



Accelerating Sterility Testing: Leveraging Pre-enumerated Reference Materials with BD BACTEC™ for Rapid Microbial Detection

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INTRODUCTION

Rapid microbiological methods (RMMs) are increasingly adopted in pharmaceutical quality control to accelerate product release while ensuring compliance with compendial standards. Traditional sterility testing as described in United States Pharmacopoeia (USP) <71> can require up to 14 days, which is not feasible for short-life or time-sensitive products. To address this, USP <72> introduces respiration-based microbiological methods, which use automated detection of microbial metabolism (e.g., CO₂ production) to deliver faster and more sensitive results.

A critical component of these rapid methods is the demonstration of media performance through growth promotion testing (GPT) and method suitability testing. USP <72> specifies that culture media must reliably support growth of compendial microorganisms when inoculated with low levels of challenge organisms (≤100 CFU for GPT and ≤10 CFU for method suitability).

The use of pre-enumerated reference cultures provides a significant advantage in this context. Unlike traditional approaches that require serial dilutions and plating to estimate inoculum levels, pre-quantified suspensions deliver precise, reproducible microbial loads. This improves accuracy in evaluating system performance, reduces variability, and enhances laboratory efficiency. Moreover, the use of pre-enumerated controls ensures alignment with compendial expectations, enabling direct comparison between traditional sterility tests and rapid detection systems.

Together, these advances support the reliable adoption of RMMs in sterility testing workflows, enabling faster, more consistent detection of compendial organisms and strengthening the scientific basis for rapid product release decisions.

METHODS

The performance of the BD BACTEC™FX system was evaluated using EZ-AccuShot™ pre-enumerated reference microorganisms in accordance with USP <72> Respiration-Based Microbiological Methods. Test organisms were hydrated and vortexed until homogeneous, and suspensions were used within 30 minutes of preparation. BD BACTEC™ Aerobic Plus, Standard Aerobic, and Anaerobic bottles were inoculated with 0.1 mL of suspension and plated in duplicate on TSA to verify inoculum levels. Growth promotion testing (GPT) and method suitability testing (MST) were performed using inocula of approximately 10-100 CFU (GPT) and 1-10 CFU (MST) per bottle, respectively. All bottles were incubated at 30-35 °C, under aerobic or anaerobic conditions depending on organism type. Microbial growth was detected automatically via CO₂ production, and time-to-detection (TTD) was recorded for each replicate. Organisms and incubation parameters for GPT and MST are summarized in Table 1 and Table 2, respectively.

Organism Name	Catalog Number	Inoculum Level	Temperature	Incubation Time	Bottle	Replicates
<i>Staphylococcus aureus</i>	0485A	100 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>Bacillus spizizenii</i>	0486A	100 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>Pseudomonas paraeruginosa</i>	0484A	100 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>Clostridium sporogenes</i>	0317A	100 CFU	30-35°C	NMT 3 days	Anaerobic	12
<i>Cutibacterium acnes</i>	0419A	100 CFU	30-35°C	NMT 3 days	Anaerobic	12
<i>Candida albicans</i>	0443A	100 CFU	30-35°C	NMT 5 days	Aerobic & Aerobic Plus	24
<i>Chaetomium globosum</i>	01094EGPT	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>Penicillium citrinum</i>	01133EGPT	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>Penicillium chrysogenum</i>	0178A	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>Aspergillus brasiliensis</i>	0392A	100 CFU	30-35°C	NMT 5 days	Aerobic & Aerobic Plus	24

Organism Name	Catalog Number	Inoculum Level	Temperature	Incubation Time	Bottle	Replicates
<i>S. aureus</i>	0485A	10 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>B. spizizenii</i>	0486A	10 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>P. paraeruginosa</i>	0484A	10 CFU	30-35°C	NMT 3 days	Aerobic & Aerobic Plus	24
<i>C. sporogenes</i>	0317A	10 CFU	30-35°C	NMT 3 days	Anaerobic	12
<i>C. acnes</i>	0419A	10 CFU	30-35°C	NMT 3 days	Anaerobic	12
<i>C. albicans</i>	0443A	100 CFU	30-35°C	NMT 5 days	Aerobic & Aerobic Plus	24
<i>C. globosum</i>	01094EGPT	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>P. citrinum</i>	01133EGPT	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>P. chrysogenum</i>	0178A	100 CFU	30-35°C	NMT 5 days	Aerobic Plus	12
<i>A. brasiliensis</i>	0392A	100 CFU	30-35°C	NMT 5 days	Aerobic & Aerobic Plus	24

Strain Number	ID	Lot	Exp Date
0317A	<i>C. sporogenes</i>	317-412-4	5/31/2026
0317A	<i>C. sporogenes</i>	317-421-5	12/31/2025
0392A	<i>A. brasiliensis</i>	392-1671-3	11/30/2026
0392A	<i>A. brasiliensis</i>	392-1697-4	11/30/2026
0419A	<i>C. acnes</i>	419-207-1	6/30/2026
0419A	<i>C. acnes</i>	419-204-4	8/31/2025
0443A	<i>C. albicans</i>	443-1638-2	9/30/2026
0443A	<i>C. albicans</i>	443-1630-2	9/30/2026
0484A	<i>P. paraeruginosa</i>	484-1742-2	11/30/2026
0484A	<i>P. paraeruginosa</i>	484-1739-5	11/30/2026
0485A	<i>S. aureus</i>	485-1291-2	10/31/2026
0485A	<i>S. aureus</i>	485-1282-4	10/31/2026
0486A	<i>B. spizizenii</i>	486-1695-3	11/31/2026
0486A	<i>B. spizizenii</i>	486-1689-5	11/31/2026
01094EGPT	<i>C. globosum</i>	PS7534-3	1/31/2026
01133EGPT	<i>P. citrinum</i>	PS-8161-2	8/31/2026
0178A	<i>P. chrysogenum</i>	178-38-1	7/31/2026
0688A	<i>K. rhizophila</i>	688-285-3	5/31/2026

RESULTS

Organism Name	Lot Number	N	% Agreement with Expected Results	Aerobic Bottle Detection Time
<i>S. aureus</i>	485-1291-2	6	100% (6/6)	13.95
	485-1282-4	6	100% (6/6)	14.0
<i>B. spizizenii</i>	486-1695-3	6	100% (6/6)	12.37
	486-1689-5	6	100% (6/6)	12.55
<i>P. paraeruginosa</i>	484-1742-2	6	100% (6/6)	17.12
	484-1739-5	6	100% (6/6)	16.44
<i>C. albicans</i>	443-1638-2	6	100% (6/6)	27.83
	443-1630-2	6	100% (6/6)	27.05
<i>A. brasiliensis</i>	392-1671-3	6	100% (6/6)	53.79
	392-1697-4	6	100% (6/6)	45.61

Organism Name	Lot Number	N	% Agreement with Expected Results	Aerobic Plus Bottle Detection Time
<i>S. aureus</i>	485-1291-2	6	100% (6/6)	15.08
	485-1282-4	6	100% (6/6)	14.95
<i>B. spizizenii</i>	486-1695-3	6	100% (6/6)	11.13
	486-1689-5	6	100% (6/6)	11.33
<i>P. paraeruginosa</i>	484-1742-2	6	100% (6/6)	16.91
	484-1739-5	6	100% (6/6)	16.81
<i>C. albicans</i>	443-1638-2	6	100% (6/6)	28.96
	443-1630-2	6	100% (6/6)	29.11
<i>A. brasiliensis</i>	392-1671-3	6	100% (6/6)	43.24
	392-1697-4	6	100% (6/6)	44.53

Tables 4 & 5 shows the detection results of the required aerobic USP <72> strains in Aerobic and Aerobic Plus bottles. Each bottle type was spiked with 10-100 CFU to simulate Growth Promotion Testing (GPT) of the bottle types. Detection time is in hours and is well below maximum incubation time for each strain during GPT (not more than 72 hours for bacteria and not more than 60 hours for fungi).

Organism Name	Lot Number	N	% Agreement with Expected Results	Aerobic Bottle Detection Time
<i>S. aureus</i>	485-1291-2	6	100% (6/6)	15.71
	485-1282-4	6	100% (6/6)	15.53
<i>B. spizizenii</i>	486-1695-3	6	100% (6/6)	13.32
	486-1689-5	6	100% (6/6)	13.70
<i>P. paraeruginosa</i>	484-1742-2	6	100% (6/6)	18.88
	484-1739-5	6	100% (6/6)	19.34
<i>C. albicans</i>	443-1638-2	6	100% (6/6)	30.82
	443-1630-2	6	100% (6/6)	32.81
<i>A. brasiliensis</i>	392-1671-3	6	100% (6/6)	68.16
	392-1697-4	6	100% (6/6)	64.17

Organism Name	Lot Number	N	% Agreement with Expected Results	Aerobic Plus Bottle Detection Time
<i>S. aureus</i>	485-1291-2	6	100% (6/6)	19.12
	485-1282-4	6	100% (6/6)	18.2
<i>B. spizizenii</i>	486-1695-3	6	100% (6/6)	12.67
	486-1689-5	6	100% (6/6)	12.48
<i>P. paraeruginosa</i>	484-1742-2	6	100% (6/6)	19.47
	484-1739-5	6	100% (6/6)	19.26
<i>C. albicans</i>	443-1638-2	6	100% (6/6)	34.08
	443-1630-2	6	100% (6/6)	35.87
<i>A. brasiliensis</i>	392-1671-3	6	100% (6/6)	54.13
	392-1697-4	6	100% (6/6)	64.49

Tables 6 & 7 shows the detection results of the required aerobic USP <72> strains in Aerobic and Aerobic Plus bottles. Each bottle type was spiked with 1-10 CFU to simulate Method Suitability Testing (MST) with each of the bottle types. Detection time is in hours, actual incubation time must be validated for each product to be examined. Use of Aerobic Plus bottles decreased detection time for most strains by at least an hour.

Organism Name	Lot Number	N	% Agreement with Expected Results	Anaerobic Bottle Detection Time
<i>C. acnes</i>	419-207-1	6	100% (6/6)	109.23
	419-204-4	6	100% (6/6)	101.05
<i>C. sporogenes</i>	317-412-4	6	100% (6/6)	17.52
	317-421-5	6	100% (6/6)	17.26

Organism Name	Lot Number	N	% Agreement with Expected Results	Anaerobic Bottle Detection Time
<i>C. acnes</i>	419-207-1	6	100% (6/6)	119.9
	419-204-4	6	100% (6/6)	113.22
<i>C. sporogenes</i>	317-412-4	6	100% (6/6)	19.67
	317-421-5	6	100% (6/6)	19.54

Tables 8 & 9 show the detection results of the required anaerobic USP <72> strain and a commonly tested anaerobe, *C. acnes*, in Anaerobic bottles. Each bottle was spiked with 10-100 CFU to simulate Growth Promotion Testing (GPT) of the bottle types and 1-10 CFU to simulate Method Suitability (MST). Detection of *C. sporogenes* is well below GPT requirement (72 hours) and is detected in a similar fashion at low levels for MST. Although not required, *C. acnes*, also detected well at both the 10-100 and 1-10 CFU inoculum ranges.

Organism Name	Target Range	N	% Agreement with Expected Results	Aerobic Plus Bottle Detection Time
<i>C. globosum</i>	1-10 CFU	6	100% (6/6)	84.37
	10-100 CFU	6	100% (6/6)	75.5
<i>P. citrinum</i>	1-10 CFU	6	66.7% (4/6)	200.86
	10-100 CFU	6	100% (6/6)	85.37
<i>K. rhizophila</i>	1-10 CFU	6	100% (6/6)	36.31
	10-100 CFU	6	100% (6/6)	32.49
<i>P. chrysogenum</i>	1-10 CFU	6	100% (6/6)*	0.00
	10-100 CFU	6	100% (6/6)*	0.00

Table 10 shows the detection results of the commonly isolated environmental isolates in Aerobic Plus bottles. Each bottle was spiked with 10-100 CFU to simulate Growth Promotion Testing (GPT) of the bottles and 1-10 CFU to simulate Method Suitability. These strains are not required per <72>, but could be used to demonstrate recovery of potential microorganisms associated with the product/manufacturing process. *P. chrysogenum* was included even though it was not expected to grow at 35°C - this strain grows optimally at 25°C.

**P. chrysogenum* is not expected to grow at 35°C

CONCLUSION

The study demonstrated that the BD BACTEC™ system, when used with EZ-AccuShot™ pre-enumerated reference microorganisms, reliably detected all compendial test organisms within compendial timeframes. Required bacterial species were detected in under 24 hours and required fungal species within 72 hours, supporting the method's suitability for rapid sterility testing. The use of pre-quantified inocula provided consistent and reproducible performance, aligning with the intent of USP <72> for accelerated microbial detection in short-life or time-sensitive products. These findings highlight the potential of this combined approach to enhance both speed and standardization in pharmaceutical microbiological quality control.

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