Instructions for Use

REF - LS0008 **Staph/Strep Selective Supplement (CNA)**

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0008 Staph/Strep Selective Supplement (CNA) is a sterile antibiotic supplement used to enhance the selective isolation of *Staphylococcus* and *Streptococcus* species on microbial media such as Columbia Agar with 7% Defibrinated Horse Blood and CNA Supplement (E&O PP1080).

2. Composition*

For one litre of prepared media

Ingredient	g/L
Colistin Sulphate	0.005
Nalidixic acid	0.0025
Aztreonam	0.00025

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Accurate quantities of Colistin Sulphate, Nalidixic Acid and Aztreonam are lyophilised and provided in individually labelled vials. Columbia Agar with 7% Defibrinated Horse Blood and CNA Supplement is intended for clinical specimens including but not limited to swabs of the throat or other infected areas, blood, urine, skin, infected material, or nasal secretions, and is recommended as a selective primary isolation media by the UK Standards for Microbiology Investigations [1 2].

4. Principle

Colistin and nalidixic acid are added to inhibit *Micrococcus* species as well as Gram-positive and Gram-negative rods. Aztreonam suppresses the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species while permitting unrestricted growth of *Staphylococcus aureus*, haemolytic streptococci, and enterococci.

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 of choice (Columbia Agar with 7% Horse Blood is recommended) prepared according to the manufacturer's instructions 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 clear liquid

7. Materials Provided

LS0008 Staph/Strep Selective Supplement (CNA) 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
LS0008-V001-500	5ml neutral clear crimped vial suitable for 500ml of media

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, quality control organisms, and sterile medium (e.g., Columbia Agar Base with 7% Horse Blood).

9. Specimens

LS0008 Staph/Strep Selective Supplement (CNA) made up as Columbia Agar with 7% Defibrinated Horse Blood and CNA Supplement is suitable for the testing of the following specimens:

- Clinical Specimens: Samples may include swabs from the infected areas, nasal secretions, skin, biopsy material, pus, bone, blood, and urine.

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

Prepare plates containing LS0008 Staph/Strep Selective Supplement and allow to come to room temperature.

Follow local, national, or international guidelines.

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

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

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 at 35-37°C in the appropriate atmosphere in accordance with the specific investigation and associated methodology being performed for 18-24 hours.

After incubation, examine agar for colonies (typical colony appearance 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)
	Columbia Agar Base with 7% Defibrinated Horse Blood and CNA Selective Supplement	
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies
S. epidermidis (NCTC 13360)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Grey/green colonies with α-haemolysis
S. pyogenes (NCTC 12696)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies with β-haemolysis
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Inhibited
P. mirabilis (NCTC 10975)	37 ± 1°C aerobically for 18-24 hours	Inhibited
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically 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

LS0008 Staph/Strep Selective Supplement (CNA) was added to Columbia Agar Base with 7% Horse Blood 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 for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C in the appropriate atmosphere as defined in the Quality Control table above for 18-24 hours. All samples showed expected recovery and morphology of the required test organisms: *Staphylococcus aureus* (NCTC 12981) *Staphylococcus epidermidis* (NCTC 13360), *Streptococcus pneumoniae* (NCTC 12977) and *Streptococcus pyogenes* (NCTC 12696) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12241), *Proteus mirabilis* (NCTC 10975) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that LS0008 Staph/Strep Selective Supplement (CNA), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Due to natural variation, some strains may grow poorly or fail to grow on the final medium.
- 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 LS0008 Safety Data Sheet

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

15. Storage conditions and Shelf life

LS0008 Staph/Strep 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. Public Health England (2021) Introduction to the preliminary identification of medically important bacteria and fungi from culture. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 1(3). London: Standards Unit, National Infection Service.

2. Public Health England (2021) Identification of Streptococcus species, Enterococcus species and morphologically similar organisms. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 4(4). London: Standards Unit, National Infection Service.

Version History*

001 08/07/2014 - New Document Created
002 24/02/2015 - Change title to Staph/Strep Selective Supplement, adjust sheet for 1 litre vials and insert new logo and layout.
003 08/09/2015 - Update to new format with IVD logo and add additional details about formulae.
004 19/12/2016 - Update with flow diagram format.
005 02/10/2023 - Updated to new format and standard
006 13/10/2025 - Update to Section 4 to include reconstitution volumes. Updated hazard statement and QC procedures to match QCR. General updates to harmonise with device file.

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

EC REP
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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	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place