Growth promotion test of aerobes, anaerobes and fungi

Test each batch of prepared medium and each batch of prepared medium from dehydrated medium or ingredients with the appropriate microorganism strains.
For fluid thioglycollate medium, use inocula less than 100 CFU of *Clostridium sporogenes** (WDCM 00008), *Pseudomonas aeruginosa** (WDCM 00026) and *Staphylococcus aureus** (WDCM 00032) using a separate portion of medium for each strain.
For soybean tryptone broth, use inocula less than 100 cfu of *Aspergillus brasiliensis*** (WDCM 00053), *Bacillus subtilis** (WDCM 00003), *Candida albicans*** (WDCM 00054) using a separate portion of medium for each strain.

Incubate for no more than 3 days for bacteria (*) and no more than 5 days for fungi (**).
The microorganisms used must not have more than 5 passages from the original strain.
The media are suitable if a clearly visible growth of the microorganisms occurs.

PRESENTATION	DESCRIPTION	PACKAGING	ART. NO.
Aspergillus brasiliensis ATCC® 16404™/ WDCM 00053	Contains 5 pellets individualized in aluminised envelope and 5 vials of hidrating liquid. (10-100 CFU/0,1 ml)	5 envelopes + 5 vials	P10001-MSC
Pseudomonas aeruginosa ATCC® 9027™/ WDCM 00026	Contains 5 pellets individualized in aluminised envelope and 5 vials of hidrating liquid. (10-100 CFU/0,1 ml)	5 envelopes + 5 vials	P10002-MSC
Staphylococcus aureus subsp. aureus ATCC® 6538™ / WDCM 00032	Contains 5 pellets individualized in aluminised envelope and 5 vials of hidrating liquid. (10-100 CFU/0,1 ml)	5 envelopes + 5 vials	P10003-MSC
Candida albicans ATCC® 10231™/ WDCM 00054	Contains 5 pellets individualized in aluminised envelope and 5 vials of hidrating liquid. (10-100 CFU/0,1 ml)	5 envelopes + 5 vials	P10005-MSC
Clostridium sporogenes ATCC® 19404™/ WDCM 00008	Contains 5 pellets individualized in aluminised envelope and 5 vials of hidrating liquid. (10-100 CFU/0,1 ml)	5 envelopes + 5 vials	P10011-MSC

Suitability test

It will be done when:
a) when the sterility test must be carried out on a new product;
b) whenever there is a change in the experimental conditions of the test.

It can be performed simultaneously with the sterility test of the product to be examined.

The suitability test is performed as described in the Product sterility test section to examine using exactly the same methods, except for the following modifications.

Membrane filtration. After transferring the contents of the container or containers to be analysed to the membrane, add the viable microorganism inoculum to the final portion of sterile diluent used to rinse the filter.

Direct inoculation. After transferring the contents of the container or containers to be analysed to the culture medium, add the inoculum of viable microorganisms.

In both cases, we used the same microorganisms as those previously known in the Aerobic, Anaerobic and Fungal Growth Promotion Test.

Perform a growth promotion test as a positive control.

Incubate all recipients containing medium for no more than 5 days.

Suitability test results:

- If a clearly visible growth of microorganisms is obtained after incubation, visually comparable to the control receptor without product, it means that the product does not have antimicrobial activity under the test conditions or said activity has been satisfactorily eliminated. The sterility test can be carried out without further modification.

- If a clearly visible growth is not obtained in the presence of the product to be analysed, visually comparable to that of control receptors without a product, it means that the product has antimicrobial activity that has not been satisfactorily eliminated under the test conditions. Modify the conditions to eliminate antimicrobial activity and repeat the method suitability test.

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CULTURE MEDIA - ORDERING INFORMATION

	DESCRIPTION	PRESENTATION	SPECIFICATIONS	ART. NO.
FTM	Fluid Thioglycollate Medium - FTM	500 g*		03-187-500
		5x500 ml		03-187BA05
		10 flasks with 40 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	0BA1026-40
		10 flasks with 100 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA1026
		6 flasks with 100 ml + ESTERIPAPEL®	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA8076
		20 tubes with 9 ml	Ink labelled tubes and metallic cap	064-TA0134
		20 tubes with 10 ml	Ink labelled tubes and metallic cap	064-TA0137
	Fluid Thioglycollate Medium w/Penase - FTM Penasa	10 flasks with 100 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA3059
TSB	TSB (Eur. Pharm.)	500 g*		02-200-500
		5x500 ml		02-200BA05
		3 bags of 3 litres**		064-BA03-3
		2 bags of 5 litres**		064-BA03-5
		10 flasks with 90 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	0BA1012-90
		10 flasks with 100 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA1012
		6 flasks with 100 ml + 2 ESTERIPAPEL®	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA8077
		10 flasks with 450 ml	Metallic-non injectable cap	064-BA2132
		6 flasks with 800 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	00BA1012-6
		20 tubes with 9 ml	Ink labelled tubes and metallic cap	064-TA2147
		20 tubes with 10 ml	Ink labelled tubes and metallic cap - non injectable cap	064-TA0113
	TSB + Penase	10 flasks with 100 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA3058
	TSB + TLHTh	10 flasks with 100 ml	Injectable cap: Plastic screw cap with injected liner of TPE with 2 injectable holes + protective outer cap with tamper-evident ring	064-BA4006
	TSB + 25% CAPITOL IV	10 flasks with 90 ml	Metallic-non injectable cap	064-BA0002
		10 flasks with 190 ml	Metallic-non injectable cap	064-BA0004
		9 flasks with 490 ml	Metallic-non injectable cap	064-BA8008
	TSB + Polisorbate + Lecitine	10 flasks with 90 ml	Metallic-non injectable cap	064-BA8009

*For dehydrated media presented in a 500 g package, we are able to offer other formats, including 5 and 25 kg. More information at: helpdesk@scharlab.com.

FILTRATION MEMBRANES - ORDERING INFORMATION

MATERIAL	Ø (mm)	PORE (µm)	STERILE	COLOUR	GRID	PACK (U.)	ART. NO.
CN	47	0,2	Yes	White	Black	100	ES47020100
CN	47	0,2	Yes	Black	White	100	ES4702010N
CN	47	0,45	Yes	White	Black	100	ES47045100
PCTE	47	0,2	Yes	White	-	100	PC47020100
PES	47	0,2	No	White	-	100	PES4702000
PES	47	0,2	Yes	White	-	100	PES4702001

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Chapter 2.6.1. Sterility test

The test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the test conditions.

Culture media and incubation temperatures

Fluid Thioglycollate Medium (FTM)
Primarily intended for the culture of anaerobic bacteria.

Composition:

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate / anhydrous	5.5 g / 5.0 g
Yeast extract	5.0 g
Casein peptone	15.0 g
Sodium thioglycollate	0.5 g
Rasazurine	0.001 g
Water R	1000 ml

pH after sterilisation 7.1 ± 0.2
Fluid thioglycollate medium is to be incubated at 30-35°C.

Soya-bean casein digest medium (TSB)
Suitable for the culture of both fungi and aerobic bacteria.

Composition:

Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate / anhydrous	2.5 g / 2.3 g
Water R	1000 ml

pH after sterilisation 7. 3 ± 0.2
Soya-bean casein digest medium is to be incubated at 20-25°C.


FTM


White screw cap with injected TPE coating and two injectable holes. Red outer shield and red tamper-proof ring specific for thioglycollate references.


White screw cap with injected coating made of TPE and two sterile injectable holes (one circular and one square).


White screw cap with injected TPE coating and two injectable holes. Blue outer shield and blue tamper evident ring specific for tryptone soy broth references.


TSB

Red and blue covers with sealing ring to show their manipulation.


Each box includes 10 self-adhesive labels with product name, batch number and the expiry date to facilitate product traceability in the lab notes.

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Test for sterility of the product to be examined. It can be applied in two different ways, by membrane filtration or by direct inoculation.

Membrane filtration technique

Applicable to filterable aqueous preparations, to alcoholic or oily preparations and to preparations miscible or soluble in aqueous or oily solvents, provided that these solvents do not have an antimicrobial effect under the test conditions.

Use membrane filters with a nominal pore size no greater than 0.45 µm. 50 mm diameter is assumed. Cellulose nitrate filters, for example, are used for aqueous, oily, and weak alcoholic solutions, and cellulose acetate filters, for example, for strongly alcoholic solutions. Filters specially adapted for certain products may be required, e.g. for antibiotics.

Appropriate negative controls must always be run.

Aqueous solutions:
- Transfer a small amount of a suitable sterile diluent, (e.g. 1 g/l casein peptone neutral solution, pH 7.1 ± 0.2) to the membrane. The diluent may contain appropriate neutralising and / or inactivating substances.
- Transfer the product to be analysed to the membrane or membranes using not less than the quantities of the product to be examined as prescribed in Table 1.
- Filter immediately.
- If the product has antimicrobial properties, wash the membrane no less than 3 times by filtering it with the sterile diluent chosen in the method suitability test. Do not exceed a wash cycle of 5 times 100 ml per filter, even if it has been shown during the method suitability test that this cycle does not completely eliminate antimicrobial activity.
- Transfer the entire membrane to the culture medium or cut it aseptically into 2 equal parts and transfer half to each of the 2 appropriate media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium to the membrane in the apparatus. Incubate the media for no less than 14 days.

Soluble solids:
- Use for each medium not less than the amount prescribed in Table 1 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injections, saline or a neutral solution of 1 g/l of peptone of casein.
- Proceed with the test as described above for aqueous solutions using an appropriate membrane for the chosen solvent.

Oils and oily solutions:
- Use for each medium not less than the quantity of the product prescribed in Table 1.
- Oils and oil solutions of sufficiently low viscosity can be filtered without dilution through a dry membrane. Viscous oils can be diluted as needed with a suitable sterile diluent, such as isopropyl myristate that shows no antimicrobial activity under test conditions. Allow the oil to penetrate the membrane under its own weight and then filter, gradually applying pressure or suction.
- Wash the membrane at least 3 times by filtering about 100 ml of a sterile neutral solution such as 1 g/l casein peptone containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability test, e.g. polysorbate 80 at a concentration of 10 g/l.
- Transfer the membrane or membranes to the culture medium or medium or vice versa as described above for aqueous solutions and incubate at the same temperatures and for the same times.

Ointments and creams:
- Use for each medium not less than the quantities of the product prescribed in Table 1.
- Ointments in a greasy base and water-in-oil type emulsions can be diluted 1% in isopropyl myristate as described above, heating, if necessary, to no more than 40°C. In exceptional cases it can be necessary to heat to no more than 44°C.
- Filter as quickly as possible and proceed as described above for oils and oily solutions.

TABLE 1 - MINIMUM QUANTITY TO BE USED FOR EACH MEDIUM

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Liquids. - Less than 1 ml. - 1-40 ml. - Greater than 40 ml and not greater than 100 ml. - Greater than 100 ml. Antibiotic liquids.	The whole contents of each container. Half the contents of each container but not less than 1 ml. 20 ml. 10 per cent of the contents of the container but not less than 20 ml. 1ml.
Insoluble preparations, creams and ointments to be suspended or emulsified.	The whole contents of each container to provide not less than 200 mg.
Solids - Less than 50 mg. - 50 mg or more but less than 300 mg. - 300 mg to 5 g. - Greater than 5 g.	The whole contents of each container. Half the contents of each container but not less than 50 mg. 150 mg. 500 mg.
Catgut and other surgical sutures for veterinary use.	3 sections of a strand (each 30 cm long).

NOTE: When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of 2 or more containers are used to inoculate the different media.

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Direct inoculation of the culture medium

Transfer the quantity of sample to be examined according to Table 1 directly to the culture medium. The volume of the product cannot exceed 10% of the volume of the medium, unless otherwise indicated.

If the product to be examined has antimicrobial activity, neutralise with a suitable neutralising substance or by dilution in a sufficient amount of culture medium. When it is necessary to use a large volume of the product, it may be preferable to use a concentrated culture medium prepared in such a way that we take into account the subsequent dilution. In which case, the concentrated medium can be added directly to the product in its container.

Appropriate negative controls must always be run.

Oily liquids:
Use media with a suitable emulsifying agent at a suitable concentration according to the method suitability test (e.g. Polysorbate 80, 10 g/L).

Ointments and creams:
Dilute 1:10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent (1 g/L neutral solution of casein peptone) and transfer the diluted product to a medium that does not contain an emulsifying agent. Incubate for no less than 14 days and observe several times during the incubation period. Gently shake the cultures containing oily products every day. However, when a fluid thioglycollate medium is used for detection of anaerobic microorganisms, shake or mix to a minimum to maintain anaerobic conditions.

Catgut and other surgical sutures for veterinary use:
Analyse the quantities of the product prescribed in Table 1. Analyse 3 sections of the strand for each culture medium and run the test on 3 sections 30 cm long (from the beginning, the middle and the end of the strand). Analyse newly opened packages. Use enough medium to cover the material to be analysed (20 ml to 150 ml).

Observation and interpretation of results

Examine at intervals during incubation and at its conclusion, pair looks for evidence of microbial growth.
If the sample makes the medium cloudy, it will make visual detection of microbial growth difficult, at 14 days of incubation, transfer the portions of not less than 1 ml of culture to flasks with the same fresh medium and incubate for not less than 4 days.
If no evidence of microbial growth is found, the product under test meets the sterility test.
If evidence of microbial growth is found, the product is a test that does not meet the sterility test, except if the test is invalid. It is considered invalid if one or more of the following conditions are met:

a) Demonstrated failure of microbiological monitoring data from the sterility test facility.
b) Failure to review the test procedure used during the test.
c) Microbial growth is detected in negative controls.
d) After determination of the identity of the micro-organisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test.
If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility.
If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.

Minimum number of items to be tested - See Table 2

TABLE 2 - MINIMUM NUMBER OF ITEMS TO BE TESTED

Number of items in the batch*.	Minimum number of items to be tested for each medium, unless otherwise justified and authorised**.
Parenteral preparations. - Not more than 100 containers. - More than 100 but not more than 500 containers. - More than 500 containers.	10 per cent or 4 containers, whichever is the greater. 10 containers. 2 per cent or 20 containers (10 containers for large-volume parenteral preparations) whichever is less.
Ophthalmic and other non-injectable preparations. - Not more than 200 containers - More than 200 containers - If the product is presented in the form of single-dose containers, apply the scheme shown above for parenteral administration.	5 per cent or 2 containers, whichever is the greater. 10 containers.
Catgut and other surgical sutures for veterinary use.	2 per cent or 5 packages whichever is the greater, up to a maximum total of 20 packages.
Bulk solid products. - Up to 4 containers. - More than 4 containers but not more than 50 containers. - More than 50 containers.	Each container. 20 per cent or 4 containers, whichever is the greater. 2 per cent or 10 containers, whichever is the greater.

*** If the batch size is not known, use the maximum number of items prescribed.*
*** If the contents of one container are enough to inoculate the 2 media, this column gives the number of containers needed for both the media together.*