

## LEGIONELLA AB SELECTIVE SUPPLEMENT

### Freeze-dried selective supplement

#### 1 - INTENDED USE

Mixture of antimicrobials to be used with Legionella BCYE Agar Base and a growth supplement for the enumeration of *Legionella* spp. in water samples, according to ISO 11731.

#### 2 - COMPOSITIONS - (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cefazolin	4.5 mg
Polymyxin B	40,000 UI
Pimaricin (natamycin)	35 mg

#### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionella AB Selective Supplement is a lyophilised mixture of antimicrobial compounds for use as an additive to BCYE Agar Base (REF 401582) supplemented with Legionella BCYE  $\alpha$  Growth Supplement (REF 423210), for the isolation and enumeration of *Legionella* spp. in water samples, according to ISO 11731.<sup>1</sup>

Polymyxin B is an inhibitor of Gram-negative bacteria, cefazolin is active against Gram-positive and some Gram-negative bacteria and pimaricin (natamycin) is used as an antimycotic agent.

#### 4- DIRECTIONS FOR MEDIA PREPARATION

Reconstitute aseptically the contents of one vial of Legionella AB Selective Supplement with 5 mL of sterile purified water. Add to 450 mL of Legionella BCYE Agar Base (REF 401582) autoclaved at 121 ° C for 15 minutes and cooled to 47-50 ° C with aseptic precautions. Also add the contents of a vial of Legionella BCYE  $\alpha$ -Growth Supplement (code 423210) reconstituted with 50 mL of sterile purified water. Mix well and distribute in sterile Petri dishes.

#### 5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	high size, white pastille
Aspect of the solution	whitish, cloudy

#### 6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Legionella AB Selective Supplement	Freeze dried supplement	423225	10 vials, each for 500 mL of medium

#### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Legionella BCYE Agar Base (REF 401582), Legionella BCYE  $\alpha$ -Growth Supplement (code 423210), autoclave, incubator and laboratory equipment as required, autoclavable flasks, sterile loops and swabs, reagents for the sample treatment.

#### 8 - SPECIMENS

The complete medium is intended for the enumeration of *Legionella* in different types of water: drinking, natural, industrial, wastewater and in water-related samples (for example biofilm, sediments, etc.). Consult the Standard ISO 11731 for sampling methods and sample handling procedures.<sup>1</sup> Apply good laboratory practices for specimen collection, transport and storage.

#### 9 - TEST PROCEDURE

Keep the plates to room temperature and allow the surface of the medium to dry.

The work procedures described in the ISO 11731 Standard differ in relation to the origin of the sample, its characteristics, the purposes of the research and in relation to the expected concentrations of the target microorganism and the contaminating flora.

Schematically, the different possibilities of treatment and inoculation of the samples involving BCYE-AB medium are summarized below.<sup>1</sup>

- For samples with a high number of Legionellae and a low number of contaminants: direct inoculation of the sample on a non-selective BCYE w/L-cysteine medium<sup>^</sup> and on a selective BCYE-AB\* medium plate.
- For samples with a low number of legionellae and a low number of contaminants: membrane filtration and positioning of the untreated filter on a BCYE w/ L-cysteine non-selective medium plate<sup>^</sup>, positioning of the filter(s) treated with acids on one or more selective or highly selective medium plates (BCYE-AB\* or BCYE-GVPC\*\* or BCYE-MWY\*\*\*); wash the untreated and acid or heat treated membrane and inoculate 0.1-0.5 mL on a non-selective medium plate and on plates of one or more selective and highly selective media (BCYE-AB\* or BCYE-GVPC\*\* or BCYE-MWY\*\*\*).

Allow the inoculum to absorb well then incubate the inverted plates in a humid atmosphere at 36 ± 2°C for 7-10 days

The procedural elements reported above are entirely schematic. For details of *Legionella* enumeration techniques in water, refer to the ISO 11731 Standard<sup>1</sup> or other applicable guidelines.

LABORATORY-PREPARED PLATES OR BIOLIFE READY-TO-USE PLATES: <sup>^</sup> 549945 LEGIONELLA AGAR (BCYE); \*549947 LEGIONELLA AB SELECTIVE AGAR; \*\*549995 or 499995 LEGIONELLA SELECTIVE AGAR-GVPC \*\*\* 549948 LEGIONELLA SELECTIVE AGAR MWY-ISO

#### 10 - READING AND INTERPRETATION

##### Examination of the plates

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristic of the colonies.

Inspect the plates for the first time either on day 2, 3, 4 or 5 followed by a final inspection at the end of the incubation period.

*Legionella* colonies, in principle, appear white-grey, with entire, shiny edges, rounded with a diameter of 1 to 4 mm. Generally, and especially in the first 2 days of incubation, the edge shows a pink or blue-green iridescence while the centre is opalescent grey with an appearance similar to ground glass. Observed under UV lamp (366 nm), some species (*L. anisa*, *L. bozemanii*, *L. cherrii*, *L. dumoffii*, *L. gormanii*, *L. gratiana*, *L. parisiensis*, *L. steigerwaltii* and *L. tucsonensis*) show a blue-white auto-fluorescence, others (*L. erythra* and *L. rubrilucens*) a bright red auto-fluorescence. *L. pneumophila* colonies appear green, opaque, often tinged with yellow. The colour of the fluorescence can help differentiate colonies in samples containing different *Legionella* species.

With the prolongation of the incubation time, the colonies become wider, the centre assumes a creamy white colour and lose much of their iridescence. A common feature of *Legionella* colonies is the difficulty in taking them with the loop from the surface of the agar.





For the details of *Legionella* spp. enumeration in water samples consult the ISO 11731.<sup>1</sup>

#### Confirmation of the colonies

Regard as *Legionella* those colonies which grow on the plate of BCYE w/cysteine but fail to grow on the plate of BCYE w/o cysteine.

Presumptive identification should be completed by Gram staining prepared from cysteine containing agar only: *Legionella* cells are Gram-negative poorly/faintly staining thin rods, which may be filamentous in older cultures.<sup>1</sup>

#### 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform his own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
<i>L. pneumophila</i>	ATCC 33152	35-37 °C / 44-48 H / A	growth
<i>L. anisa</i>	ATCC 35292	35-37 °C / 3-5 days / A	growth
<i>E. coli</i>	ATCC 25922	35-37 °C / 3 days / A	partially inhibited
<i>E. faecalis</i>	ATCC 319433	35-37 °C / 3 days / A	totally inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

#### 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of Legionella AB Selective Supplement (Test Batch-TB), is tested for productivity and selectivity, by comparing the results with the non-selective BCYE Agar (Reference Batch-RB).

Productivity is tested by a quantitative method, with the following strains: *L. pneumophila* ATCC 33152, *L. anisa* ATCC 35292. Test Batch and Reference Batch are inoculated with decimal dilutions in water of the colonies' suspensions and incubated at 35-37°C for 44-48 hours (*L. pneumophila*) and 3-5 days (*L. anisa*). The colonies are enumerated on both batches and the productivity ratio ( $Pr = \text{CFU}_{\text{TB}} / \text{CFU}_{\text{RB}}$ ) is calculated. If  $Pr$  is  $\geq 0,5$  and if the colonies morphology is typical, the results are considered acceptable and conform to the specifications. Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 18804. After incubation at 35-37°C for 72 hours the growth of non-target strain is observed and recorded: *E. faecalis*, *E. coli* and *C. albicans* are totally inhibited, while *S. aureus* and *P. aeruginosa* are partially inhibited.

#### 13 - LIMITATIONS OF THE METHOD

- Colonies of *Legionella* grown on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
- Do not incubate the medium with CO<sub>2</sub> concentrations higher than 2.5% due to the possibility that *L. pneumophila* growth may be inhibited.
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non-selective and selective media is strongly recommended.<sup>2</sup>
- The plates with characteristic growth and with colonies presumptively identified as *Legionella*, must undergo confirmation tests with biochemical, immunological, molecular or mass spectrometry techniques.

#### 14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- AB Supplement is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- AB Supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- When handling *Legionella* spp., it is important to avoid aerosol formation. Thoroughly clean and disinfect all working areas.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use AB Supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

#### 15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes) and the applied storage conditions (temperature and packaging). According ISO 11731<sup>1</sup> the prepared plates with AB supplement may be stored at  $5 \pm 3$  °C in airtight containers in the dark for up to 3 months.
















### 16 – REFERENCES

1. ISO 11731:2017 Water quality — Enumeration of Legionella
2. Kusnetsov JM, Jousimies-Somer HR, Nevalainen AI, Martikainen PJ. Isolation of Legionella from water samples using various culture methods. J Appl Bacteriol. 1994 76(2):155-62.

### TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Use by	 Fragile, handle with care	 Manufacturer
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Store away from direct light	 This side up

### REVISION HISTORY

Version	Description of changes	Date
Revision 0	First issue	2024/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

