COLD

Dehydrated Culture Media

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FILTERABLE TRYPTONE SOYA BROTH (TSB)
(SOYBEAN CASEIN DIGEST MEDIUM USP)

Code: CM1065

A gamma-irradiated, cold filterable Tryptone Soya Broth suitable for microbiological Media Fill Trials (MFT) for the pharmaceutical industry.

Typical Formula

	gm/litre
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	2.5
Glucose	2.5

pH 7.3 ± 0.2 @ 25°C

Download product literature on cold filterable Tryptone Soya Broth (192KB) in PDF format.

Directions
Cold Filterable TSB should be used at a working dilution of 30g in 1 litre of distilled water (3% w/v). The medium may be made at this concentration and then sterilised by filtration or by autoclaving at 121°C for 15 minutes.

Incubation of media fills is usually carried out for 14 days[6] at both 20-25°C and 30-35°C. Visual inspection of the units should be carried out on a daily or every second day basis. Micro-organisms from any contaminated units should be sub-cultured, purified and identified to species level. Refer to the appropriate regulatory body for full guidelines[2,3,4,5].

Description
Tryptone Soya Broth is highly nutritious, general purpose medium which can support the growth of a wide range of bacteria, yeasts and moulds when incubated under the appropriate conditions[1].

The formulation of Cold Filterable TSB conforms to that stated in the European Pharmacopoeia 4th Edition 2002[2], the British Pharmacopoeia 2003[3], the US Pharmacopoeia 27 NF22 2004[4] and the Japanese Pharmacopoeia XIV 2001[5]. Each component of this medium has been specially screened and selected to give a highly filterable solution.

Packs of Cold Filterable TSB have been given a sterilising dose of gamma-irradiation (minimum 25KGy) validated as a lethal dose for all yeasts, moulds and bacteria including bacterial spores and mycoplasmas.

Aqueous liquid products that are required to be sterile but cannot be terminally sterilised due to the heat-sensitive nature of one or more component, may be produced by filtering-sterilising the dissolved solution and maintaining sterility by filling and closing the product under aseptic conditions.

The purpose of MFT is to provide a measure of the likelihood of microbiological contamination occurring in a particular aseptic process. Cold Filterable TSB can be used as a substitute for filter-sterilised drug products and is processed in a manner identical to that in which the product would be processed i.e. filtering, filling and closing. The medium is then incubated and the number of contaminated units is scored versus those that are un-contaminated. A decision following pre-determined guidelines can be made based on the proportion of contaminated units and the identity of the micro-organisms recovered[6].

For solid presentations where a sterile end product is required, aseptic production processes can be monitored by adding medium to a suitable placebo. The placebo chosen should allow the aseptic

process to be simulated exactly and the pre-sterilised TSB is added downstream of the processing[6].

For laboratory use, Oxoid standard Tryptone Soya Broth (CM0129) may be more appropriate.

Technique
'Sterile for use' liquid drugs often contain heat-sensitive components which means that terminal sterilisation by autoclaving is not an option. Sterilisation by filtering (for soluble liquids) followed by filling under aseptic conditions is the method for preparation of these types of drug. The purpose of MFT is to provide a measure of the likelihood of microbiological contamination arising in a particular aseptic process.

Typically, the composition of a liquid injectable drug means that that a very large volume can be filtered before blocking of that filter occurs. Due to the biological nature of TSB, filters will block sooner which will mean that the medium will have to be heated or filters changed during a MFT. Oxoid pre-screen and select the raw materials that go into Cold Filterable TSB so that every batch of product will have a high V_{cap} value. V_{cap} is the theoretical maximum volumetric throughput for the filter under test. With this information the maximum filterable volume of TSB may be calculated before starting a MFT.

At Oxoid, a filter management system is used with a test filters to determine V_{cap} values for each batch of Cold Filterable TSB. The final filterable volume of TSB will depend on the membrane type, pore size and area of the process filter used. Each batch of Oxoid Cold Filterable TSB will have a minimum V_{cap} of 2,800 litres/m^2 for the three filter types tested (0.2µm pore size).

Typical V_{cap} values for Oxoid Cold filterable TSB:

Filter membrane	V_{cap} (litres/m^2)	V_{cap} (ml) 47mm disc (area 14cm^2)
Polyvinylidene fluoride (PVDF)	4,909	3,506
Polyethersulfone (PES)	6,700	4,786
Nylon (NR)	4,561	3,258

Vcap is the extrapolation to a "flow = zero" point; the time to this point may be very long. Therefore Vcap is good for comparative analysis but is not practical for MFT where time for a process is limited. A more useful value is V90 which is calculated as 68% of Vcap and is the point at which flow has decayed to 10% of the initial rate. Contact your filter manufacturer for guidance.

N.B.: Cold Filterable TSB should not be used to validate the suitability of the chosen filtration system for it's ability in providing a sterile drug product. The components of TSB will be quite different to those found in an aqueous drug formulation and validation for this purpose should be carried out on the drug preparation itself.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium in the dark at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Straw coloured solution

Quality control

Positive controls:
Expected results
Staphylococcus aureus ATCC® 6538* Turbid growth
Pseudomonas aeruginosa ATCC® 9027*
Turbid growth
Bacillus subtilis ATCC® 6633*
Flocculent/surface growth
Aspergillus brasiliensis ATCC® 16404* White mycelia, black spores or no spores
Candida albicans ATCC® 10231* Flocculent/surface growth
Negative control:

Uninoculated medium
No change

*** This organism is available as a Culti-Loop®**

Reference
1. Oxoid Manual 8th Edition, 1998, p2-208
2. European Pharmacopoeia 4th Edition 2002
3. British Pharmacopoeia 2003
4. US Pharmacopoeia 27 NF22 2004
5. Japanese Pharmacopoeia XIV 2001
6. Microbiological Media Fills Explained by Nigel Halls, 2002. Sue Horwood Publishing Ltd., UK.