TSB 3P™ Animal Peptones
Ref. 51101 / 51102

TSB 3P™ Vegetable peptones
Ref. 51103 / 51104

Mycoplasma validation

Mycoplasmas, a potential risk in aseptic filling manufacturing

The bacteria of the genus Mycoplasma (trivial name: mycoplasmas) are characterized by a lack of cell wall. There are over 100 recognized species of the genus Mycoplasma, they belong to the Mollicutes bacterial class. Mollicutes are parasites of animals (including humans) and plants. Several species are known to be pathogenic to humans.
Mycoplasma species are often found in laboratories as contaminants in cell culture due to contamination from contaminated culture medium ingredients. Mycoplasma cells are physically small – less than 1 µm – and can pass through 0.2µ filters because of the absence of cell wall that provide bending properties. Cultivation is difficult due to the complex growth requirements and the growth still remains slow (more than 14 days).
Mycoplasmas can represent a potential risk of contamination for an aseptic line during a Media Fill Test (MFT).

Absence of mycoplasma in culture media

bioMérieux TSB 3P dehydrated culture media were controlled for the absence of Mycoplasma in the final product, and the irradiation cycle showed an efficiency of 10^8 reduction of mycoplasmas following an artificial contamination study. Two methods were used to detect mycoplasmas in the media*:

❶ Control before irradiation

The method used to detect mycoplasmas in the media dehydrated powder was an indirect culture growth method with indicator cells followed by a PCR-based method with a genetic amplification.
The protocol was performed following this culture chronology: Day 0 Vero Cells growth, Day 2 Co-incubation of sample, positive control and negative control with Vero cells, Day 3 Vero cells subculture, Day 6 Vero cells subculture on slides; qPCR test, Day 7 Hoecht DNA staining and epifluorescence reading.
Three (3) grams of dehydrated culture medium were mixed with 100 ml of de-ionized water, the solution was filtered on 0.45 µm and centrifuged. The sample (1 ml) was co-incubated with indicatives Vero cells at 37°C ± 2°C and in a 5% ± 1% CO2 atmosphere. *Mycoplasma hyorhinis* was introduced in a Vero cells flask as positive control and a NaCl 0.9% solution aliquot was also incubated with Vero cells as negative control. Vero cells were removed from their 25cm² flask with trypsin at day 6, then DNA was extracted. PCR tests were performed with Q|Aamp DNA minikit. The mycoplasmas detection was realized with generic primers.

Results

No mycoplasma was found in the different dehydrated raw materials

❷ Control after irradiation / Spiking method

The “over-kill” effect of irradiation for mycoplasmas was validated by checking the absence of mycoplasmas after irradiation at 25kGy (lowest irradiation dose).
This study was performed on the basis of European Pharmacopoeia and 21 CFR 610.30 recommendations for direct culture of mycoplasmas.
Twelve biological indicators (6 mycoplasmas, 2 lyophilised vials per strains) were used for the spiking protocol. One BI of each strain was placed inside both dehydrated culture media and subjected to the gamma irradiation cycle. Six non irradiated mycoplasma strains were used as positive growth control and an inoculum of NaCl 0.9% as a negative growth control. After irradiation, each lyophilisate was rehydrated with 1ml of suitable culture medium. Serial dilutions were realized. An aliquot of 200µl of these suspensions was inoculated on 3 Petri dishes (solid medium for mycoplasma, Sigma). Plates were incubated in aerobic, microaerophilic or anaerobic conditions according to the researched mycoplasma.
Numerations were performed between 7^{th} and 21^{th} day.

Results

The irradiation is mycoplasmacidal

No viable mycoplasma found after irradiation
Logarithmic destruction up to 8 Log

** All results of the reports can be available upon request*
Mycoplasmas researched by the external laboratory: Acholeplasma laidlawii ATCC 23206, Mycoplasma gallisepticum ATCC 19610, Mycoplasma hyorhinis ATCC 17981, Mycoplasma orale ATCC 23714, Mycoplasma pneumoniae ATCC 15531, Mycoplasma synoviae ATCC 25204

BIOMÉRIEUX INDUSTRY