



EZ-CFU™ One Step Verification Protocol Guide

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Verification Protocol Guide

1. Introduction

The purpose of this Verification Guide is to help customers smoothly and efficiently verify that EZ-CFU™ One Step microorganism strains are suitable for use in the Growth Promotion Test described in the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeias (JP). EZ-CFU™ One Step microorganisms are lyophilized, standardized, stable suspensions that deliver less than 100 CFU (colony forming units) when used as directed. Once verified, EZ-CFU™ One Step microorganism strains can be used as an easy and precise alternative to the seed-lot system and the time-consuming process of inoculum preparation.

EZ-CFU™ One Step microorganism strains meet the following pharmacopeial requirements:

1. Each strain delivers less than 100 CFU per 0.1 mL when used as directed.
2. Each strain is derived from a reference culture strain recommended by the harmonized pharmacopeias for growth promotion testing. The strains are traceable to the American Type Culture Collection (ATCC®) or National Collection of Industrial, Food and Marine Bacteria Ltd. (NCIMB). Microbiologics® is licensed by ATCC® to sell derivatives of ATCC® original microorganism strains.
3. Each strain is less than five passages from reference culture (the original master strain).
4. The identity of each strain has been confirmed by Microbiologics®.

In addition to meeting pharmacopeial requirements, EZ-CFU™ One Step strains have the following benefits:

1. Microorganism suspensions can be used for eight hours if refrigerated after rehydration with the exception of *B. fragilis*, 0320Z (ATCC® 25285™*), and *C. perfringens*, 0318Z (ATCC® 13124™*).
2. A Certificate of Assay is provided with every lot. The certificate lists the mean assay value (CFU concentration) obtained at Microbiologics®.
3. A Certificate of Analysis can be downloaded for every lot from the Microbiologics® website. The certificate lists such information as reference number, number of passages from reference culture, and macroscopic, microscopic and phenotypic characteristics when appropriate.


The Verification Guide has been divided into three parts.

Part 1: Verification Plan

- The laboratory determines the scope of the verification.
- The laboratory determines acceptance criteria.
- The plan is approved by management.

Part 2: The laboratory verifies the following for each EZ-CFU™ One Step strain:

- Traceability. Strains should be traceable to an original reference culture strain recommended by the pharmacopeias and should be less than five passages from the original master strain.
- Counts. Counts should be less than 100 CFU per agar plate, when product is used as directed.
- Purity. Contamination should not be seen on agar plates inoculated with the EZ-CFU™ One Step product.
- Stability. Once made, microorganism suspensions should be stable for 8 hours, if refrigerated, with the exception of *B. fragilis*, 0320Z (ATCC® 25285™*), and *C. perfringens*, 0318Z (ATCC® 13124™*). Labs do not need to test stability if intending to use EZ-CFU™ One Step microorganism suspensions within 30 minutes.
- Identity. Identification methods can range from confirming microscopic and macroscopic microorganism characteristics to performing biochemical or molecular tests.

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Part 3: Verification Summary

- The laboratory summarizes the data and determines if EZ-CFU™ One Step strains meets acceptance criteria.
- The laboratory submits the Verification Protocol to management for approval.

1.1 Safety Guidelines

EZ-CFU™ One Step preparations contain potentially harmful microorganisms. All microorganisms produced by Microbiologics® are BioSafety Level 1 or 2. Guidelines for handling microorganisms can be found on the Microbiologics® website in a Technical Bulletin called “Microorganism Biosafety Levels”. Directions for handling a microorganism spill can be found in the EZ-CFU™ One Step product insert.

Before using the EZ-CFU™ One Step product the MSDS should be downloaded and filed electronically or on site. There are two MSDS sheets available for the product. One MSDS is for Lyophilized Microorganism Preparations and the other MSDS is for Microorganism Hydrating Fluid.

1.2 Available Technical Information

The following documents are available to help with your validation.

- MSDS for Lyophilized Microorganism Preparations, MSDS.244
- MSDS for Microorganism Hydrating Fluid, MSDS.208
- “Microorganism Biosafety Levels”, TIB.072
- EZ-CFU™ One Step Product Insert, PI.209
- EZ-CFU™ One Step Illustrated Instructions, LIT. 217
- “Growth Promotion Test Guide for EZ-CFU™, EZ-CFU One Step™ and EZ-Accu Shot™”, TIB.264
- ATCC® 16404™ Name Change Letter, LIT.256 The Name Change Letter explains why the name of ATCC® 16404™ was changed to *Aspergillus brasiliensis*.

Documents are available at www.microbiologics.com. An easy way to obtain the documents is to use the search box. Search for documents by entering key words such as MSDS or biosafety.

1.3 References

The references used to create this guide are listed below.

1. The United States Pharmacopeia-National Formulary. 2010. <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests, *First Supplement to the USP33-NF28, Reissue*
2. The United States Pharmacopeia-National Formulary. 2010. <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms, *First Supplement to the USP33-NF28, Reissue*
3. The United States Pharmacopeia-National Formulary. 2010. <71> Sterility Tests, *First Supplement to the USP33-NF28, Reissue*
4. The United States Pharmacopeia-National Formulary. 2010. <1226> Verification of Compendial Procedures, *First Supplement to the USP33-NF28, Reissue*
5. The United States Pharmacopeia-National Formulary. 2010. <1111> Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use, *First Supplement to the USP33-NF28, Reissue*

Acknowledgements



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 NCIMB is the National Culture of Industrial, Food and Marine Bacteria Ltd.

2. Verification Plan

2.1 Intended Location of Use

Company Name: _____

Address: _____

Department: _____


Signature: _____ Date: _____

2.2 Microorganisms to be Tested

In Table 1, check (✓) EZ-CFU™ One Step microorganisms included in the verification study. Extra rows are available for additional microorganisms, if needed.

Table 1: EZ-CFU™ One Step Microorganisms

Microorganism	Reference Culture #	EZ-CFU™ One Step Product #	Check (✓)if included in study
<i>Aspergillus brasiliensis</i>	ATCC® 16404™*	0392Z	
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	ATCC® 6633™*	0486Z	
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	
<i>Candida albicans</i>	ATCC® 10231™*	0443Z	
<i>Clostridium sporogenes</i>	ATCC® 19404™*	0317Z	
<i>Clostridium sporogenes</i>	ATCC® 11437™*	0487Z	
<i>Clostridium sporogenes</i>	NCIMB 12343	0580Z	
<i>Escherichia coli</i>	ATCC® 8739™*	0483Z	
<i>Escherichia coli</i>	NCIMB 8545	0581Z	
<i>Pseudomonas aeruginosa</i>	ATCC® 9027™*	0484Z	
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	ATCC® 6538™*	0485Z	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	

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2.3 Scope of Verification

Briefly summarize the purpose of the Verification.

2.4 Verification Tests

List the Tests which the laboratory will perform in order to verify the EZ-CFU™ One Step product.

2.5 Acceptance Criteria

Describe acceptance criteria for determining suitability of EZ-CFU™ One Step microorganisms.



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2.6 Technician(s) Performing Verification

Technicians who perform the Verification Study should enter their information and sign below.

Name (print) _____ Title _____

Signature _____ Date _____

Department _____

Company Name _____

Address _____

Name (print) _____ Title _____

Signature _____ Date _____

Department _____

Company Name _____

Address _____

Name (print) _____ Title _____

Signature _____ Date _____

Department _____

Company Name _____

Address _____

Name (print) _____ Title _____

Signature _____ Date _____

Department _____

Company Name _____

Address _____

2.7 Approval of Plan

Signature(s) of management staff

Name (print) _____ Title _____

Signature _____ Date _____

Name (print) _____ Title _____

Signature _____ Date _____

Name (print) _____ Title _____

Signature _____ Date _____

Name (print) _____ Title _____

Signature _____ Date _____

3. Verification of Traceability, Counts, and Purity

3.1 Materials Needed for Verification

- 3.1.1 The following materials are needed for testing counts and purity.
- EZ-CFU™ One Step lyophilized microorganisms (two pellets per test)
 - Hydrating Fluid (received with EZ-CFU™ One Step kit)
 - Calibrated pipetter and sterile tips
 - Media such as Soybean Casein Agar (Tryptic Soy Agar) and Sabouraud Dextrose Agar
 - Vortex Mixer
 - Sterile Spreaders
 - Incubator at 20-25°C
 - Incubator at 30-35°C
 - Glass slides
 - Gram Stain Kit for staining bacteria
 - Lactophenol Blue Stain and clear tape for staining *Aspergillus brasiliensis*
- 3.1.2 Macroscopic characteristics should match the description of the microorganisms provided in Table 10. If your protocol requires further identification testing, you will need a manual or automated biochemical or molecular system.

3.2 Medium Information

In Table 2, record the lot number and expiration date of the agar used for growing the microorganisms. Certificates of Analysis for each medium should be attached to the Verification paper work. Check (✓) if Certificate of Analysis is attached.

Table 2: Media used for Verification


Medium	Manufacturer	Catalog Number	Lot Number	Expiry Date	Certificate of Analysis (✓)	Recorded By
Sabouraud Dextrose Agar						
Soybean Casein Agar (Tryptic Soy Agar)						
Columbia Agar						

3.3 Documentation of Traceability to Reference Culture

In Table 3, record the lot number and the expiration date of each microorganism strain tested. Record the Hydrating Fluid lot number and the expiry date. Check the appropriate column after the Certificates of Assay and Analysis have been attached to the Verification paperwork. Both certificates document traceability to the original reference culture and the passage number from reference culture.

Table 3: Documentation of Microorganism and Hydrating Fluid Lot Numbers

Microorganism	Reference Culture #	Catalog #	Lot #	Expiry Date	Hydrating Fluid Lot Number	Expiry Date	Certificate of Assay (✓)	Certificate of Analysis (✓)	Recorded by
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z							
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC® 6633™*	0486Z							
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z							
<i>C. albicans</i>	ATCC® 10231™*	0443Z							
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z							
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z							
<i>C. sporogenes</i>	NCIMB 12343	0580Z							
<i>E. coli</i>	ATCC® 8739™*	0483Z							
<i>E. coli</i>	NCIMB 8545	0581Z							
<i>P. aeruginosa</i>	ATCC® 9027™*	0484Z							
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z							
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC® 6538™*	0485Z							
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z							

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3.4 Counts and Purity

For each EZ-CFU™ One Step strain, do the following:


1. Remove a vial of Hydrating Fluid and a vial of lyophilized microorganism pellets from refrigerated storage.
2. Allow the vial of pellets to equilibrate to room temperature.
3. Pre-warm the Hydrating Fluid to 35°C.
4. Label the Hydrating Fluid vial with the microorganism lot number.
5. Remove screw top and stopper from the vial of Hydrating Fluid and the screw top from vial containing the lyophilized pellet.
6. Use a sterile forceps to transfer two lyophilized microorganism pellets into the 2 mL vial of Hydrating Fluid. Do not remove the desiccant from the vial of pellets.
7. Immediately stopper and recap the pellet vial and return it to 2° to 8°C.
8. Incubate the hydrated material at 35°C for 30 minutes.
9. Vortex until the suspension is homogenous.
10. Dispense 0.1 mL (100 µl) of suspension per agar plate. Perform a total of five replicates.
11. Spread inoculum evenly across plate with sterile spreader.
12. Incubate agar plates. Follow directions in Table 4 for temperature and length of time for incubation. Record incubator identity, incubator temperature range, date and time when incubation begins and initials in Table 5.
13. Recap the microorganism vial and store the suspension at 2-8°C if intending to verify 8 hour stability. This step is not necessary if the laboratory never plans to store the suspension. Record refrigerator identity, refrigerator temperature range, date and time suspension is placed in the refrigerator, and initials in Table 7 in Section 3.5, Verification of Stability. Catalog numbers 0320Z and 0318Z must be used within 30 minutes.
14. Record in Table 5 the date and time when incubation ends. Record initials.
15. Count the colonies on each replicate plate and record the number in Table 6. Determine and record average count.
16. Inspect the plates for purity. If no contamination is seen, record S in the Purity Column in Table 6. If contamination is seen, record U. Date and initial.

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Table 4: Incubation Instructions

EZ-Accu Shot™ Microorganism	Reference Culture #	Catalog #	Media	Temp. Range	Pharmacopeia Incubation Instructions	Check Macroscopic Characteristics	Atmosphere
<i>A. brasiliensis</i>	ATCC® 16404™*	0392A	Soybean Casein Agar ¹	30-35°C	≤5 days	4-5 days	Aerobic
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	Sabouraud Dextrose Agar	20-25°C	≤5 days	4-5 days	Aerobic
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC® 6633™*	0486Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>C. albicans</i>	ATCC® 10231™*	0443Z	Soybean Casein Agar ¹	30-35°C	≤5 days	48 hours	Aerobic
<i>C. albicans</i>	ATCC® 10231™*	0443Z	Sabouraud Dextrose Agar	20-25°C	≤5 days	48 hours	Aerobic
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z	Columbia Agar	30-35°C	48 hours	48 hours	Anaerobic
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z	Columbia Agar	30-35°C	48 hours	48 hours	Anaerobic
<i>C. sporogenes</i>	NCIMB 12343	0580Z	Columbia Agar	30-35°C	48 hours	48 hours	Anaerobic
<i>E. coli</i>	ATCC® 8739™*	0483Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>E. coli</i>	NCIMB 8545	0581Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>P. aeruginosa</i>	ATCC® 9027™*	0484Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC® 6538™*	0485Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	Soybean Casein Agar ¹	30-35°C	≤3 days	24 hours	Aerobic

¹ Soybean Casein Agar is also known as Tryptic Soy Agar

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Table 5: Incubation Data

Microorganism Name	Reference Culture #	Catalog #	Agar ¹	Incubator Identity	Incubator Temperature Range	Date/Time Incubation Begins	By	Date/ Time Incubation ends	By
<i>A. brasiliensis</i>	ATCC® 16404™**	0392Z	SCA						
<i>A. brasiliensis</i>	ATCC® 16404™**	0392Z	SDA						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC®6633™**	0486Z	SCA						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	SCA						
<i>C. albicans</i>	ATCC® 10231™**	0443Z	SCA						
<i>C. albicans</i>	ATCC® 10231™**	0443Z	SDA						
<i>C. sporogenes</i>	ATCC® 19404™**	0317Z	CA						
<i>C. sporogenes</i>	ATCC® 11437™**	0487Z	CA						
<i>C. sporogenes</i>	NCIMB 12343	0580Z	CA						
<i>E. coli</i>	ATCC® 8739™**	0483Z	SCA						
<i>E. coli</i>	NCIMB 8545	0581Z	SCA						
<i>P. aeruginosa</i>	ATCC®9027™**	0484Z	SCA						
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™**	0363Z	SCA						
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC®6538™**	0485Z	SCA						
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	SCA						

¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar



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Table 6: Microorganism Counts and Purity

Microorganism	Reference Culture #	Catalog #	Agar ¹	CFU Count					Avg. Count	Purity S/U	Date	By
				1	2	3	4	5				
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	SCA									
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	SDA									
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC® 6633™*	0486Z	SCA									
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	SCA									
<i>C. albicans</i>	ATCC® 10231™*	0443Z	SCA									
<i>C. albicans</i>	ATCC® 10231™*	0443Z	SDA									
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z	CA									
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z	CA									
<i>C. sporogenes</i>	NCIMB 12343	0580Z	CA									
<i>E. coli</i>	ATCC® 8739™*	0483Z	SCA									
<i>E. coli</i>	NCIMB 8545	0581Z	SCA									
<i>P. aeruginosa</i>	ATCC® 9027™*	0484Z	SCA									
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z	SCA									
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC® 6538™*	0485Z	SCA									
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	SCA									

¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar

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3.5 Stability

For each EZ-CFU™ One Step strain, do the following:

1. Remove suspension from refrigerator after 8 hours (or less if laboratory is planning for shorter storage duration). Record date and time when the suspension is taken out of the refrigerator and record initials in Table 7.
2. Remix suspension on vortex mixer.
3. Dispense 0.1 mL of suspension per plate. Set up a total of five replicates.
4. Spread inoculum evenly across plate with sterile spreader.

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5. Incubate second set of agar plates at the same temperature and for the same length of time as the first set of agar plates. Record incubator identity, incubator temperature range, date and time incubation begins, and initials in Table 8.
6. Record the date and time when incubation ends and initials in Table 8.
7. Count the colonies on each replicate plate. Record the number in Table 9. Determine and record average count. Record date and initials.

Table 7: Refrigeration Data

Microorganism	Reference Culture #	Catalog #	Refrigerator Identity	Refrigerator Temperature Range	Date and Time Refrigeration Begins	By	Date and Time Refrigeration Ends	By
<i>A. brasiliensis</i>	ATCC® 16404™**	0392Z						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC® 6633™**	0486Z						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z						
<i>C. albicans</i>	ATCC® 10231™**	0443Z						
<i>C. sporogenes</i>	ATCC® 19404™**	0317Z						
<i>C. sporogenes</i>	ATCC® 11437™**	0487Z						
<i>C. sporogenes</i>	NCIMB 12343	0580Z						
<i>E. coli</i>	ATCC® 8739™**	0483Z						
<i>E. coli</i>	NCIMB 8545	0581Z						
<i>P. aeruginosa</i>	ATCC® 9027™**	0484Z						
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™**	0363Z						
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC® 6538™**	0485Z						
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z						



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Table 8: Incubation Data for Refrigerated Suspensions

Microorganism	Reference Culture #	Catalog #	Agar ¹	Incubator Identity	Incubator Temperature Range	Date and Time Incubation Begins	By	Date and Time Incubation Ends	By
<i>A. brasiliensis</i>	ATCC® 16404™*	0392A	SCA						
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	SDA						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC®6633™*	0486Z	SCA						
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	SCA						
<i>C. albicans</i>	ATCC® 10231™*	0443Z	SCA						
<i>C. albicans</i>	ATCC® 10231™*	0443Z	SDA						
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z	CA						
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z	CA						
<i>C. sporogenes</i>	NCIMB 12343	0580Z	CA						
<i>E. coli</i>	ATCC® 8739™*	0483Z	SCA						
<i>E. coli</i>	NCIMB 8545	0581Z	SCA						
<i>P. aeruginosa</i>	ATCC®9027™*	0484Z	SCA						
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z	SCA						
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC®6538™*	0485Z	SCA						
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	SCA						

¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar


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Table 9: Microorganism counts after refrigeration

Microorganism	Reference Culture #	Catalog #	Agar ¹	CFU Count					Avg. Count	Date	By
				1	2	3	4	5			
<i>A. brasiliensis</i>	ATCC® 16404™**	0392Z	SCA								
<i>A. brasiliensis</i>	ATCC® 16404™**	0392Z	SDA								
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC®6633™**	0486Z	SCA								
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	SCA								
<i>C. albicans</i>	ATCC® 10231™**	0443Z	SCA								
<i>C. albicans</i>	ATCC® 10231™**	0443Z	SDA								
<i>C. sporogenes</i>	ATCC® 19404™**	0317Z	CA								
<i>C. sporogenes</i>	ATCC® 11437™**	0487Z	CA								
<i>C. sporogenes</i>	NCIMB 12343	0580Z	CA								
<i>E. coli</i>	ATCC® 8739™**	0483Z	SCA								
<i>E. coli</i>	NCIMB 8545	0581Z	SCA								
<i>P. aeruginosa</i>	ATCC®9027™**	0484Z	SCA								
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™**	0363Z	SCA								
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC®6538™**	0485Z	SCA								
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	SCA								

¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar

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3.6 Macroscopic and Microscopic Characteristics

1. After incubation examine colonies from all strains macroscopically. Colony appearance should agree with descriptions listed in Table 10. Enter S (satisfactory appearance) or U (unsatisfactory appearance) in appropriate column. Record date and initials.
2. Use the Gram Stain method to stain bacterial colonies from each strain. Examine all strains microscopically. Microscopic appearance should agree with descriptions in Table 10. Enter S (satisfactory appearance) or U (unsatisfactory appearance) in appropriate column. Record date and initials.
3. Examine *Aspergillus brasiliensis* microscopically after spores have formed (this may take 5-7 days). To do this, firmly press the sticky side of transparent tape to the surface of the fungal culture. Gently place tape on a drop of Lactophenol Blue on a glass slide. Examine slide microscopically (a magnification of 400X works well). Microscopic appearance should agree with descriptions in Table 10. Enter S (satisfactory appearance) or U (unsatisfactory appearance) in appropriate column. Record date and initials.

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4. If SOP calls for further testing, subculture colonies from each strain onto fresh agar plates (the new plates are the fifth passage from Reference Culture).
5. After incubation, perform identification tests, if required to do so by SOP. Describe identification method in Section 3.7.
6. Record identification in Table 11. Enter S (satisfactory results) or U (unsatisfactory results) in appropriate column. Record date and initials. Attach worksheets and results.
7. Autoclave contaminated articles or dispose of them in a biohazard container.


Table 10: Macroscopic and Microscopic Characteristics

Strain	Cat. #	Agar ¹	Macroscopic description	S/U	Date	By	Microscopic description	S/U	Date	By
<i>A. brasiliensis</i> ATCC® 16404™	0392Z	SCA, 30-35°C, 4-5 days.	By 4 days, rapidly growing colonies which are initially white or pale yellow, quickly become black with conidia (spore) production. Reverse is pale yellow.				Lactophenol Blue Stain: chains of small conidia which arise from short sterigmata arranged radially over the surface of the vesicle.			
<i>A. brasiliensis</i> ATCC® 16404™	0392Z	SDA, 20-25°C, 4-5 days.	By 4 days, rapidly growing white colonies which quickly become black with conidia (spore) production.				Lactophenol Blue Stain: chains of small conidia which arise from short sterigmata arranged radially over the surface of the vesicle.			
<i>B. subtilis</i> subsp. <i>spizizenii</i> ATCC®6633™	0486Z	SCA, 30-35°C, 24 hours.	Large, irregular, flat, undulate edge, cream and wrinkled with ground glass appearance; slight yellow coloring may appear in wrinkles by 48 hours.				Straight, gram positive rod, with an ellipsoidal, central, or terminal endospore.			
<i>B. subtilis</i> subsp. <i>spizizenii</i> NCIMB 8054	0582Z	SCA, 30-35°C, 24 hours.	Large, irregular, flat, undulate edge, cream and wrinkled with ground glass appearance; slight yellow coloring may appear in wrinkles by 48 hours.				Straight, gram positive rod, with an ellipsoidal, central, or terminal endospore.			
<i>C. albicans</i> ATCC® 10231™	0443Z	SCA, 30-35°C, 48 hours.	Large, white, circular, convex, dull colonies.				Gram positive, spherical, budding yeast cells.			
<i>C. albicans</i> ATCC® 10231™	0443Z	SDA, 20-25°C, 48 hours.	Large, white, circular, convex, dull colonies.				Gram positive, ovoid, budding yeast cells.			
<i>C. sporogenes</i> ATCC® 19404™	0317Z	CA, anaerobic condition s, 30- 35°C, 48 hours.	Large, irregularly circular, with coarse rhizoid edge, slightly raised center, gray, beta hemolytic, swarms, adheres to agar.				Gram positive straight rods. Spores, when seen, are oval, central or subterminal, and distend the cell slightly.			
<i>C. sporogenes</i> ATCC® 11437™	0487Z	CA, anaerobic condition s, 30- 35°C, 48 hours.	Large, irregularly circular, with coarse rhizoid edge, slightly raised center, gray, beta hemolytic, swarms, adheres to agar.				Gram positive straight rods. Spores, when seen, are oval, central or subterminal, and distend the cell slightly.			
<i>C. sporogenes</i> NCIMB 12343	0580Z	CA, anaerobic condition s, 30- 35°C, 48 hours.	Large, irregularly circular, with coarse rhizoid edge, slightly raised center, gray, beta hemolytic, swarms, adheres to agar.				Gram positive straight rods. Spores, when seen, are oval, central or subterminal, and distend the cell slightly.			

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Strain	Cat. #	Agar ¹	Macroscopic description	S/U	Date	By	Microscopic description	S/U	Date	By
<i>E. coli</i> ATCC® 8739™**	0483Z	SCA, 30-35°C, 24 hours.	Medium to large, creamy, low convex, slightly rough, slightly erose.				Gram negative straight rod.			
<i>E. coli</i> NCIMB 8545	0581Z	SCA, 30-35°C, 24 hours.	Medium to large, creamy, low convex, slightly rough, slightly erose.				Gram negative straight rod.			
<i>P. aeruginosa</i> ATCC® 9027™**	0484Z	SCA, 30-35°C, 24 hours.	Medium to large, flat, circular to irregular shaped, cream.				Straight or slightly curved gram negative rod.			
<i>Salmonella</i> <i>enterica</i> subsp. <i>enterica</i> serovar Typhimurium ATCC® 14028™**	0363Z	SCA, 30-35°C, 24 hours.	Large, convex, entire edge, smooth, cream, opaque.				Gram negative straight rod.			
<i>S. aureus</i> subsp. <i>aureus</i> ATCC®6538™**	0485Z	SCA, 30-35°C, 24 hours.	Medium, convex, circular, glistening, smooth, creamy, opaque, light gold colonies.				Gram positive cocci occurring singly, in pairs, and in irregular clusters.			
<i>S. aureus</i> subsp. <i>aureus</i> NCIMB 9518	0579Z	SCA, 30-35°C, 24 hours.	Medium, convex, circular, glistening, smooth, creamy, opaque, light gold colonies.				Gram positive cocci occurring singly, in pairs, and in irregular clusters.			


¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar

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3.7 Biochemical and/or Molecular Identity (If Required)

Table 11: Identification Test Results

Microorganism	Reference Culture #	Catalog #	Biochemical/Molecular Identification	S/U	Date	By
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z				
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC®6633™*	0486Z				
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z				
<i>C. albicans</i>	ATCC® 10231™	0443Z				
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z				
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z				
<i>C. sporogenes</i>	NCIMB 12343	0580Z				
<i>E. coli</i>	ATCC® 8739™*	0483Z				
<i>E. coli</i>	NCIMB 8545	0581Z				
<i>P. aeruginosa</i>	ATCC®9027™*	0484Z				
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z				
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC®6538™*	0485Z				
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z				

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4. Summary and Conclusion

Determine if each EZ-CFU™ One Step lyophilized microorganism strain meets acceptance criteria.


4.1 Summary of Data

Enter S (Satisfactory) or U (Unsatisfactory) for each category. Date and initial.

Table 12: Summary

Microorganism	Reference Culture #	Cat. #	Agar ¹	Traceability S/U	CFU count S/U	8 hour Stability S/U	Purity S/U	Identity S/U	Date	By
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	SCA							
<i>A. brasiliensis</i>	ATCC® 16404™*	0392Z	SDA							
<i>B. subtilis</i> subsp. <i>spizizenii</i>	ATCC®6633™*	0486Z	SCA							
<i>B. subtilis</i> subsp. <i>spizizenii</i>	NCIMB 8054	0582Z	SCA							
<i>C. albicans</i>	ATCC® 10231™	0443Z	SCA							
<i>C. albicans</i>	ATCC® 10231™	0443Z	SDA							
<i>C. sporogenes</i>	ATCC® 19404™*	0317Z	CA							
<i>C. sporogenes</i>	ATCC® 11437™*	0487Z	CA							
<i>C. sporogenes</i>	NCIMB 12343	0580Z	CA							
<i>E. coli</i>	ATCC® 8739™*	0483Z	SCA							
<i>E. coli</i>	NCIMB 8545	0581Z	SCA							
<i>P. aeruginosa</i>	ATCC®9027™*	0484Z	SCA							
<i>S. enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC® 14028™*	0363Z	SCA							
<i>S. aureus</i> subsp. <i>aureus</i>	ATCC®6538™*	0485Z	SCA							
<i>S. aureus</i> subsp. <i>aureus</i>	NCIMB 9518	0579Z	SCA							

¹SCA = Soybean Casein Agar, SDA = Sabouraud Dextrose Agar, CA = Columbia Agar

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4.2 Comments

By _____ Date _____

4.3 Conclusion

By _____ Date _____

4.4 Review and Approval

Name	Title	Signature	Date