Add the following:

▲⟨60⟩ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS —TESTS FOR *BURKHOLDERIA CEPACIA* COMPLEX

INTRODUCTION

The tests described in this chapter will allow determination of the absence of *Burkholderia cepacia* complex (Bcc), which can be detected under the conditions described.

The tests are designed to determine whether a substance or preparation complies with an established specification for microbiological quality and/or to evaluate whether products—especially those for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use—contain members of the Bcc.

GROWTH-PROMOTING AND INHIBITORY PROPERTIES OF THE MEDIA AND SUITABILITY OF TESTS FOR ABSENCE OF Bcc

Test each batch of ready-prepared medium and each batch of medium prepared from either dehydrated medium or ingredients.

Preparation of Test Strains

Use standardized stable suspensions of test strains (see *Table 1*) NMT 5 passages removed from the original strain culture.

Table 1. Test Strains of Microorganisms for Growth Promotion and Suitability Testing

Microorganism	Standard Strain
Burkholderia cepacia	ATCC 25416, NCTC 10743, or CIP 80.24
Burkholderia cenocepacia	ATCC BAA-245 or LMG 16656
Burkholderia multivorans	ATCC BAA-247, LMG 13010, CCUG 34080, CIP 105495, DSM 13243, or NCTC 13007
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, or NBRC 13275
Staphylococcus aureus	ATCC 6538, NCIMB 9518, CIP 4.83, or NBRC 13276

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Microorganisms

Grow each of the test strains separately in *Soybean–Casein Digest Broth* or on Soybean–Casein Digest Agar at 30°–35° for 18–24 h.

Use *Buffered Sodium Chloride–Peptone Solution pH 7.0* or *Phosphate Buffer Solution pH 7.2* to make the test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2°–8°. If purchased, follow the supplier's instructions. If self-prepared cultures are used, follow a validated procedure (such as in *Microbial Enumeration Tests* ⟨61⟩) for preparation. Use a challenge inoculum of NMT 100 colony-forming units (cfu) for growth promotion and suitability testing.

NEGATIVE CONTROLS

Include a negative control to verify the testing conditions. There must be no growth of microorganisms. A negative control is also performed when testing the products as described in *Testing of Products*.

TEST FOR GROWTH-PROMOTING PROPERTIES, SOLID MEDIA

Perform the *Surface-Spread Method* (see *Microbial Enumeration Tests* ⟨61⟩*, Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Recovery of Microorganisms in the Presence of Product, Plate-Count Methods*), inoculating each plate with a small number (NMT 100 cfu) of the appropriate microorganism (see *Table 2*). Incubate at the specified temperature for NMT the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Table 2. Microorganisms for the Growth-Promoting, Inhibitory, and Indicative Properties of the Media

Medium	Property	Microorganism
Burkholderia cepacia selective agar	Growth-Promoting and Indicative	*Burkholderia cepacia*, *Burkholderia cenocepacia*, or *Burkholderia multivorans*
	Inhibitory	*Pseudomonas aeruginosa*, *Staphylococcus aureus*

TEST FOR INHIBITORY PROPERTIES, SOLID MEDIA

Inoculate the appropriate medium with at least 100 cfu of the appropriate microorganism. Incubate at the specified temperature for NLT the longest period of time specified in the test. Inhibition of growth of the indicated microorganisms occurs (see *Table 2*).

TEST FOR INDICATIVE PROPERTIES

Perform the *Surface-Spread Method* (see *Microbial Enumeration Tests* ⟨61⟩*, Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Recovery of Microorganisms in the Presence of Product, Plate-Count Methods*), inoculating each plate with a small number (NMT 100 cfu) of the indicated microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium (see *Table 2*).

Suitability of the Test Method

The ability of the test to detect Bcc in the presence of the product to be tested must be established. The incubation time for the method suitability should not exceed the shortest incubation period specified. Suitability must be confirmed if there is a change in testing performance or a change in the product that may affect the outcome of the test.

For each new product to be tested, perform the sample preparation as described in *Testing of Products*. At the time of mixing, add each test strain in the prescribed growth medium. Inoculate the test strains individually. Use a number of microorganisms equivalent to NMT 100 cfu in the inoculated test preparation.

Perform the test as described in *Testing of Products*, using the shortest incubation period prescribed. Bcc microorganisms must be detected with the indication reactions described in *Interpretation*.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see *Microbial Enumeration Tests* ⟨61⟩*, Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Neutralization/Removal of Antimicrobial Activity*).

TESTING OF PRODUCTS

Sample Preparation and Pre-Incubation

Prepare a sample using a 1-in-10 dilution of NLT 1 g of the product to be examined. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in *Suitability of the Test Method*) of *Soybean–Casein Digest Broth* or an appropriate dilution of *Soybean–Casein Digest Broth* as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Then mix and incubate at 30°–35° for 48–72 h.

Selection and Subculture

Subculture by streaking on a plate of *Burkholderia cepacia* selective agar (BCSA), and incubate at 30°–35° for 48–72 h.

Interpretation

The possible presence of Bcc is indicated by the growth of greenish–brown colonies with yellow halos, or white colonies surrounded by a pink–red zone on BCSA. Any growth on BCSA is confirmed by identification tests. See *Microbial Characterization, Identification, and Strain Typing* ⟨1113⟩ for additional information.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

RECOMMENDED CULTURE MEDIA

[NOTE—This section is given for information.]

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the tests in this Pharmacopeia. Other media may be used provided that their suitability can be demonstrated.

Stock Buffer Solution

Transfer 34 g of potassium dihydrogen phosphate to a 1000-mL volumetric flask, dissolve in 500 mL of *Purified Water*, adjust with sodium hydroxide to a pH of 7.2 ± 0.2, add *Purified Water* to volume, and mix. Dispense in containers, and sterilize. Store at a temperature of 2°–8°.

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Phosphate Buffer Solution pH 7.2

Prepare a mixture of *Purified Water* and *Stock Buffer Solution* (800:1 v/v), and sterilize.

Buffered Sodium Chloride–Peptone Solution pH 7.0

Prepare *Buffered Sodium Chloride–Peptone Solution pH 7.0* as directed in *Table 3*. Sterilize in an autoclave using a validated cycle.

Table 3

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g (equivalent to 0.067 M of phosphate)
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified Water	1000 mL

Soybean–Casein Digest Broth

Prepare *Soybean–Casein Digest Broth* as directed in *Table 4*. Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Table 4

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Dibasic hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Purified Water	1000 mL

Burkholderia cepacia Selective Agar

Prepare BCSA as directed in *Table 5*. When preparing media in-house, first prepare the base ingredients without the antibiotics. Adjust the pH so that after sterilization it is 6.8 ± 0.3 at 25°. Sterilize in an autoclave using a validated cycle. Cool the base medium to 45°–50° and add a 1% solution of the sterile filtered antibiotics, mix, and pour into the plates.

Table 5

Casein peptone	10.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Yeast extract	1.5 g
Phenol red	0.08 g
Gentamicin	10.0 mg
Vancomycin	2.5 mg
Crystal violet	2.0 mg
Polymyxin B	600,000 U
Agar	14.0 g
Demineralized water	1000 mL▲ (USP 1-Dec-2019)

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