TECHNICAL LEAFLET
MEDI-MEDIA-FILL KIT
(Code: MR-25)

ASEPTIC PROCEDURE SIMULATION TEST WITH PERSONNEL AND ENVIRONMENTAL MICROBIOLOGICAL MONITORING

KIT COMPONENTS:

- TSBSolution 75 ml: 3 vials
- Sterile Test Vial 50 ml: 6 vials
- Sterile Vacuum Test Vial 8 ml: 6 vials
- Test Agar Plate: 5 plates
- Cleaning Swab: 6 pieces
- Label for simulation test: 12 pieces
- Data Log Sheet: 1 piece
- Technical Leaflet: 1 piece

Additional reagents, disposables, supplies and equipments needed to be supplied by the user:
- Needle (18-gauge recommended to Protocol or other size chosen by the user)
- Syringe (10 ml recommended to Protocol or other volume size chosen by the user)
- Disinfectant solution e.g. 70 % (v/v) alcohol / isopropanol solution
- Incubator cabinet or incubation room with controlled temperatures from +15 °C up to +40 °C
- Laminar airflow workbench or biological safety cabinet or barrier isolator conformity to the specification of ISO Class 5 with antearaes up to ISO Class 8

KIT APPLICATION AREAS

The MEDI-MEDIA-FILL Kit can be applied for the validation of aseptic techniques and procedures of radioactive and non-radioactive compounding for medical use, (re)qualification of personnel, who (will) perform the procedure, and for the routine compounding, and simultaneously the qualification of some operational conditions of equipments during aseptic simulation test. The kit provides a complex tool for testing the personnel competency and proficiency during an aseptic procedure at low or medium risk combining with basic monitoring of environmental and personnel hygiene status.

The kit is recommended for routine use in the monitoring of aseptic procedures used in Compounding Sterile Preparations (CSPs). Each kit contains materials designed for one operator to perform the regular low and medium-risk aseptic medium challenge testing as specified in USP Chapter <797> for the environmental and personnel hygiene monitoring during the procedure (1,2,3).

CSPs include compounded biologics, diagnostics, drugs, nutrients and radiopharmaceuticals that must be sterile, non-pyrogen or contain acceptable level of bacterial endotoxin contamination. The bacterial endotoxin limit is product specific (see in Monographs for compounds, products in pharmacopoeias).

Each kit includes sterile components (TSB and vials) with low endotoxin content that provides an optimal and necessary tool for testing sterility and bacterial endotoxin contents of the product prepared in the aseptic process simulation test.

The low and medium risk levels for aseptic processes are defined and detailed in USP Chapter <747>. At low risk level the compounding procedure involves only a few closed system, not more than three sterile components, basic and simple aseptic transfers and manipulations. Most of radiopharmaceuticals belong to low risk CSPs. Medium risk level compounding involves complex aseptic manipulation, multiple pooled sterile product for many patients or used with multiple injection time, and the procedure takes over a prolonged period of time because of its complexity.

The MEDI-MEDIA-FILL kit can not be applied for high risk level CSPs.

MATERIALS

Tryptic Soy Broth (TSB) presented in the kit is a general and widely used medium for the cultivation of microorganisms from environmental sources. It supports the growth of the majority of bacteria and fungi, and therefore, one of the recommended medium used for Media Fill tests. Tryptic Soy Broth conforms to the formula given by the European Pharmacopoeia and USP.

Composition of TSB Solution

Ingredients per liter for injection:
- Tryptic Soy Broth: 50 g
- Glucose: 10 g
- Bovine serum: 1 ml
- Phosphate buffer solution: 10 ml

Clear, light amber solution

PH 7.3 ± 0.2 at 25 °C

Sterile

Bacterial endotoxin content is indicated on the Quality Control Certificate.

Test Agar Plate

RODAC (Replication Organism Detection and Counting) Petri dishes are applied in contact plate form for the detection and enumeration of microorganisms present on surfaces to monitor the cleanliness of the environment and the operators. RODAC plates contain Trypticase Soy Agar supplemented with neutralizers, lectine and polysorbate 80, which can detect live microorganisms. These plates are suitable for collecting microbial samples from various surfaces and evaluating the microbial contamination.

The grid on the plate bottom makes the counting of microbial growing colonies easy for the analyst. Counts of CFU (colony forming unit) is usually used as an approximate measure of the microbial bioburden.

Sterile Test Vial and Sterile Vacuum Test Vial

Colourless Ph.Eur. type I, glass vials 50H (50 ml) and 8R (8 ml) are closed with sterile rubber stopper and sterile plastic-aluminum caps with turned up edge. The empty vials in the kit are sterile, and have low bacterial endotoxin content. The 8 ml vials are vacuum vials.

PERSONNEL AND WORKING CONDITIONS

The competent operators (e.g. technicians, nurses, pharmacists) participating in CSPs processes must be (re)assessed on at least an annual basis after extensive and/or regular training.

Similarly to the aseptic CSPs processing, the aseptic procedure simulation test should be performed in an ISO Class 5 (equivalent to Grade A and Grade B or Class 100) air quality environment (e.g. laminar airflow workbenches or biological safety cabinets or barrier isolator).

At the beginning, meantime and at the end of the aseptic procedure simulation test sampling with RODAC agar plate should be done from critical surfaces of the instruments and the operator. Finger prints from at least one hand covered with sterile gloves and the sterile garb are considered as critical sampling sites to monitor personal hygiene. Cleanliness of the critical areas under the laminar flow or isolator should be checked by contact plate during the procedure. Critical sampling sites are the frequently touched sites, e.g. shielded and unshielded areas during the preparations and manipulations of radiopharmacuticals.

PROTOCOL

The procedure described below that complies with the routine compounding of medium risk level CSPs according to USP <797>, may be modified by the user.

1. ASEPTIC PROCEDURE SIMULATION TEST

a) Label the vials before the test performance.
b) Sterilize the vials with sterile alcohol / isopropanol solution (70 % v/v) before place into the sanitized working area (e.g. laminar airflow workbenches or barrier isolator).
c) Divide the six empty 50 ml vials into three pairs and tear off the cap and wipe the rubber closures with cleansing swab before the first injection.
d) Transfer 5 ml sterile TSB solution into each of 50 ml sterile empty vials with 5 ml aliquots using 10 ml syringe and 18-gauge needle. It will be six 50 ml vials filled with 25 ml TSB solution, in each.
e) Transfer 5 ml aliquot of TSB solution from the first vial into the second vial within the pair.
f) Shake the second vial for 10 seconds and then aseptically remove a 5 ml aliquot and return into the first vial in the pair.
g) Shake the first vial for 10 seconds and then aseptically remove a 5 ml aliquot from the first vial into the second vial.
h) After the media solution exchange, perform step f) to g) three times within the pair.
i) Remove a 5 ml aliquot from each container and transfer into empty 8 ml test vials.
j) Repeat step e) to h) for the other two remaining pairs.
k) Cover the vials with sterile adhesive seal, wrap and transport immediately to the incubation facility for sterility testing (see section 2).
l) To document the procedure simulation test use the Data Log Sheet of MEDI-MEDIA-FILL Kit enclosed to this Technical Leaflet.

2. MICROBIOLOGICAL EVALUATION (STERILITY TEST)

a) Incubate the test vials at 20 °C - 25 °C and/or 30 °C - 35 °C for 14 days in an incubator or a temperature-controlled room. If two temperatures are chosen for incubation, incubate for 7 days at each temperature.
b) Examine the medium for turbidity or cloudiness in the test vials meantime and at the end of the incubation. Mix the vials before checking turbidity.
c) The result must be negative or positive for microbial contamination. If there is no visible turbidity or cloudiness in the test vial, the result is negative, there is no microbial growth indicating a sterile preparation.

d) Record and evaluate in „No Growth“ and „Pass“ columns on Data Log Sheet.

If the TSB solution in a test vial is turbid or opaque or cloudy, this test vial must be contaminated with microbes during the procedure, and considered as positive, there is microbial growth indicating a non-sterile preparation.

d) Record and evaluate in „Growth“ and „Fail“ columns on Data Log Sheet.

d) Bacterial endotoxin contamination in test vials can be examined after the sterility test evaluation. Bacterial endotoxin content must not exceed the value specified for TSB solution (see in Quality Control Certificate of Medi-Media-Fill Kit).

Note: Each test vial must be negative in sterility test if the procedure conducted properly.
This microbiological monitoring test is connecting to Section 1 of Protocol.

a. **Sanitize the package foil of RODAC agar plates before put into the working area.**

b. **Open the lid of the plates and touch slightly the agar gel into the selected surfaces, then close, wrap and transfer them to the incubation facility immediately after the sampling.**

c. **Incubate the contact plates at +30 – +35 °C and 48 hours then +20 – +25 °C for 48-72 hours in an incubator.**

d. Count the separate colonies visible by naked eyes on the agar surface at the end of the incubation time. CFU (Colony Forming Unit) is the number of colonies.

e. **Record CFU values on Data Log Sheet.**

f. **Interpret the results based on the recommended specifications shown in Table 1.**

The values for CFU not exceeding the limit value indicate appropriate environmental and/or personnel hygiene status.

- **Record and evaluate in „Fail” column on Data Log Sheet.**

The values for CFU not exceeding the limit value indicate appropriate environmental and/or personnel hygiene status.

- **Record and evaluate in „Pass” column on Data Log Sheet.**

Overall interpretation of results:

If each test vial passes the sterility test and the bioburden in each hygiene monitoring sample is below the limit value, the aseptic procedure monitoring does not pass, the simulation test is unsuccessful.

**QUALITY CONTROL**

**MEDI-MEDIA-FILL kit** manufactured and certified in compliance with GMP [3].

The empty vials and TSB filled vials are tested for sterility and bacterial endotoxin contamination according to European Pharmacopoeia and USP. Before the kit release, each batch of TSB solution is examined in growth promotion test to verify the suitability of the fluid medium. Each lot of RODAC agar plates in the kit possess Certificate of Analysis. The following microorganisms strains are used for quality control of TSB solution in growth promotion test:

- **Staphylococcus aureus** ATCC 6538
- **Pseudomonas aeruginosa** ATCC 9027
- **Bacillus subtilis** ATCC 6633
- **Candida albicans** ATCC 10231
- **Aspergillus brasiliensis** ATCC 16404

The bacterial endotoxin content is determined by the kinetic turbidimetric method according to European Pharmacopoeia and USP.

**Table 1** Recommended Limits for Viable Particle in Aseptic Areas

<table>
<thead>
<tr>
<th>Classification (Grade or Class)</th>
<th>Contact plates CFU/plate</th>
<th>Gloveprint CFU/5 fingers</th>
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<tr>
<td><strong>A</strong> ISO Class 5 (Laminar air flow)</td>
<td>Class 100</td>
<td>&lt; 1</td>
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The CFU values shown in Table 1 are for guidance only.

**References:**

1. **European Pharmacopoeia 7th Edition published by EDGM** (European Directorate for the Quality of Medicines & HealthCare) in July 2010

   Chapter 2.6.1 Sterility; Chapter 2.6.12. Microbiological Examination of Non-sterile Products: Total Viable Aerobic Count; Chapter 2.6.14. Bacterial Endotoxins

   Website: [http://www.edgm.eu](http://www.edgm.eu)


   Chapter <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests; Chapter <71> Sterility Tests; Chapter <85> Bacterial Endotoxins Test; Chapter <97> Pharmaceutical Compounding-Sterile Preparations

   Website: [http://www.uspnf.com](http://www.uspnf.com)


   Website: [http://www.iaea.org/](http://www.iaea.org/)


**TECHNICAL SUPPORT AND ORDERING**

The kit was developed and is manufactured by [MEDI-RADIOPHARMA Co., Ltd., H-23-521, Erd, Szamos u., 10-12., Hungary](http://order@mediradiopharma.hu) or to the above phone/fax numbers.

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**Classified according to ISO Guides (ISO-14644-1, ISO-14698-2):**

- **Germ Class 1** (Swiss class 100000, US class 4)
- **Germ Class 2** (Swiss class 10000, US class 3)
- **Germ Class 3** (Swiss class 1000, US class 2)
- **Germ Class 4** (Swiss class 100, US class 1)
- **Germ Class 5** (Swiss class 10, US class 0)
- **Germ Class 6** (Swiss class 1, US class 0)
- **Germ Class 7** (Swiss class 1, US class 0)

For further advice please contact the manufacturer via E-mail at: [order@mediradiopharma.hu](mailto:order@mediradiopharma.hu)

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