

Instructions for Use

Bacitracin Selective Supplement

Freeze-Dried Supplement

REF - LS0012

1. Intended Use

For *in vitro* diagnostic use. LS0012 Bacitracin Selective Supplement is an antibiotic supplement used to enhance the selective isolation of *Haemophilus* species.

2. Composition* For one litre of prepared media

<u>Ingredient</u>	<u>mg/L</u>
Bacitracin	120.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Bacitracin Selective Supplement is formulated to be used with E&O KM0001 Columbia Agar Base to form Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L).

Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L) is based on the original Columbia Agar formulation described by Ellner *et al.* from Columbia University ⁽¹⁾. *Haemophilus influenzae* is a common cause of infections of the upper respiratory tract. Although culture remains the primary means of microbiological isolation and identification of *Haemophilus* spp., it may be hindered by overgrowth with commensal microbial flora. Finland and Wilcox investigated the action of antibiotics on strains of *H. influenzae* and found all strains tested to be resistant to 750µg/ml of bacitracin ⁽²⁾. Hovig and Aandahl used chocolate agar with 300µg/ml of bacitracin and reported improved recovery of *Haemophilus* spp. when compared to isolation on sheep blood agar with a streak of *Staphylococcus aureus* ⁽³⁾.

Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L) made with LS0012 Bacitracin Selective Supplement is recommended as a selective primary isolation media by the UK Standards for Microbiology Investigations ⁽⁴⁾ and tested in accordance with the principals of ISO 11133:2014 ⁽⁵⁾.

4. Principle

Bacitracin inhibits a broad spectrum of non-target microorganisms and facilitates isolation of *Haemophilus* spp. when added to Chocolate agar.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water:

Format	Volume of sterile deionised water to add
V001	5ml

Close the vial and gently agitate to assist reconstitution – avoid frothing. The resulting solution will be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium, KM0001 Columbia Agar Base prepared as Chocolate Agar according to the instructions provided and allowed to cool to 45°C- 50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	White Solid Pellet	Colourless liquid

7. Materials Provided

LS0012 Bacitracin Selective Supplement is supplied in boxes of 10 vials (lyophilised). Each vial is labelled with product name, product code, lot number and expiry date.

Product code	Product format
LS0012-V001-500	5ml neutral clear crimped vial suitable for 500ml of media

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms, sterile media (e.g., Chocolate agar prepared from Columbia Agar Base (E&O KM0001)).

9. Specimens

LS0012 Bacitracin Selective Supplement can be used with the following specimens when added to KM0001 Columbia Agar Base and Chocolate Horse Blood to form Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L):

- Clinical Specimens: Samples of blood, sputum or spinal fluid are most used to test for *Haemophilus* spp.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Clinical Specimens ⁽⁴⁾:

Based on sample type and information provided, determine whether the clinical specimen requires pre-enrichment prior to inoculation to Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L). Chocolate Agar with 7% Horse Blood and Bacitracin (120mg/L) may be used for direct inoculation with the sample or subculture after incubation in enrichment broth.

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 37 ± 1°C in 5% CO₂ for 18-24 hours.

After incubation, examine the agar for colonies (typical colony appearance is outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>H. influenzae</i> (NCTC 12699)	37 ± 1°C in 5% CO ₂ for 18-24 hours	Growth: Grey colonies
<i>S. pneumoniae</i> (NCTC 12977)	37 ± 1°C in 5% CO ₂ for 18-24 hours	Inhibited
<i>S. pyogenes</i> (NCTC 12696)	37 ± 1°C in 5% CO ₂ for 18-24 hours	Inhibited
<i>N. gonorrhoeae</i> (NCTC 8375)	37 ± 1°C in 5% CO ₂ for 18-24 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium produced, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify the performance of LS0012 Bacitracin Selective Supplement, supplement samples were added to sterilised and cooled Chocolate Adar prepared from Columbia Agar Base (E&O KM0001) and defibrinated horse blood, and resulting plates tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at 37 ± 1°C in 5% CO₂ for 18-24 hours. All samples grew and showed good recovery and the correct morphology of the required test organism: *Haemophilus influenzae* (NCTC 12699). Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10⁴ - ≤1.5x10⁵ cfu of the required non-target test organisms: *Streptococcus pneumoniae* (NCTC 12977), *Streptococcus pyogenes* (NCTC 12696) and *Neisseria gonorrhoeae* (NCTC 8375), which were inhibited and gave a selectivity factor of ≥ 3. Therefore, it can be concluded that LS0012 Bacitracin Selective Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Media

- Due to natural variation, some strains may grow poorly or fail to grow on the final medium.
- Plates of the final selective medium should not be overdried before inoculation.
- Perform further biochemical, serological, molecular, or mass spectroscopy (MALDI-TOF) testing to confirm identity of presumptive positive isolates. Refer to standard method or relevant local guidelines.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to LS0012 Bacitracin Selective Supplement Safety Data Sheet prior to use.

Do not use if supplement is not specified colour, has been stored inappropriately or the packaging has been damaged. Once reconstituted, any unused supplement should be discarded.

15. Storage conditions and Shelf life

LS0012 Bacitracin Selective Supplement must be stored at 2°-8°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements.

16. References

1. Ellner, P., Stoessel, C., Drakeford, E. and Vasi, F. (1966) A New Culture Medium for Medical Bacteriology. Am J Clin Pathol,, 45(4): 502-504.
2. Finland, M. and Wilcox, C. (1950) In vitro susceptibility of Hemophilus influenzae to seven antibiotics: (penicillin, streptomycin, bacitracin, polymyxin, aerosporin, aureomycin and chloromycetin). Am. J. Clin. Pathol., 20(4): 335-340.
3. Hovig, B. and Aandahl, E. (1969) A selective method for the isolation of Haemophilus in material from the respiratory tract. Acta Pathol Microbiol Scand, 77(4): 676-684.
4. UK Standards for Microbiology Investigations (2021). Identification of Haemophilus species and the HACEK group of organisms. ID 12, Issue 4.
5. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. Geneva, ISO.

Version History*

- 001 18/02/2014 - New document.
- 002 29/12/2015 - Update document with new format & place PDF on system.
- 003 02/08/2016 - Update with flow diagram.
- 004 04/12/2023 - Updated to new format and standard
- 005 13/10/2025 - Update to Section 4 to include reconstitution volumes. Updated *H. influenzae* strain in line with updates Quality Control Record.

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













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TABLE OF APPLICABLE SYMBOLS

IFU/LS0012 REV. 005

 REF Catalogue number	 LOT Batch code	 IVD In vitro Diagnostic Medical Device	 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place