EO Labs
Microbiological Culture Media

UK CA IVD CE

Instructions for Use

REF - LS0001 **LCAT Selective Supplement**

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0001 LCAT Selective Supplement is an antibiotic supplement used to enhance the selective isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

2. Composition*

For one litre of prepared media

Ingredient	mg/L
Colistin	7.5
Lincomycin	3.0
Amphotericin B	2.0
Trimethoprim	5.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS0001 LCAT Selective Supplement is formulated to be used with E&O KM0016 GC Agar, BM0478 Suplex Solution, and 7% Laked Horse Blood to form GC Selective Agar with LCAT.

GC Selective Agar with LCAT is based on the formulation described by Thayer and Martin [1] that was modified from the original formulation development by Johnson [2].

Neisseria species are fastidious, Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. *Neisseria spp.* are facultatively intracellular and typically appear in pairs (diplococci), they are non-spore forming and require reduced oxygen to grow. In humans, *N. gonorrhoeae* is the causative agent of gonorrhoea and *N. meningitidis* is one cause of bacterial meningitis.

The probability of success in the isolation and identification of pathogenic *Neisseria* spp. from clinical specimens is related to several factors:

- Time and care taken in obtaining specimens.
- Transport of the specimen to the laboratory and inoculation onto the chosen culture medium.
- The culture medium chosen must be capable of growing a small inoculum of demanding strains.
- Selective agents present in the medium to prevent overgrowth of commensal organisms must not inhibit the growth of *Neisseria* species or interfere with confirmatory tests to identify the isolated species.

It is a wise precaution to inoculate GC media with and without antibiotics in parallel because some strains of *Neisseria* species may fail to grow in the presence of antibiotics, especially vancomycin [3].

Diagnosis of gonorrhoea is achieved by microscopic examination and cultivation of material from infected sites. GC Selective Agar with LCAT is recommended as a selective agar by the UK Standards for Microbiology Investigations [4, 5] for a variety of clinical specimens and falls under the category of selective media for pathogenic *Neisseria spp*. according to the Clinical and Laboratory Standards Institute [6]. Presumptive identification should be confirmed by biochemical, serological, molecular, or mass spectrometry methods [4].

4. Principle

Lincomycin and colistin inhibit the growth of most non-target bacteria, trimethoprim is added to inhibit *Proteus* spp. and amphotericin B to prevent the growth of yeasts and fungi [7].

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 should be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium, KM0016 GC Agar, BM0478 Suplex Solution, and 7% DHB Defibrinated Horse Blood prepared according to the instructions provided and allow to cool to 45-50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	Yellow Solid Pellet	Yellow liquid

7. Materials Provided

LS0001 LCAT Selective Supplement is supplied 10 vials per box (lyophilised). Each bottle is labelled with (abbreviated) product name, product code, lot number, expiry date and as being suitable for addition to the appropriate volume of prepared media. The vials will have freeze dried neutral rubber stoppers and flip off seal caps.

Product Code	Product Format
LS0001-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, a sterile medium (e.g., GC Agar made up from KM0016 with other specified supplements).

9. Specimens

LS0001 LCAT Selective Supplement can be used with the following specimens when added to E&O KM0016 GC Agar with the other supplements to make GC Selective Agar with LCAT:

- Clinical Specimens: Swabs from the infected areas, biopsy material, pus, spinal fluid, and blood.

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 made to come to room temperature.

Follow local, national, or international guidelines.

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Humidity is essential for the successful isolation of gonococci. If the prepared plates look dry, moisten the surface with a few drops of sterile broth and allow it to soak into the agar before inoculation. Do not flood the plate with broth.

Clinical Specimens [3]:

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 36 ± 1°C in 5% CO_2 (with high humidity) for 40-48 hours.

After incubation, it is recommended that the following criteria are used for the presumptive identification of *Neisseria spp*.:

- The colonies isolated must have characteristic colour, size and appearance when examined (typical colony appearance outlined in Quality Control table below).
- The organisms should show characteristic Gram morphology.
- The organisms should exhibit positive oxidase results.

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)
	GC Selective Agar with LCAT	
N. gonorrhoeae (NCTC 8375)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Growth: Grey colonies
N. gonorrhoeae (NCTC 12700)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Growth: Grey colonies
P. mirabilis (NCTC 10975)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Inhibited
C. albicans (NCPF 3179)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Inhibited
S. epidermidis (NCTC 13360)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Inhibited
E. coli (ATCC 25922)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 hours	Inhibited
N. sicca (DSMZ 23539)	37 ± 1°C in 5% CO_2 (with high humidity) for 48 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 LS0001 LCAT Selective Supplement performance, supplement samples were added to sterilised KM0016 GC Agar, BM0478 Suplex Solution, and 7% Laked 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% CO_2 with high humidity for 48 hours. All samples grew and showed good recovery and the

correct morphology of the required test organisms: *Neisseria gonorrhoeae* (NCTC 8375) and *Neisseria gonorrhoeae* (NCTC 12700) Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10^4 - ≤1.5x10^5 cfu of the required non-target test organisms: *Proteus mirabilis* (NCTC 10975), *Candida albicans* (NCPF 3179), *Staphylococcus epidermidis* (NCTC 13360), *Escherichia coli* (ATCC 25922) and *Neisseria sicca* (DSMZ 23539), which were inhibited and have a selectivity factor of ≥ 3. Therefore, it can be concluded that LS0001 LCAT Selective Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to nutritional 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 LS0001 LCAT 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

LS0001 LCAT Selective Supplement must be stored at 2-8°C in the dark. 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. Thayer, J. and Martin, J. (1966) Improved Medium Selective for Cultivation of N. gonorrhoeae and N. meningitidis. Public Health Rep. (1896-1970), 81(6), 559-562.
2. Johnston, J. (1945) Comparison of gonococcus cultures read at 24 and 48 hours. J Vener Dis Inf., 26, 239-241.
3. Faur Y., Weisburd, M. and Wilson, M. (1978) The selectivity of vancomycin and lincomycin in NYC medium for the recovery of N. gonorrhoeae from clinical specimens. Health Lab. Sci. 15(1), pp.22-26.
4. Public Health England (2015) UK Standards for Microbiology Investigations: Identification of Neisseria species. ID6.4. London: Standards Unit, Microbiology Services.
5. Standards Unit, United Kingdom Health Security Agency (UKHSA) (2025) UK Standards for Microbiology Investigations: Infectious syndromes affecting the genitourinary tract and reproductive organs. S6 Issue No. 2.
6. Clinical and Laboratory Standards Institute (2004) CLSI M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media. 3rd Edition. Wayne, PA: CLSI.
7. Young H. (1978) Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. Br J Vener Dis, 54(1), 36-40.

Version History*

001 08/07/2014 - New document.
002 28/01/2015 - Addition of new logo.
003 08/12/2016 - Update to new flow diagram format.
004 19/10/2023 - Updated to new format and standard
005 13/10/2025 - Update to Section 4 to include reconstitution volume. Update to Section 10 to bring incubation temperature in line with UKHSA SMI S6 and state time range for incubation. Inclusion of UKHSA SMI S6 into reference section. General updates to harmonise with device file. Updated formula to be for one litre of final prepared medium.

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

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TABLE OF APPLICABLE SYMBOLS

REF o 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