control were tested once per week. In addition, an *M. tuberculosis* 25177 positive control was tested every other week for the duration of the study. Overall QC results were found to be acceptable. Instances where unacceptable QC results were observed were found to be due to technical errors (i.e., colony counts out of range, contaminated bottles, etc.).

d. Detection limit:

Limit of Detection (LoD)

An in-house LoD study was conducted with seeded samples to determine the LoD of mycobacteria in the adjusted MP Culture Bottle in the presence of reconstituted AS, representing the reagent system as a whole. Testing with reconstituted AS was conducted to represent the worst-case scenario, as the antimicrobials in AS can negatively impact the growth of some mycobacterial species. For this study, the species listed in **Table 3** were grown on solid mycobacteria media until confluence was achieved. Organisms were then vortexed to break up clumps. Subsequently, a portion of the vortexed culture was used to generate a 100 CFU/bottle suspension in mycobacteria media. The 100 CFU/bottle suspension was plated in triplicate and used

to determine final concentrations in each dilution (i.e., 0, 10, 25, 75, and 100 CFU/bottle). After inoculating the species denoted in **Table 3** into adjusted MP Culture Bottles, they were incubated on the BTA 3D instrument. Three lots of each adjusted reagent (MP Culture Bottle, NS, and AS) were included. A minimum of 40 adjusted MP Culture Bottles were tested for each species at each inoculum level. All bottles flagged positive by the instrument were subcultured for purity. Results were considered valid if subcultures had no evidence of contamination and morphology was consistent with the expected organism. The lowest inoculum level with a recovery rate $\geq$ 95% was defined as the LoD for that species. Results are illustrated in **Table 3**.

Table 3. LoD Study Testing Results for the MP Reagent System

Microorganism	Strain ID	LoD (CFU/bottle)
Mycobacterium avium	ATCC 25291	$\leq$ 15
Mycobacterium kansasii	ATCC 12478	$\leq$ 4
Mycobacterium tuberculosis	ATCC 25177	$\leq$ 14

Percent Recovery (Detection) Study

An in-house seeded study was conducted to evaluate percent recovery (detection) of mycobacterial species in the adjusted MP Culture Bottle supplemented with Nutrient Supplement or reconstituted AS (Antimicrobial Supplement and Nutrient Supplement). A diverse set of 20 clinically and environmentally relevant mycobacterial species were evaluated using three lots of each adjusted reagent (MP Culture Bottle, AS, and NS). The species tested are listed in **Table 4b**. All organisms were seeded into adjusted MP Culture Bottles at a target inoculum of 10^2 and 10^6 CFU/bottle to represent challenging concentrations and those commonly seen in clinical environments. Actual inoculums for the 10^2 and 10^6 targets ranged from 30-183 CFU/bottle and 3.0 x 10^5-1.82 x 10^6 CFU/bottle, respectively. All seeded bottles were incubated on the BTA 3D System.

Performance was considered acceptable if $\geq$ 95% of adjusted MP Culture Bottles yielded a positive result for each organism/condition evaluated and overall.

The overall detection rate for the adjusted MP Culture Bottle was 100%, regardless of inoculum level or supplementation (**Table 4a**). Calculation of the overall detection rate excluded results for *M. haemophilum* and *M. ulcerans*, which were not recovered in the adjusted MP Culture Bottle (**Table 4b**). Failure to recover these organisms was not unexpected, as they have optimal temperature requirements outside the BTA 3D instrument range, as indicated by a limitation in the package insert for the MP Reagent System and predicate device. Percent recovery was the unaffected by adjusted MP Culture Bottle lot.

Table 4a. Percent Recovery in the Adjusted MP Culture Bottle Stratified by Inoculum Level and Supplement

Target Inoculum	Supplement	Percent Recovery	95% CI
10^2	NS	100.0% (229/229)	98.4%, 100.0%
	NS + AS	100.0% (148/148)	97.5%, 100.0%
10^6	NS	100.0% (230/230)	98.4%, 100.0%
	NS + AS	100.0% (137/137)	97.3%, 100.0%

NS – Nutrient Supplement
AS – Antimicrobial Supplement

Table 4b. Percent Recovery Stratified by Organism, Inoculum Level, and Supplementation

Organism	10^2 Target Inoculum (CFU/bottle)			10^6 Target Inoculum (CFU/bottle)		
	Actual Inoculum (CFU/bottle)	% Recovery (NS)	% Recovery (NS + AS)	Actual Inoculum (CFU/bottle)	% Recovery (NS)	% Recovery (NS + AS)
M. abscessus (1 strain)	134	100.0 (10/10)	100.0 (6/6)	1.34×10^6	100.0 (10/10)	100.0 (6/6)
M. africanum (1 strain)	64	100.0 (10/10)	100.0 (6/6)	6.4×10^5	100.0 (10/10)	100.0 (6/6)
M. avium (2 strains)	42-182	100.0 (20/20)	100.0 (12/12)	4.2×10^5- 1.82×10^6	100.0 (20/20)	100.0 (12/12)
M. bovis (1 strain)	77	100.0 (10/10)	100.0 (11/11)	7.7×10^5	100.0 (10/10)	100.0 (6/6)
M. canetti (1 strain)	111	100.0 (10/10)	100.0 (6/6)	1.11×10^6	100.0 (10/10)	100.0 (6/6)
M. fortuitum (1 strain)	54	100.0 (10/10)	100.0 (6/6)	5.4×10^5	100.0 (10/10)	100.0 (6/6)
M. gordonae (1 strain)	53	100.0 (10/10)	100.0 (6/6)	5.3×10^5	100.0 (10/10)	100.0 (5/5)[1]
M. intracellulare (2 strains)	48-75	100.0 (20/20)	100.0 (12/12)	4.8×10^5 - 7.5×10^5	100.0 (20/20)	100.0 (12/12)
M. kansasii (1 strain)	32	100.0 (9/9)[1]	100.0 (6/6)	3.2×10^5	100.0 (10/10)	100.0 (6/6)
M. microti (1 strain)	75	100.0 (10/10)	100.0 (6/6)	7.5×10^5	100.0 (10/10)	100.0 (6/6)
M. pinnipedii (1 strain)	63	100.0 (10/10)	100.0 (6/6)	6.3×10^5	100.0 (10/10)	100.0 (6/6)
M. scrofulaceum (1 strain)	92	100.0 (10/10)	100.0 (6/6)	9.2×10^5	100.0 (10/10)	100.0 (6/6)
M. simiae (1 strain)	48	100.0 (10/10)	100.0 (6/6)	4.8×10^5	100.0 (10/10)	100.0 (6/6)

M. tuberculosis (4 strains)	39-60	100.0 (40/40)	100.0 (29/29)	3.9×10^5 - 6.0×10^5	100.0 (40/40)	100.0 (24/24)
M. chelonae (1 strain)	118	100.0 (10/10)	100.0 (6/6)	1.18×10^6	100.0 (10/10)	100.0 (6/6)
M. haemophilum[2] *(1 strain)*	64	0.0 (0/9)[3]	0.0 (0/6)	6.4×10^5	0.0 (0/10)	0.0 (0/6)
M. malmoense (1 strain)	141	100.0 (10/10	100.0 (6/6)	1.41×10^6	100.0 (10/10	100.0 (6/6)
M. marinum (1 strain)	87	100.0 (10/10	100.0 (6/6)	8.7×10^5	100.0 (10/10	100.0 (6/6)
M. ulcerans[2] *(1 strain)*	30	0.0 (0/10)	0.0 (0/6)	3.0×10^5	0.0 (0/10	0.0 (0/6)
M. xenopi (1 strain)	94	100.0 (10/10	100.0 (6/6)	9.4×10^5	100.0 (10/10	100.0 (6/6)

1. One bottle was removed due to failure to inoculate
2. *M. haemophilum* and *M. ulcerans* were not recovered in the adjusted MP Culture Bottle. Failure to recover these organisms was not unexpected, as they have optimal temperature requirements outside the BTA 3D instrument range
3. One bottle was removed from analysis due to an early FP call

Time-To-Detection (TTD) Study

An in-house seeded study was conducted to evaluate Time-To-Detection (TTD) (in days), for the MP Reagent System. For this study, the organisms listed in **Table 5** were evaluated. All organisms were seeded into adjusted MP Culture Bottles at a target inoculum of 10^2 and 10^6 CFU/bottle to represent challenging concentrations and those commonly seen in clinical environments. Actual inoculums for the 10^2 and 10^6 CFU/bottle targets ranged from 30-183 CFU/bottle and 3.0×10^5 - 1.82×10^6 CFU/bottle, respectively. Three lots of each reagent of the MP Reagent System were included in testing. Mean TTD results from the three lots, stratified by organism and inoculum level, are shown in **Table 5**. When multiple strains were tested for a single species, mean TTD was calculated by pooling strain data.

Mean TTD was considered acceptable if a given species was identified in $\leq$ 42-days. The mean TTD was < 42-days for all evaluated species and conditions.

Table 5. Mean Time-To-Detection for the Adjusted MP Bottle Stratified by Organism and Inoculum Level*

Species	Target Inoculum (CFU/bottle)	Nutrient Supplement		Nutrient Supplement and Antimicrobial Supplement	
		# Positive Bottles	Mean TTD (in Days)	# Positive Bottles	Mean TTD (in Days)
M. abscessus (1 strain)	10^2	10	3.7	6	3.4
	10^6	10	2.1	6	1.6
M. africanum (1 strain)	10^2	10	18.8	6	23.1
	10^6	10	5.2	6	6.9
M. avium (2 strains)	10^2	20	9.8	12	10.5
	10^6	20	4.5	12	4.8
M. bovis (1 strain)	10^2	10	15.2	11	15.0
	10^6	10	4.8	6	4.9
M. canetti (1 strain)	10^2	10	10.9	6	11.1
	10^6	10	4.5	6	4.6
M. fortuitum (1 strain)	10^2	10	3.5	6	4.3
	10^6	10	1.2	6	1.4
M. gordonae (1 strain)	10^2	10	15.3	6	18.5
	10^6	10	5.2	5	7.1
M. intracellulare (2 strains)	10^2	20	10.5	12	10.9
	10^6	20	4.0	12	4.1
M. kansasii (1 strain)	10^2	9	10.5	6	11.1
	10^6	10	4.7	6	4.8
M. microti (1 strain)	10^2	10	28.6	6	30.3
	10^6	10	6.4	6	6.3
M. pinnipedii (1 strain)	10^2	10	16.8	6	19.8
	10^6	10	4.8	6	6.0
M. scrofulaceum (1 strain)	10^2	10	11.7	6	12.3
	10^6	10	5.5	6	5.6
M. simiae (1 strain)	10^2	10	8.2	6	11.1
	10^6	10	4.2	6	4.3
M. tuberculosis (4 strains)	10^2	40	15.8	29	16.4
	10^6	40	4.6	24	4.6
M. chelonae (1 strain)	10^2	10	8.4	6	6.7
	10^6	10	2.5	6	2.5
M. malmoense (1 strain)	10^2	10	14.4	6	16.1
	10^6	10	6.7	6	7.8
M. marinum (1 strain)	10^2	10	12.1	6	12.4
	10^6	10	2.6	6	2.8
M. xenopi	10^2	10	34.9	6	34.6
	10^6	10	11.7	6	11.2

*TTD was not calculated for *M. haemophilum* or *M. ulcerans*, as they were not recovered in the adjusted MP Culture Bottle

To ensure that changes to the MP Reagent System formulation do not negatively impact mean TTD, a comparison study was conducted with adjusted and current MP Culture Bottles. For this study, the *M. tuberculosis* complex strains listed in **Table 6** were inoculated at concentrations of ≤100 CFU/bottle into adjusted MP Culture Bottle. Mean TTD results for 77 adjusted MP Culture Bottles were compared to historical data from 2009 for 35 predicate MP Culture Bottles inoculated with the same organisms at similar concentrations. Adjusted MP Culture Bottles were supplemented with AS reconstituted with NS, while predicate bottles evaluated in the historical study were supplemented with AS reconstituted with RF (reconstitution fluid).

The overall mean TTD was 15.6 days for the adjusted MP Culture Bottle and 18.7 days for the predicate MP Culture Bottle, with a mean difference in TTD of 3.1 days (**Table 6**). All evaluated mycobacterial strains favored the adjusted MP Culture Bottle compared to the predicate MP Culture Bottle.

Table 6. Mean Time-To-Detection for the Adjusted and Predicate MP Culture Bottles

Organism	**Strain ID**	**Mean TTD (in Days)**		**Mean TTD difference (Adj-Predicate) (in Days) and 95% CI[1]**
		Adjusted Bottle (N = 11)	**Predicate Bottle (N = 5)**	
M. bovis	CDC423	14.1	20.7	-6.6 [-8.1, -5.0%]
	CDC8131	15.0	20.2	-5.2 [-6.5, -3.8%]
M. tuberculosis	ATCC25177	17.1[2]	18.2	-1.1 [-1.7, -0.5%]
	CDC2663	13.7	17.4	-3.7 [-4.3, -3.1%]
	CDC2677	19.0	20.3	-1.3 [-1.9, -0.7%]
	ATCC27294	14.5	16.3	-1.8 [-2.6, -0.9%]
	ATCC35822	15.9	18.1	-2.2 [-2.7, -1.7%]
Overall		15.6	18.7	-3.1 [-3.8, -2.4%]

1. The difference in Mean TTD was calculated by subtracting the mean TTD for the predicate bottle from the mean TTD for the adjusted bottle. Negative values are indicative of shorter TTDs in the adjusted bottle compared to the predicate bottle
2. Twenty-two adjusted bottles were evaluated for *M. tuberculosis* ATCC25177

Contaminating Respiratory Flora (CRF) Suppression Study

An in-house study was conducted to evaluate the ability of the adjusted MP Culture Bottle, in the presence of Antimicrobial Supplement, to suppress breakthrough (growth) of common respiratory flora (CRF) found in sputum. For this study, the 35 CRF strains listed in **Table 7** were inoculated independently at a target concentration of 2000 CFU/bottle into simulated sputum. This concentration was chosen because it represents the worst-case scenario encountered by inadequately digesting-decontaminating non-sterile samples. Note that spiked, simulated sputum samples were not processed (i.e., digested-decontaminated) before bottle inoculation. Ten adjusted MP Culture Bottles were inoculated for each CRF organism in addition to three positive control bottles (NS supplementation only). Three lots of adjusted reagents (MP Culture Bottle, AS, and NS) were evaluated during the study.

Table 7. Common Respiratory Flora Evaluated in the Suppression Study

CRF Test Panel			
Organism	**Strain**	**Organism**	**Strain**
A. baumanii	Sputum isolate (SPT) 2	*S. maltophilia*	106418
C. albicans	11006	*S. oralis*	12975[1]
	302876		11663
	SPT 15	*S. sanguinis*	10556
E. faecalis	29212		SPT 27[1]
	8340 (VRE)	*N. sicca*	501593
E. faecium	35667	*P. mirabilis*	33946
	8353		SPT 31
P. aeruginosa	27853	*S. pyogenes*	19615[1]
	106159		11616[1]
	SPT 19	*C. krusei*	303824
S. anginosus	2052[1]	*C. tropicalis*	13803
S. aureus	6538		SPT 14
	8832 (MRSA)	*E. coli*	25922
	MRSA SPT 7	*K. pneumoniae*	109241
S. constellatus	2104[1]	*S. pneumoniae*	6305[1]
B. cepacia	STL 102628		
C. neoformans	14116		
N. abscessus	23824		

1. *Streptococcus* species removed from analysis due to poor or no growth in the adjusted MP Culture Bottle positive controls

Acceptable performance was demonstrated by no growth (breakthrough) in $\geq$ 80% of non-processed (i.e., non-digested-decontaminated) bottles in $\leq$ 10 days. Acceptable performance was shown for all organisms except *A. baumanii* (Sputum 2), *B. cepacia* (STL102628*), E. faecium* (35667 and 8353), *S. sanguinis* (10556), and *N. abscessus* (23824). *N. abscessus* breakthrough was expected as *Nocardia* are partially acid-fast and known to grow in mycobacteria media. Seven *Streptococcus* species (denoted by

a footnote in **Table 7**) were removed from analysis as they demonstrated no or poor growth in the MP Reagent positive control bottles.

For the CRF organisms that exhibited breakthrough in the adjusted MP Culture Bottle, simulated sputum spiked with organisms that broke through were digested-decontaminated prior to bottle inoculation to demonstrate that processing can suppress breakthrough. *N. abscessus* was not included in this evaluation. After processing, no breakthrough was observed for *A. baumanii* (Sputum 2), *B. cepacia* (STL102628*), E. faecium* (35667 and 8353), or *S. sanguinis* (10556), demonstrating that sputum processing (i.e., digestion-decontamination) can prevent breakthrough contamination.

Antimicrobial Supplement (AS) Vial Stopper Equivalency Study
Studies were conducted to evaluate the effect of replacing the current chlorobutyl AS stopper with a bromobutyl AS stopper. For this study two lots of antimicrobial supplement vials, with each stopper type, were evaluated at release and end of shelf-life (20 months). In addition, thermally shocked bottles were tested at the end of shelf-life to evaluate the worst-case scenario. At each time point physical parameters, growth performance (GP), false positive (FP) rate, and suppression of contaminating respiratory flora (CRF) were evaluated.

Results of testing indicated that all physical parameters were equivalent between the lots tested. Additionally, all functional testing (GP, FP, and CRP) met all acceptance criteria for all tested mycobacterial species. The study demonstrated that the bromobutyl stopper is equivalent to the chlorobutyl stopper.

e. Analytical specificity:

Not Applicable

Assay cut-off:

f. Prozone/Hook Effect:

Not Applicable

2. Comparison studies:
   a. Method comparison with predicate device:

A multi-center prospective clinical study was conducted at three different geographic sites in the U.S. and India to compare performance of the BACT/ALERT MP Reagent System and BACT/ALERT MP Culture Bottle for non-sterile (e.g., sputum) and normally sterile (e.g., tissues, fluids, etc.) specimens collected from patients suspected of mycobacterial infection. A total of 1,488 bottle pairs were obtained from 1,162 patients. There were a total of 1,435 non-sterile specimens and 53 sterile specimens obtained

during the study. Non-sterile specimens were subjected to digestion/decontamination (processing) using two different methods. 1,294 specimens were digested/decontaminated by the NALC-NaOH method, while 141 were digested/decontaminated using the NaOH modified Petroff method. For processed specimens, reconstituted Antimicrobial Supplement (AS) was added to the adjusted and current MP Culture Bottles. For sterile specimens, Nutrient Supplement (NS) and Reconstitution Fluid (RF) were added to the adjusted and current bottles, respectively. A primary solid media culture and primary AFB smear were performed for each patient specimen. Subcultures were performed for any bottle flagged positive by the instrument. For subcultures that recovered mycobacteria, identification at the complex or genus/species level was performed by standard biochemical/phenotypic methods or an FDA-cleared method (e.g., DNA sequencing). A culture bottle was determined to be a "True Positive" if the culture was flagged positive by the BACT/ALERT 3D System and resulted in growth of a mycobacterial species upon bottle subculture. Clinical isolates recovered from each subcultured bottle were classified as mycobacteria positives or breakthrough contaminants.

A culture bottle was determined to be a false negative if the bottle was flagged negative by the instrument and resulted in growth of a mycobacterial species upon bottle subculture.

A culture bottle was determined to be a false positive if the bottle was flagged positive by the instrument, but no viable organisms were present in the bottle as confirmed by subculture.

The false negative rate for the adjusted and predicate bottle were 0.13% (2/1,488) and 0.27% (4/1,488), respectively, with a difference in the false negative rate of -0.14%. The false positive rate for the adjusted and predicate bottle were 2.28% (34/1,488) and 4.77% (71/1,488), respectively, with a difference in the false positive rate of -2.49%. These results illustrate that the false negative and positive rates for the adjusted bottle are lower, and therefore, equivalent to the predicate bottle.

To compare performance between the bottle types, Sensitivity and Specificity were determined. Sensitivity was calculated as the number of bottles flagged as positive by the instrument with mycobacteria recovered by subculture, out of the total number of samples with a positive patient infected status. Specificity was calculated as the number of bottles flagged negative by the instrument without mycobacteria isolated by subculture, out of the total number of samples with a negative patient infected status. Positive patient infected status was assigned to each patient specimen if the following criteria were met: at least one of the paired bottle subcultures was positive *or* the primary solid media culture recovered a mycobacterial species.

In total, 200 adjusted MP Culture Bottles and 188 predicate Culture Bottles flagged positive by the BTA 3D instrument were positive for at least one mycobacterial species by subculture. Compared to 231 specimens with a positive patient infected status, the Sensitivity of the adjusted MP Culture Bottle was 86.6% (200/231) and 81.4% (188/231)

for the predicate MP Culture Bottle, with a difference in Sensitivity of 5.2%. **Table 8** illustrates the performance of the adjusted and predicate MP Culture Bottles relative to positive patient infected status. The ratio of true positives for the adjusted and predicate MP Culture Bottles, relative to positive patient infected status, is 1.064 (95% CI: 0.999, 1.129), indicating that Sensitivity of the adjusted MP Culture Bottle is improved compared to the predicate. **Table 8a** illustrates the ratio of true positives for the adjusted vs. predicate MP Culture Bottles relative to positive patient infected status.

Table 8. Performance of the Adjusted and Predicate MP Culture Bottle as Determined Relative to Positive Patient Infected Status*

			Predicate MP Culture Bottle				Total Bottles
			Instrument Signal Positive		Instrument Signal Negative		
			Subculture Positive	Subculture Negative	Subculture Positive	Subculture Negative	
Adjusted MP Culture Bottle	Instrument Signal Positive	Subculture Positive	176	5	2	17	200
		Subculture Negative	0	0	0	0	0
	Instrument Signal Negative	Subculture Positive	0	0	1	1	2
		Subculture Negative	12	1	1	15	29
Total Bottles			188	6	4	33	231
Sensitivity (Adjusted Bottle) [95% CI]			86.6% (200/231), [81.6%, 90.4%]				
Sensitivity (Predicate Bottle) [95% CI]			81.4% (188/231), [75.9%,85.9%)				
Difference in Sensitivity (Adjusted Bottle - Predicate Bottle) [95% CI]			5.2% [-1.5%, 11.9%]				

*A specimen with a positive patient infected status was defined as a specimen for which either the adjusted or predicate bottle subculture was positive (recovered mycobacteria) *or* the primary solid medium culture was positive for a mycobacterial species

Table 8a. Ratio of True Positives by Adjusted and Predicate MP Culture Bottles Relative to Positive Patient Infected Status

Number of True Positives by Adjusted MP Culture Bottle	Number of True Positives by Predicate MP Culture Bottle	True Positive Ratio (Adjusted/Predicate) (95% CI)
200	188	1.064 (0.999, 1.129)

A total of 1,013 adjusted MP Culture Bottles and 982 predicate MP Culture bottles were flagged negative by the BTA 3D instrument and by subculture. Compared to 1,047 specimens with a negative patient infected status, the Specificity of the adjusted bottle was 96.8% (1,013/1,047) and 93.8% (982/1047) for the predicate bottle, with a difference in Specificity of 3.0%. **Table 9** illustrates the performance of the adjusted and predicate bottles relative to negative patient infected status. The ratio of true negatives for the adjusted and predicate MP Culture Bottles, relative to positive patient infected status, is 1.032 (95% CI: 1.014, 1.050), indicating that Specificity of the adjusted MP Culture Bottle is improved compared to the predicate. **Table 9a** illustrates the ratio of true negatives for the adjusted vs. predicate MP Culture Bottles relative to negative patient infected status.

Table 9. Performance of the Adjusted and Predicate MP Culture Bottle Relative to Negative Patient Infected Status*

			Predicate MP Culture Bottle				Total Bottles
			Instrument Signal Positive		Instrument Signal Negative		
			Subculture Positive	Subculture Negative	Subculture Positive	Subculture Negative	
Adjusted MP Culture Bottle	Instrument Signal Positive	Subculture Positive	0	0	0	0	0
		Subculture Negative	0	11	0	23	34
	Instrument Signal Negative	Subculture Positive	0	0	0	0	0
		Subculture Negative	0	54	0	959	1013
Total Bottles			0	65	0	982	1047
Specificity (Adjusted Bottle) [95% CI]			96.8% (1013/1047), [95.5%, 97.7%]				
Specificity (Predicate Bottle) [95% CI]			93.8% (982/1047), [92.2%, 95.1%)				
Difference in Specificity (Adjusted Bottle - Predicate Bottle) [95% CI]			3.0% [1.1%, 4.8%]				

*A specimen with a negative patient infected status was defined as a sample for which the adjusted bottle subculture, predicate bottle subculture and primary solid medium culture were all negative (i.e., no mycobacterial growth)

Table 9a. Ratio of True Negatives by Adjusted and Predicate MP Culture Bottles Relative to Negative Patient Infected Status

Number of True Negatives by Adjusted MP Culture Bottle	Number of True Negatives by Predicate MP Culture Bottle	True Negative Ratio (Adjusted/Predicate) (95% CI)
1013	982	1.032 (1.014, 1.050)

Percent Recovery

Percent recovery (detection) for the adjusted and predicate bottle was determined by calculating the number of adjusted *or* predicate bottles flagged positive by the BTA 3D System with mycobacteria recovered by subculture, out of the total number of bottles flagged positive by the instrument with mycobacteria recovered by subculture.

Percent recovery was 94.3% for the adjusted bottle and 88.7% for the predicate bottle, with a difference in percent recovery of 5.6%. **Table 10** illustrates the number of adjusted and predicate bottles that were positive by the BTA 3D instrument and recovered mycobacteria by subculture, stratified by site. **Table 11** contains percent recovery results stratified by specimen type and mycobacterial species. During the clinical study, three dual mycobacterial infections were recovered by both the adjusted and predicate bottles.

Table 10. Percent Recovery of Mycobacteria in the Adjusted and Current Bottle Stratified by Site for all Specimen Types Combined

Site	Percent Recovery		Diff. in % Recovery (Adj-Predicate)[a]	95% CI of the Diff. in % Recovery (Adj-Predicate)
	Adjusted Bottle	Predicate Bottle		
1	91.2 (31/34)	85.3 (29/34)	5.9%	-12.5%, 24.2%
2	98.4 (62/63)	88.9 (56/63)	9.5%	-0.7%, 20.7%
3	93.0 (107/115)	89.6 (103/115)	3.4%	-4.7%, 11.8%
Total	94.3 (200/212)	88.7 (188/212)	5.6%	-0.04%, 11.49%

[a] Positive values indicate that % recovery is higher in the adjusted bottle compared to the predicate bottle

Table 11. Percent Recovery of Mycobacteria in the Adjusted and Current Bottles Stratified by Specimen Type and Species

Specimen Type	Species	Percent Recovery		Diff. in % Recovery (Adjusted-Predicate) [a]	95% CI of the Diff. in % Recovery (Adjusted - Predicate)
		Adjusted Bottle	Predicate Bottle		
Aspirate fluid	*M. tuberculosis* complex	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
Bronchial lavage	*M. abscessus*	90.9 (10/11)	90.9 (10/11)	0.0	-34.9%, 34.9%
	M. avium complex	100.0 (3/3)	33.3 (1/3)	66.7	-21.0%, 98.4%
	M. chimaera	100.0 (3/3)	100.0 (3/3)	0.0	-69.1%, 69.1%
	M. fortuitum	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
	M. intracellulare	100.0 (6/6)	100.0 (6/6)	0.0	-48.3%, 48.3%
	M. tuberculosis	100.0 (10/10)	80.0 (8/10)	20.0	-18.2%, 55.8%
	M. tuberculosis complex	100.0 (10/10)	80.0 (8/10)	20.0	-18.2%, 55.8%
Corneal ulcer	*M. chelonae*	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
Endotracheal tube	*M. gordonae*	50.0 (1/2)	50.0 (1/2)	0.0	-66.9%, 66.9%
Pus	*M. abscessus*	100.0 (1/1)	0.0 (0/1)	100.0	-33.7%, 115.2%
	M. tuberculosis complex	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
Sputum	*M. abscessus*	88.5 (23/26)	88.5 (23/26)	0.0	-21.5%, 21.5%
	M. abscessus / *M. chelonae*	50.0 (1/2)	100.0 (2/2)	-50.0	-97.6%, 43.1%
	M. avium complex	88.6 (31/35)	80.0 (28/35)	8.6	-11.0%, 27.7%
	M. bolleti	100.0 (1//1)	0.0 (0//1)	100.0	-33.7%, 115.2%
	M. chimaera	87.5 (7/8)	87.5 (7/8)	0.0	-42.5%, 42.5%
	M. chimaera / *M. yongonense*	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
	M. colombiense	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
	M. fortuitum	50.0 (1/2)	100.0 (2/2)	-50.0	-97.6%, 43.1%
	M. gordonae	85.7 (6/7)	57.1 (4/7)	28.6	-25.0%, 67.9%
	M. intracellulare	97.5 (39/40)	97.5 (39/40)	0.0	-12.5%, 12.5%

	M. intracellulare / *M. yongonense*	100.0 (3/3)	100.0 (3/3)	0.0	-69.1%, 69.1%
	M. kansasii	100.0 (2/2)	100.0 (2/2)	0.0	-80.4%, 80.4%
	M. simiae	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
	M. tuberculosis	100.0 (20/20)	100.0 (20/20)	0.0	-20.1%, 20.1%
	M. tuberculosis complex	100.0 (9/9)	88.9 (8/9)	11.1	-27.5%, 49.3%
	M. yongonense	50.0 (1/2)	100.0 (2/2)	-50.0	-97.6%, 43.1%
Lung tissue	*M. abscessus*	100.0 (1/1)	100.0 (1/1)	0.0	-95.2%, 95.2%
Tissue biopsy	*M. tuberculosis*	100.0 (2/2)	100.0 (2/2)	0.0	-80.4%, 80.4%
	M. tuberculosis complex	100.0 (2/2)	100.0 (2/2)	0.0	-80.4%, 80.4%
Total		94.3 (200/212)	88.7 (188/212)	5.6%	-0.04%, 11.49%

[a] Positive value indicates that % recovery is higher in the adjusted bottle compared to the current bottle

Time-To-Detection (TTD)

To determine if modifications to the formulation of the adjusted reagents (MP Culture Bottle, AS, and NS) alter mean TTD for mycobacterial species, mean TTD (in Days) was determined for each paired set of adjusted and predicate bottles for which the instrument signaled positive and pure, mycobacterial growth was recovered by subculture.

The study demonstrated that mean TTD for the MTB group for all specimen types, pooled, was 11.6 days in the adjusted bottle and 14.4 in the predicate bottle, for a mean TTD difference of 2.7 days. Additionally, the study demonstrated that mean TTD for all specimen types, pooled, for the NTM-rapid growers was 7.3 days in the adjusted bottle and 7.4 days in the predicate bottle, with a mean TTD difference of 0.1 days. The mean TTD for all specimen types, pooled, for the NTM-slow growers was 11.5 days in the adjusted bottle and 11.8 days in the predicate bottle, with a mean TTD difference of 0.3 days. Results are illustrated in **Table 12**.

Table 12. Time-to-Detection Study Results for Clinical Specimens

Organism Group	Number of Isolates	Mean Time-to-Detection (In Days)		Mean TTD Diff. (Adj-Predicate) (in Days) (95% CI)[1]	% Diff in TTD
		Adjusted Bottle	Predicate Bottle		
MTB	50	11.6	14.4	-2.7 [-4.2, -1.3]	-19.1%
NTM-rapid growers	30	7.3	7.4	-0.1 [-0.7, 0.5]	-1.9%
NTM -slow growers	83	11.5	11.8	-0.3 [-1.3, 0.7]	-2.5%

MTB: Includes isolates identified as *M. tuberculosis* and *M. tuberculosis* complex
NTM rapid: Includes isolates identified as *M. fortuitum*, *M. abscessus*, and *M. abscessus/chelonae*
NTM slow: Includes isolates identified as *M. avium*, *M. avium* complex, *M. chimaera*, *M. chimaera/yongonense*, *M. colombiense*, *M. simiae*, *M. yongonense*, *M. kansasii*, *M. gordonae*, *M. intracellulare*, and *M. intracellulare/yongonense*
[1] Negative values are indicative of shorter TTDs in the adjusted bottle compared to the predicate bottle

Breakthrough Contamination Rate

The breakthrough contamination rate, which is defined as the number of adjusted or predicate bottles with a positive instrument signal that recovered a non-mycobacterial organism by subculture and/or staining, was calculated for each bottle type. The breakthrough contamination rate was calculated independently for sterile and non-sterile specimens. For non-sterile specimens, results were stratified by the digestion/decontamination method evaluated during the clinical study (i.e., NALC-NaOH Method and NaOH Modified Petroff Method). Of the 1,435 non-sterile specimens evaluated in the clinical study, 90.1% (1,293/1,435) were processed using the NALC-NaOH Method and 9.8% (141/1,435) were processed using the NaOH Modified Petroff Method. Results are illustrated in **Table 12a**, stratified by specimen type and digestion/decontamination method.

Table 12a. Breakthrough Contamination Rate by Specimen Type and Digestion/Decontamination Method

Specimen Type	Processing Method	Number of specimens	Breakthrough Contamination Rate	
			Adjusted Bottle	Predicate Bottle
Non-Sterile	NALC-NaOH Method	1,293[a]	7.1% (92/1294)	8.9% (115/1,294)
	NaOH Modified Petroff Method	141	3.5% (5/141)	1.4% (2/141)
Sterile	NA	53	1.9%	0.0%

			(1/53)	(0/53)

[a.] One non-sterile specimen was removed from analysis as the digestion/decontamination method employed for this specimen was not recorded

Percent Recovery in Seeded Simulated Sputum Samples

Due to the low prevalence of *M. tuberculosis* in the U.S. and difficulty obtaining NTM specimens during the prospective trial, the clinical study was augmented with a seeded study conducted at one U.S. site. For this study, seeded samples were generated from the following clinically prevalent mycobacterial strains: two strains of *M. tuberculosis* (ATCC25177 and a clinical isolate) and eight NTM strains (*M. avium* ATCC25291, *M. fortuitum* ATCC6841, *M. intracellulare* ATCC13950, *M. kansasii* ATCC12478, *M. malmoense* ATCC29571, *M. scrofulaceum* ATCC19981, *M. szulgai* clinical isolate, and an *M. xenopi* clinical isolate). Seeded samples were produced by inoculating each strain into simulated sputum matrix, taking into consideration the percent loss of cells during digestion-decontamination. Considering cell loss during processing, simulated sputum sample was inoculated with a single mycobacterial strain at a target CFU/bottle of 300, 500, and 1000 CFU/bottle. Actual inoculums for the 300, 500, and 1000 CFU/bottle target inoculums ranged from 0.5-240, 0.5-390.5, and 1.5-500 CFU/bottle, respectively. To represent a challenging clinical scenario, seeded samples were also inoculated with common respiratory flora (CRF) representative of organisms found in either immunocompetent or immunocompromised patient sputum. Immunocompetent CRF group 1 consisted of *E. faecalis, N. sicca, S. epidermidis*, and *S. oralis*, while immunocompromised CRF group 2 consisted of *A. baumanii, C. albicans, E. coli, P. aeruginosa*, and *S. aureus*. Actual inoculum levels for these organisms ranged from 70-156 CFU/bottle. Seeded samples were processed identically as was described for prospectively collected clinical specimens. In total, 52 bottles (paired design) were evaluated, 6 for each *M. tuberculosis* species (12 bottles in total) and 5 for each NTM species (40 bottles in total). The adjusted and predicate bottles were found to be equivalent, with each exhibiting 100% recovery for all seeded species regardless of addition of CRF group 1 or CRF group 2.

Percent Recovery in Seeded Tissue and CSF Samples

During the prospective clinical study, it was difficult to procure non-sterile tissue samples and cerebrospinal fluid (CSF) samples. To determine the recovery rate of mycobacteria from these uncommon sample types, a seeded study was performed. Seeded samples were produced by spiking pooled CSF (from living donors) or homogenized tissue samples (from cadavers), with the *M. tuberculosis* and NTM strains listed in **Table 13**. Inoculums of 30-300 CFU/bottle were targeted for both CSF and tissue samples, factoring in cell loss from digestion-decontamination. The actual inoculums ranged from 16-1136 CFU/bottle. Each CSF pool was tested individually, while tissue samples were tested in triplicate. The adjusted and predicate bottles were equivalent, with 100% of seeded species, regardless of sample type. Results are shown in **Table 13**, stratified by sample type and species.

Table 13. Results of Seeded CSF and Tissue Study Stratified by Sample Type

Sample Type	Species	Cadaver Number	Average CFU/bottle	Adjusted Bottle		Predicate Bottle	
				# of Positives	# of Specimens	# of Positives	# of Specimens
CSF	*M. avium* ATCC 25291	NA	264	4	4	4	4
	M. intracellulare ATCC 13950	NA	184	4	4	4	4
	M. tuberculosis ATCC 25177	NA	924	4	4	4	4
Lymph node	*M. tuberculosis* ATCC 25177	4	244	3	3	3	3
	M. avium ATCC 25291	4	576	3	3	2	2
	M. intracellulare ATCC 13950	4	222	3	3	3	3
	M. scrofulaceum ATCC 19981	4	16	3	3	3	3
	M. malmoense ATCC 29571	4	380	3	3	3	3
Bone	*M. tuberculosis* ATCC 25177	1	1068	3	3	3	3
	M. intracellulare ATCC 13950	3	568	3	3	3	3
	M. avium ATCC 25291	2	48	3	3	3	3
Skin	*M. tuberculosis* ATCC 25177	1	628	3	3	3	3
	M. abscessus ATCC 199977	2	24	3	3	3	3
	M. fortuitum ATCC 6841	3	536	3	3	3	3
Lung	*M. tuberculosis* ATCC 25177	1	368	3	3	3	3
	M. avium ATCC 25291	1	1136	3	3	3	3
	M. intracellulare ATCC 13950	2	140	3	3	3	3
	M. kansasii ATCC 12478	3	1096	3	3	3	3
Percent Recovery				100%		100%	

b. Matrix comparison:

Simulated Sputum Matrix Equivalency Study

An in-house study was conducted to demonstrate equivalency between simulated sputum (i.e., 1% methylcellulose with egg solution) and remnant patient sputum for use in mycobacteria seeded studies. To ensure that simulated sputum samples were representative of patient sputum samples, simulated sputum was supplemented with human cell debris and mouth flora on oral swabs. In addition, CRF flora (e.g., *E. faecalis*-ATCC29212, *S. epidermidis*-ATCC12228, and *S. oralis*-ATCC11663) were added at a concentration of 2.5 x 10^6 CFU/mL per organism in order to achieve a concentration of ~ 1 x 10^7 CFU/mL typically found in clinical sputum samples. Remant patient sputum and simulated sputum were seeded with the organisms in **Table 14** at target mycobacteria concentrations, post digestion-decontamination, of 300 and 1000 CFU/bottle to represent a low inoculum (~3x LoD) and an incolum close to the sensitivity of a positive AFB smear. After digestion-decontamination, actual inoculums for the 300 CFU/bottle and 1000 CFU/bottle targets ranged from 31-336 CFU/bottle and 170-1165 CFU/bottle, respectively. Remnant and simulated sputum samples were added to adjusted MP Culture Bottles supplemented with reconstituted AS and incubated on the BTA 3D System. Percent recovery and mean TTD were calculated.

Table 14. Simulated Sputum Equivalency Study Organism Test Panel

Category	Organism
M. tuberculosis complex (Slow grower)	*M. tuberculosis* (ATCC 25177)
M. avium-intracellulare complex	*M. avium* (ATCC 25291)
	M. intracellulare (ATCC 13950)
Non-Tuberculosis Mycobacteria (NTM)	*M. scrofulaceum* (ATCC 19981)
	M. kansasii (ATCC 12478)
Rapid Growers	*M. foruitum* (ATCC 6841)

Equivalence for remnant sputum and simulated sputum was demonstrated by $\geq$ 95% recovery for each mycobacteria species in each sputum type. Regardless of inoculum level or sputum type, percent recovery was 100% for all species. It was noted that mean TTDs varied by no greater than one day between the matrices for all evaluated species. These results demonstrate that simulated sputum is equivalent to remnant sputum for use in seeded mycobacteria studies.

3. Clinical studies:

Not Applicable

a. Clinical Sensitivity:

b. Clinical Specificity:

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The expected positive percentage of culture bottles will vary based on factors such as patient population, specimen type, prevalence of organisms, site location, and contamination rates. The expected values show below are based on the clinical study conducted to evaluate the MP Reagent System:

   - Percent mycobacterial positive cultures were observed to be 9.4% (range 0% - 14.3%) for isolates recovered from sterile specimens from three clincal trial sites in the MP Reagent System.

   - Percent mycobacterial positive cultures were observed to be 13.6% (range 4.0%-41.0%) for isolates recovered from non-sterile specimens from three clinical trial sites in the MP Reagent System.

   - The breakthrough contamination rate for the MP Reagent System was observed to be 7.1% (range 2.6%-10.0%) for non-sterile specimens recovered from two clinical trial sites that were digested/decontaminated using the NALC-NaOH Method.

   - The breakthrough contamination rate for the MP Reagent System was observed to be 3.5% for non-sterile specimens recovered from one clinical trial site that were digested/decontaminated using the NaOH Modified Petroff Method.

   - The breakthrough contamination rate for the MP Reagent system was observed to be 1.9% for sterile specimens recovered from three clinical trial sites.

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports substantial equivalence decision.