Growth Promotion Test Guide for Media Used in Tests for *Burkholderia cepacia* complex

Tests for Burkholderia cepacia complex

The purpose of the Tests for *Burkholderia cepacia* complex is to determine if *Burkholderia cepacia* complex (Bcc) microorganisms can be detected in a nonsterile aqueous based pharmaceutical product or component and exceeds the limits established by the pharmacopeia. Examples of product categories to be tested are those for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use. Before a product can be tested, the laboratory must know whether the medium it is using for the test will grow the specified microorganism if it is present in small numbers.

The objective of the Growth Promotion Test is to demonstrate that the media is suitable to grow and will show the correct indicative reactions of the specified organism. Laboratories perform the test by inoculating new batches of media with a small number of microorganisms. The microorganisms will grow and exhibit the correct inhibitory properties if the media is suitable.

The Media

The media used in the tests are designed to isolate microorganisms in the *Burkholderia cepacia* complex. The media are listed in Table 2.

The Microorganisms

Table 1 lists the reference culture strains that are used for the Growth Promotion Test. The list is based on the United States Pharmacopeia (USP). At this time the Tests for *Burkholderia cepacia* complex is only official in the United States Pharmacopeia (USP).

The Microbiologics products listed in Table 1 are lyophilized microorganism preparations that are 3 passages or fewer from the reference culture. For more information about the products, visit our website at www.microbiologics.com. The microorganisms are offered in the following 2 formats:

1. **EZ-Accu Shot™** kits include: 5 vials of a single enumerated microorganism (1 lyophilized pellet per vial) and 5 vials of Hydrating Fluid (1.2 ml in each vial). When processed as directed, each 0.1ml inoculum will deliver 10-100 CFU. Ten tests can be performed with each pellet for a total of 50 tests per kit.
2. **Bcc Select™** kits include: vials each containing a lyophilized pellet of a different microorganism (1 vial of each of 5 strains) and 5 vials of Hydrating Fluid (1.2 ml in each vial). When processed as directed, each 0.1ml inoculum will deliver 10-100 CFU. Ten tests can be performed with each pellet for a total of 50 tests per kit.
3. **Epower™** kits include: 1 vial of a single enumerated microorganism (10 lyophilized pellets per vial). It is available in a variety of strains with concentrations ranging from 10^2 to 10^8 CFU per pellet.

Table 1: Microbiologics Catalog Numbers

Microorganism Name	Microbiologics Catalog Number
Burkholderia cepacia derived from ATCC® 25416™*	0488
Burkholderia cenocepacia derived from ATCC® BAA-245™*	01269
Burkholderia multivorans derived from ATCC® BAA-247™*	01270
Psuedomonas aeruginosa derived from ATCC® 9027™*	0484
Staphylococcus aureus subsp. *aureus* derived from ATCC® 6538™*	0485
Bcc Select	7009

Requirements of the Test

1. Perform the Growth Promotion Test on each new batch of purchased ready-prepared medium, dehydrated medium or medium prepared from components in the laboratory.
2. Inoculate medium with ≤100 CFU for growth promoting and indicative properties. Inoculate medium with ≥100 CFU for inhibitory properties.
3. Use the microorganism strains recommended by the pharmacopeia. The strains should be no more than 5 passages from the reference culture.
4. Test new medium in parallel with previously approved medium. Inoculate the previously approved, non-selective agar to determine the number of CFU in the inoculum. The number of CFU in the inoculum is the standard value. When parallel testing is used, the new and previously approved batches of the medium must be inoculated with the same inoculum, by the same technician, and are subjected to identical incubation conditions. The only variable is the medium.
5. Test each medium with only 1 microorganism strain at a time.
6. Test in duplicate.

Materials

- **EZ-Accu Shot™** kits, **Bcc Select™** kits or **Epower™**: The **EZ-Accu Shot™** and **Bcc Select™** kits contain the lyophilized microorganisms and Hydrating Fluid.
- New batch of medium
- Previously approved batch of medium
- Non-selective agar if testing liquid medium
- Calibrated micropipette
- Spreader for distributing the inoculum
- Vortex mixer
- **Epower™** if testing the inhibitory properties of solid and/or liquid medium
- Sterile Phosphate buffer pH 7.2 if testing the inhibitory properties of solid and/or liquid medium

Test Procedures

Growth Promoting and Indicative Properties of Burkholderia Selective Agar

1. Prepare an inoculum for each of the required microorganisms by following the **EZ-Accu Shot™** Instructions for Use.
    a. If using **Epower™**, hydrate 1 pellet in 10ml of pH 7.2 phosphate buffer and serially dilute to obtain a total dilution factor of 1:10,000 in the final pH 7.2 phosphate buffer tube
    b. A 0.1ml inoculum from the 1:10,000 dilution will yield 10-100 CFU
2. Inoculate the new and previously approved batches of the medium with 0.1 ml of the microorganism suspension. A control is recommended to verify the 0.1 ml of microorganism suspension contains ≤100 CFU. The control is the non-selective agar.
3. Use a spreader to disperse the inoculum evenly across the agar.
4. Follow pharmacopeia directions for incubation temperature and length of incubation for each microorganism tested (refer to Table 2).
5. Determine if the new medium is suitable for use by using the acceptance criteria below.

Acceptance Criteria

1. **Solid Medium**
   Average the number of colonies from the new batch of medium and the number of colonies from the previously approved batch of medium. For the new batch of medium to be approved, the following acceptance criteria must be met for each microorganism tested:
    - The average number of colonies on the new batch of the medium must be “comparable” to the average number of colonies on the previously approved batch. A quantitative definition of “comparable” is not established by the USP.
    - There must be ≤100 colonies on the control (non-selective agar).

2. **Indicative Reactions**
   Visually compare the colonies on the agar plates to the colonies on the previously approved batch of medium. The colonies should be similar in appearance. Expected indicative reactions are described in Table 2.

Table 2: Growth Promotion Test Requirements for *Burkholderia cepacia* complex

Type of Medium	Microorganism	Properties	Temperature	Incubation Period	Colony Morphology
Burkholderia cepacia Selective Agar (BCSA)	*Burkholderia cepacia*	Growth Promoting and Indicative	30°C-35°C	48 hours	Greenish-brown colonies with yellow halos, or white colonies surrounded by a pink-red zone
	Bukholderia cenocepacia	Growth Promoting and Indicative	30°C-35°C	48 hours	Greenish-brown colonies with yellow halos, or white colonies surrounded by a pink-red zone
	Burkholderia multivorans	Growth Promoting and Indicative	30°C-35°C	48 hours	Greenish-brown colonies with yellow halos, or white colonies surrounded by a pink-red zone
	Psuedomonas aeruginosa	Inhibitory	30°C-35°C	72 hours	Inhibition
	Staphylococcus aureus	Inhibitory	30°C-35°C	72 hours	Inhibition

Inhibitory Properties of Solid Media

1. Using **Epower**™ E3 or E7, prepare an inoculum of each of the required microorganisms.
2. Rehydrate one **Epower™** E3 pellet in 1.0 ml or one **Epower™** E7 pellet in 10ml of hydrating fluid as described in the **Epower**™ Instructions for Use document.
    a. An **Epower™** E3 pellet contains 1000-9999 CFU. One ml of the **Epower™** microorganism suspension contains 1000-9999 CFU per ml. This will yield a concentration of 100-999 CFU/0.1ml
    b. An Epower™ E7 pellet contains 10,000,000 to 99,999,999 CFU per pellet. One ml of the **Epower™** microorganism suspension contains 1,000,000 to 9,999,999 CFU per ml. Dilute further to get to 100-999 CFU/0.1ml in the final dilution
3. Inoculate the new and previously approved batches of the medium with 0.1 ml of the microorganism suspension.
4. A control is recommended to verify the 0.1 ml of microorganism suspension contains ≥100 CFU. The control is the non-selective agar.
5. Use a spreader to disperse the inoculum across the agar.
6. Follow pharmacopeia directions for incubation temperature and length of incubation for each microorganism tested (refer to Table 2).
6. Determine if the new medium is suitable for use by using the acceptance criteria below.

Acceptance Criteria

1. The test microorganism should be inhibited on the new batch of agar.
2. There should be ≥100 colonies on the non-selective control agar.

Best Practices for Growth Promotion Tests

Preparation

Use the microorganism strains recommended by the pharmacopeia. The cultures should be traceable to and no more than 5 passages from the reference culture.

Equipment

- Use automatic calibrated micropipette. Routinely verify its accuracy.
- Calibrate thermometers and incubators yearly.

Control

A negative control (diluent[5]) is recommended for the Growth Promotion Test. Microbiologics has performed a Sterility Test on the hydrating fluid and a Certificate of Analysis stating the results is available upon request. If additional hydrating fluid is required, Microbiologics offers hydrating fluid sold separately from the kits.

The Test

- Allow the medium and the vial of pellets to equilibrate to room temperature before use.
- If using **Epower™** the hydrating fluid and any dilution fluids should warm for 30 minutes at 35˚C before use.
- If using the Pour Plate Method, add 0.1 ml of the microorganism suspension to a sterile Petri dish. Pour molten agar over the inoculum and mix well by swirling the contents in the plate. Invert and incubate the agar after it has solidified.
- The molten medium must be cooled to 44°C-46°C before it is poured. It should not be left in the molten state for more than 4 hours. Unused medium should not be re-solidified and used again. It may be necessary to double the inoculum when using selective agar. If this is the case, inoculate non-selective and selective agar in parallel. There must be ≤100 colonies on the non-selective agar.

Incubation

Due to pour plates requiring longer incubation periods and the variability of colony sizes, the use of a backlit colony counter is recommended.

[5] The diluent is the Hydrating Fluid catalog number HF0611. If using **Epower™**, the diluent is phosphate buffer pH 7.2.

Other

- Recovery on BCSA may be less than on non-selective media.
- Microbiologics has validated the procedures for using **EZ-Accu Shot™ Bcc Select™** and **Epower™** on TSA for the following microorganism strains:
    - *Burkholderia cepacia derived from ATCC® 25416™**
    - *Burkholderia cenocepacia derived from ATCC® BAA-245™**
    - *Burkholderia multivorans derived from ATCC® BAA-247™**
    - *Pseudomonas aeruginosa* derived from ATCC® 9027™*
    - *Staphylococcus aureus* derived from ATCC® 6538™*
- A customer qualification study is recommended to verify that the product works for the chosen manufacturer of BCSA media, the company procedures, equipment, etc.
- To ensure end-user safety, a pharmaceutical product may need to be tested for microorganisms other than those mentioned in the Tests for *Burkholderia cepacia* complex. Visit our website, www.microbiologics.com, for a complete list of **EZ-Accu Shot™**, **EZ-CFU™ One Step**, and **EZ-CFU™** microorganisms that can be used in growth promotion testing.
- Wild-type microorganism strains found in the manufacturing environment can contaminate pharmaceutical products. To ensure the environmental strains can grow on new batches of culture media used in the sterility test, include them when performing the growth promotion test. Your environmental isolates can be professionally characterized, preserved and manufactured in a convenient, ready-to-use format using a program called Microbiologics Custom Solutions. Contact your Microbiologics sales representative if you would like more information about the program.

References

United States Pharmacopeia <60> Microbial Examination of Nonsterile Products: Test for *Burkholderia cepacia* complex, The United States Pharmacopeial Convention. Rockville, MD.

Acknowledgements

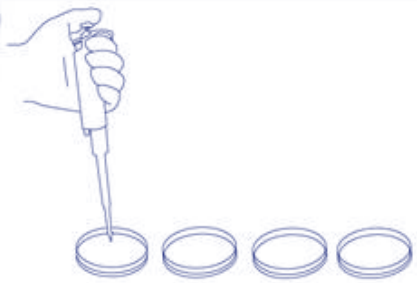
Illustrated Instructions

Instructions for testing the growth promoting and indicative qualities of new batches of solid medium to be used in Tests for Specified Microorganisms.

1

Prepare an inoculum for each of the required microorganisms by following the **EZ-Accu Shot™** Instructions for Use document.

2



Inoculate the new previously approved batches of the medium with 0.1 ml of the microorganism suspension.

3

A control is recommended to verify the 0.1 ml of microorganism suspension contain ≤ 100 CFU. The control is the non-selective agar.

4

Use a spreader to disperse the inoculum across the agar.

5

Follow the pharmacopeia directions for the incubation temperature and the length of incubation for each microorganism being tested (refer to Table 2).

6

Determine if the new medium is suitable for use by using the acceptance criteria.

ILLUSTRATED INSTRUCTIONS

Instructions for testing inhibitory qualities of new batches of solid medium using Epower E3™ and Epower E7™. Note: Although the illustrations illustrate solid medium, they can also be used for liquid medium.