

REF - LS0002

Instructions for Use

VCAT Selective Supplement

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use only. LS0002 VCAT 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

<u>Ingredient</u>	<u>mg/L</u>
Colistin	7.5
Vancomycin	3.0
Amphotericin B	2.0
Trimethoprim	5.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS0002 VCAT 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 VCAT.

GC Selective Agar with VCAT 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 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 target 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 Agar is recommended as a selective agar by the UK Standards for Microbiology Investigations ⁽⁴⁾ for a variety of clinical samples and falls under the category of selective media for pathogenic *Neisseria* spp. according to the Clinical and Laboratory Standards Institute ⁽⁵⁾. Presumptive identification should be confirmed by biochemical, serological, molecular, or mass spectrometry methods ⁽⁴⁾.

4. Principle

Vancomycin 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 ⁽⁶⁾.

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, 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	Pale Yellow Solid Pellet	Pale Yellow clear liquid

7. Materials Provided

LS0002 VCAT 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
LS0002-V001-500	5ml neutral clear crimped vial

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, quality control organisms, a sterile medium (e.g., GC Agar made up from KM0016 with other specified supplements).

9. Specimens

LS0002 VCAT 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 VCAT:

- 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, check to see if the specimen needs to be pre-enriched 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:

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 5% CO₂ (with high humidity) at 37 ± 1°C for 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 VCAT	
<i>N. gonorrhoeae</i> (NCTC 8375)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Growth: Grey colonies
<i>N. gonorrhoeae</i> (NCTC 12700)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Growth: Grey colonies
<i>P. mirabilis</i> (NCTC 10975)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Inhibited
<i>C. albicans</i> (NCPF 3179)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Inhibited
<i>S. epidermidis</i> (NCTC 13360)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Inhibited
<i>E. coli</i> (NCTC 12241)	37 ± 1°C in 5% CO ₂ and high humidity for 48 hours	Inhibited
<i>N. sicca</i> (ATCC 9913)	37 ± 1°C in 5% CO ₂ and 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

LS0002 VCAT Selective Supplement was added to GC Agar Base manufactured from KM0016 following the methods described and samples were 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 (with high humidity) for 48 hours. All samples showed expected recovery and 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 10⁴ - ≤1.5x10⁵ cfu of the required non-target test organisms: *Proteus mirabilis* (NCTC 10975), *Candida albicans* (NCPF 3179), *Staphylococcus epidermidis* (NCTC 13360), *Escherichia coli* (NCTC 12241) and *Neisseria sicca* (ATCC 9913) which were inhibited and have a selectivity factor of ≥ 3. Therefore, it can be concluded that LS0002 VCAT Selective Supplement, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- 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 LS0002 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

VCAT Selective Supplement (LS0002) 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. 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. Clinical and Laboratory Standards Institute (2004) CLSI M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media. 3rd Edition. Wayne, PA: CLSI.
6. Young H. (1978) Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. Br J Vener Dis, 54(1), 36-40.

Version History*

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| 001 | 17/06/2014 - New Document Created |
| 002 | 28/01/2015 - Update to new format with header & footer. |
| 003 | 27/08/2015 - Update with IVD logo. |
| 004 | 02/03/2016 - Update to new marketing format PDF |
| 005 | 09/11/2016 - Update format to flow diagram. |
| 006 | 05/07/2023 - Fully updated IFU to new standard |
| 007 | 13/10/2025 - Update to Section 4 to include reconstitution volumes. Product name updated to match other records. |
- General updates to harmonise with device file.

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



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TABLE OF APPLICABLE SYMBOLS

IFU/LS0002 REV. 007

 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