Microbiological Examination of *Burkholderia cepacia* Complex in Non-Sterile Products

Introduction

The tests described here will allow determination of the absence of *Burkholderia cepacia* complex (Bcc), that may be detected under the conditions described.

This test are carried out to find out 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.

Following procedures are carried out as described under *Total aerobic viable count* in *Microbial contamination in nonsterile products* (2.2.9).

i) The preparation of sample.

ii) Inactivation of antimicrobial property, if present, in the product.

iii) Confirmation that the surface active substances if used are not toxic for the test-organisms and are compatible with the inactivating agent.

Growth promotive, Inhibitory and indicative properties of the media and validity of the test

Test each batch of medium prepared either from the dehydrated medium or from the ingredients described for growth promotion. Use the already tested and approved medium prepared from dehydrated medium or from the ingredients as a positive control. Verify the properties of relevant media as described in Table 1.

Table 1-Growth promoting, inhibitory and indicative properties of media

Test/Medium	Property	Test Strains
Test for *Burkholderia cepacia* complex (Bcc)		
Burkholderia cepacia selective Agar	Growth promoting + Indicative	*Burkholderia cepacia, Burkholderia cenocepacia or Burkholderia multivorans*
	Inhibitory	*Pseudomonas aeruginosa, Staphylococcus aureus*

Preparation of the inoculum

Microorganisms used. *Burkholderia cepacia* ATCC 25416, ATCC BAA-245, *Burkholderia multivorans* ATCC BAA-247, *Staphylococus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027.

In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages made from the original culture. One passage is defined as inoculation and growth of the organisms from existing culture to a fresh medium.

Grow each of the aerobic bacterial strains separately in Casein soyabean digest broth or on Casein soyabean digest agar and incubate them at 30º to 35º for 18 to 24 hours. After incubation, re-suspend the growth of each of the organisms separately in *buffered sodium chloride-peptone solution pH 7.0.* Use suspension of these organisms within 2 to 4 hours. The suspension may be stored at 2º to 8º for a validated period of time.

Negative control. To test the sterility of the medium and the diluent, use the diluent, *buffered sodium chloride-peptone solution pH 7.0* (without organisms) as a negative control. There should not be any growth of microorganisms in this control. If a negative control fails (microorganisms grow in the control), its cause should be investigated.

Tests for growth promotion by solid media. Using the surface spread method as described under *Total viable aerobic count* in *Microbial contamination in nonsterile products* (2.2.9), inoculate each plate with not more than 100 CFU of appropriate microorganism (Table 1). Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth obtained on the medium should be comparable to that on the same medium previously approved.

Tests for growth inhibition in solid media. Inoculate the appropriate medium with not less than 100 CFU of the appropriate microorganism (Table 1). Incubate at the specified temperature for not less than the longest period of time specified in the test. There should not be any growth of the microorganism on this medium.

Tests for indicative properties. Using the surface spread method as described under *Total aerobic viable count* in *Microbial contamination in nonsterile products* (2.2.9). Inoculate each plate with not more than 100 CFU of appropriate microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colony morphology and indication reactions should be similar to that obtained with the previously approved batch of medium.

Validity 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 prepare a sample as described below in the pertinent paragraph under Testing of Products. At the time of mixing add each test organism in the prescribed growth medium. Inoculate not more than 100 CFU of the test organisms individually. Carry out the test as described under Testing of Products, using the shortest incubation period prescribed. The specified microorganism must be detected with the colony morphology and indication reaction as described.

If the product has antimicrobial activity then it should be inactivated as mentioned in *inactivation of antimicrobial activity under Appropriateness of enumeration method in presence of product in Microbial contamination in nonsterile products* (2.2.9).

Testing of Products

Sample Preparation and Pre-Incubation

Using Casein soyabean digest broth (Medium 1) as a diluent, make 1 in 10 dilution of more than 1 g of the product to be examined as mentioned under *Total aerobic viable count* in *Microbial contamination in nonsterile products* (2.2.9) and use 10 ml or the quantity corresponding to 1 g or 1 ml of the product to inoculate a suitable amount (determined as under *Validity of the test method*) of Casein soyabean digest broth, mix and incubate at 30º to 35º for 48 to 72 hours.

Sub-culture on a plate of *Burkholderia cepacia* selective Agar (BCSA) and incubate at 30º to 35º for 48 to 72 hours. A greenish-brown colonies with yellow halos, or white colonies surrounded by a pink–red zone on BCSA indicates the possibility of presence of *Burkholderia cepacia* Complex. This should be confirmed by identification tests.

If there is no growth of such type of colonies, or identification tests are negative it indicates absence of *Burkholderia cepacia* Complex and the product passes the test.

Recommended solutions and culture media

The following solutions and culture media have been found to be satisfactory for the purpose for which they are prescribed in the Pharmacopoeia. Other media may be used provided their suitability can be demonstrated.

Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dehydrate	7.2 g
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified water	1000 ml

Sterilise in an autoclave.

Casein soyabean digest broth

Pancreatic digest of casein	17.0 g
Papaic digest of soyabean	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Dextrose monohydrate	2.5 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 $\pm$ 0.2.

Burkholderia cepacia Selective Agar

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
Purified water	1000 ml

Add the ingredients without the antibiotics. Adjust the pH so that after sterilization it is 6.8 ± 0.3 at 25° temperature. Sterilize in an autoclave, cool the medium to 45°to 50°. Before use add a 1 per cent solution of the sterile filtered antibiotics, mix, and pour into the plates.