

Specification

Liquid medium for fungal isolation according to the Pharmacopeial Harmonized Methods.

Formula * in g/L

Casein peptone 5.0
Meat peptone 5.0
D(+)-Glucose 20.0

Final pH 5.6 ±0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30 g of powder in 1 L of distilled water, heating only if necessary. Dispense into suitable containers and sterilize in a preheated autoclave for 15 minutes at 121°C. Avoid overheating, since it may brown the glucose.

Description

This medium is especially adapted to the culture of fungi and acidophilic bacteria.

Sabouraud USP Broth is formulated according to the US Pharmacopoeia, US NF and the 21 CFR guidelines. In the latest editions of these methods Tryptone Soy Broth may be used for sterility checking in pharmaceutical products for injection. This formulation is similar to Antibiotic Medium No. 13 by Grove and Randall and the 21 CFR guideline.

This medium is not selective, but the low pH inhibits the growth of non acidophilic microorganisms. Special measures must be taken while reconstituting and heating the medium due to this strong acid reaction and the high content of glucose. It is important to preheat the autoclave and thereby reach the sterilization temperature as soon as possible otherwise the glucose becomes caramelized turning the medium dark and reducing its effectiveness.

Technique

This medium is recommended for use in many tests and assays, but for a long time has been the medium of choice for verifying the sterility of sterile pharmaceutical products.

The efficacy of the medium and absence of fungistatic products is verified by using *Candida albicans* positive control. A loop of a 1:1000 dilution of a fresh 24 hours culture is added to the control tube and incubated appropriately. Sterility testing must be carried out in a controlled and verified medium. To check fungistatic activity of a product, prepare a control culture inoculum as mentioned above and inoculate two series of tubes of Sabaroud Broth as follows:

- To one set of tubes add a specified amount of product to be tested. This is the test series.
- To another series add only the inoculum, and incubate both series of tubes simultaneously.
- Incubation of both series of tubes must be carried out at 22 °C for 10 days. After this period compare the results.

If the assay series has less growth than the control series, the product has fungistatic activity. If the growth is equal or more, then it has no fungistatic properties. For the quantitative assay of fungistatic activity, perform the assay with several series of different concentrations until a point of equal growth in both control and test series.

Quality control

Incubation temperature: 20-25°C

Incubation time: 48 h- 3 - 5 days

Inoculum: Practical range 50-100 CFU (productivity), according to Ph. Eur.

Microorganism	Growth	Remarks
<i>Aspergillus brasiliensis</i> ATCC® 16404	Good	5 days, black sporulation
<i>Candida albicans</i> ATCC® 10231	Good	-
<i>Saccharomyces cerevisiae</i> ATCC® 9763	Good	-

References

- AJELLO, L. (1957) Cultural Methods for Human Pathogenic Fungi J. Chron. Dis. 5:545-551.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC. USA.
- EUROPEAN PHARMACOPOEIA 8.0 (2014) 8th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- MARSHALL, R.T. (1992) Standard methods for the examination of dairy products. 16th ed. APHA. Washington. DC. USA.
- SABOURAUD, R. (1910) Les Teignes. Masson. Paris.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.
- USP 33 - NF 28 (2011) <81> Antibiotics - Microbial Assays. USP Corp. Inc. Rockville. MD. USA.

Storage

Keep tightly closed, away from light, in a dry place (4-30 °C).