Instructions for Use

REF - LS0018 **Bordetella Supplement with Cephalexin (40mg/L)**

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0018 Bordetella Supplement with Cephalexin (40mg/L) is an antibiotic supplement used to enhance the selective isolation of *Bordetella pertussis*.

2. Composition*

For one litre of prepared media

Ingredient	mg/L
Cephalexin monohydrate	40.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Bordetella Supplement with Cephalexin (40mg/L) is formulated to be used with E&O KM0185 Charcoal Agar and Defibrinated Horse Blood to form Charcoal Agar with 10% Horse Blood and Cephalexin (40mg/L).

Whooping cough is caused by *Bordetella pertussis* and *Bordetella parapertussis* which are among the most fastidious bacteria known and require special culture techniques [1]. A charcoal-based medium was found to be an efficient substitute for Bordet-Gengou agar in the production of *B. pertussis* vaccines [2] and can be used as a maintenance medium for stock cultures of Bordetella species [3]. Proom demonstrated nicotinic acid is an essential growth factor for *Bordetella* spp. [4]. Sutcliffe and Abbott added 40 µg/ml of cephalexin to charcoal agar to suppress the growth of commensal nasopharyngeal flora and compared its inhibitory effect with that of penicillin [5]. Stauffer *et al*. compared the inhibitory effects on *Bordetella* spp. of charcoal agar with cephalexin to that of charcoal agar with penicillin and methicillin [6]. Both groups reported agar supplemented with cephalexin was superior to supplementation with other antibiotics for selective recovery of *B. pertussis*. In 1989, Hoppe *et al*. reported that supplementation with horse blood was superior to supplementation with sheep or human blood for recovery of *Bordetella* spp. [7].

Charcoal Agar with 10% Horse Blood and Cephalexin (40mg/L) is recommended as a primary isolation medium by the UK Standards for Microbiology Investigations [8 9] and tested in accordance with ISO 11133:2014 [10].

4. Principle

The addition of cephalexin inhibits accompanying unwanted nasopharyngeal flora often present in specimens submitted for the isolation of *B. pertussis*.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water. 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, KM0185 Charcoal Agar and Defibrinated Horse Blood, prepared according to instructions and allowed 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	White Solid Pellet	Colourless liquid

7. Materials Provided

LS0018 Bordetella Supplement with Cephalexin (40mg/L) 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**
LS0018-V001-1000	5ml neutral clear crimped vial suitable for 1L of media
LS0018-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, quality control organisms, and sterile medium (e.g., Charcoal Agar with 10% Horse Blood and Cephalexin (40mg/L) made from KM0185 Charcoal Agar and Defibrinated Horse Blood)

9. Specimens

LS0018 Bordetella Supplement with Cephalexin (40mg/L) can be used with the following specimens when added to KM0185 Charcoal Agar and Defibrinated Horse Blood to form Charcoal Agar with 10% Horse Blood and Cephalexin (40mg/L):

- Clinical Specimens: nasopharyngeal swabs or aspirates.

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

Follow relevant guidelines.

Allow the plates to come to room temperature.

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

Clinical Specimens:

Process the clinical specimen as required by local guidelines.

Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate the plates at 36 ± 1°C aerobically for 7 days (further incubation up to 12 days has been shown to improve recovery of clinical *Bordetella* species). It is recommended to check plates at the 4- and 7- day marks.

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)
B. pertussis (NCTC 10739)	37 ± 1°C aerobically for 72-120 hours	Growth: Grey colonies
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 72-120 hours	Inhibited
K. pneumoniae (NCTC 9633)	37 ± 1°C aerobically for 72-120 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 LS0018 Bordetella Supplement with Cephalexin (40mg/L), supplement samples were added to sterilised KM0185 Charcoal Agar and Defibrinated Horse Blood. Resulting plates 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 aerobically for 72-120 hours. All samples showed good recovery and the correct morphology of the required test organism: *Bordetella pertussis* (NCTC 10739). 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: *Staphylococcus aureus* (NCTC 12981) and *Klebsiella pneumoniae* (NCTC 9633), which were inhibited with a selectivity factor of ≥ 3. Therefore, it can be concluded that LS0018 Bordetella Supplement with Cephalexin (40mg/L) 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 this medium.
- Plates of the final selective medium should not be overdried before inoculation.
- Perform further biochemical, serological, molecular, or mass spectroscopy (MALDI-TOF) analysis 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 LS0018 Bordetella Supplement with Cephalexin (40mg/L) Safety Data Sheet prior to use.

Do not use if the supplement is not the 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

LS0018 Bordetella Supplement with Cephalexin (40mg/L) should 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. Isenberg. H. (2004) Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.
2. Ensminger, P., Culbertson, C. and Powell, H. (1953) Antigenic Hemophilus Pertussis Vaccines Grown on Charcoal Agar. J. Infect Dis, 93(3): 266-268.
3. Mishulow, L., Sharpe, L. and Cohen, L. (1953) Beef-heart charcoal agar for the preparation of pertussis vaccines. Am. J. Public Health, 43(11): 1466-1472.
4. Proom, H. (1955) The minimal nutritional requirements of organisms of the genus Bordetella López. J. Gen. Microbiol., 12(1): 63-75.
5. Sutcliffe, E. and Abbott, J. (1972) Selective medium for the isolation of Bordetella pertussis and parapertussis. J. Clin. Pathol., 25(8): 732-733.
6. Stauffer, L. R., Brown, D.R. and Sandstrom, R.E. (1983) Cephalexin-supplemented Jones-Kendrick charcoal agar for selective isolation of Bordetella pertussis: comparison with previously described media. J. Clin. Microbiol., 17(1):60-62.
7. Hoppe, J.E. and Schlagenhauf, M. (1989) Comparison of three kinds of blood and two incubation atmospheres for cultivation of Bordetella pertussis on charcoal agar. J. Clin. Microbiol. 27(9):2115-2117.
8. Public Health England (2020) Identification of Bordetella species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 5(4). London: Standards Unit, Microbiology Services.
9. Public Health England (2018) Investigation of whooping cough. UK Standards for Microbiology Investigations. Bacteriology, B 6(9). London: Standards Unit, Microbiology Services.
10. 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.

Revision

001	08/07/14 - New document
002	15/04/15 - Update with new logo and alter layout etc.
003	01/08/16 - Update to new format with diagram.
004	10/11/23 - Updated to new format and standard

*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