

## Instructions for Use

REF - KM0016

GC Agar

### Dehydrated Culture Media

#### 1. Intended Use

For *in vitro* diagnostic use only. KM0016 GC Agar may be supplemented with blood and other enrichment factors and is recommended primarily for the isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*, but is also capable of supporting the growth of most fastidious micro-organisms from clinical specimens.

#### 2. Composition\*

| <u>Ingredient</u>               | <u>g/L</u> |
|---------------------------------|------------|
| Peptone                         | 15.0       |
| Starch                          | 1.0        |
| Sodium chloride                 | 5.0        |
| Di-potassium hydrogen phosphate | 4.0        |
| Potassium di-hydrogen phosphate | 1.0        |
| Agar                            | 10.0       |

\*Adjusted/supplemented as needed to meet performance requirements

#### 3. Summary and Explanation

This medium is based on the formulation described by Thayer and Martin <sup>(1)</sup> that was modified from the formulation development by Johnson <sup>(2)</sup> for the isolation of gonococcus cultures and is recommended as a primary and selective agar by the UK Standards for Microbiology Investigations <sup>(3-8)</sup> 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 <sup>(9)</sup>.

*Neisseria* species are fastidious, Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. 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* from clinical specimens is related to several factors. The time and care taken in obtaining specimens. The transport of the specimen to the laboratory and inoculation onto the chosen culture medium. The type of culture medium chosen must be capable of growing small inoculum of demanding strains.

The inclusion of selective agents in the medium which can prevent overgrowth of commensal organisms, but which will not inhibit the growth of the species required and the confirmatory tests to identify the isolated species.

Diagnosis of gonorrhoea is achieved by microscopic examination and cultivation of material from infected sites. Presumptive gonococci have then to be distinguished from other organisms of the *Neisseria* group which will grow on the media and have a similar morphology and staining characteristics <sup>(3)</sup>.

#### 4. Principle

Peptone supplies the required nitrogen, carbon, and vitamins. Sodium chloride maintains the osmotic balance in the medium and starch is present to absorb toxic metabolites that may inhibit growth of target species. Phosphate buffers minimise pH changes during incubation.

Enrichment of KM0016 GC Agar is usually attained using lysed blood but haemoglobin powder or chocolate blood are suitable alternatives. Further enrichment may be provided by the addition of Suplex Supplement (E&O BM0478) which contains yeast extract and glucose. Selective plated media variants of KM0016 GC Agar, may be prepared by the addition of various selective supplements such as LCAT Selective Supplement (E&O LS0001) or VCAT Selective Supplement (E&O LS0002). These supplements suppress most microbiota present in clinical specimens. Vancomycin, lincomycin and colistin will inhibit many contaminating bacteria, amphotericin will inhibit yeasts, and the trimethoprim will restrict the swarming of *Proteus* species.

#### 5. Preparation Instructions

Suspend 36.0 of dehydrated culture medium in 1 litre of deionised/purified water and allow to soak with frequent agitation for 10 minutes.

Sterilise at 121°C for 15 minutes before cooling to 45-50°C.

##### Chocolating Blood

Chocolating blood is a process that heats added blood after addition to sterilised media to allow red blood cells to lyse and release extra nutrients that facilitates enhanced growth of fastidious organisms.

- Blood must be added when media is cooled to 45-50°C
- Heat the medium back up to 70°C **slowly** using a waterbath or hotplate
- When the process is complete, the colour of the agar will be a chocolate-brown
- Cool to 42-46°C

Aseptically add any final selective supplements if required and gently homogenize the final medium before dispensing the specified volume into appropriate sterile containers and allowing to cool.

## 6. Physical Characteristics

|                       | Dehydrated Medium      | Prepared Medium                |
|-----------------------|------------------------|--------------------------------|
| Appearance and Colour | Pale straw fine powder | With lysed blood: red firm gel |
| pH                    | N/A                    | 7.2 ± 0.2                      |

## 7. Materials Provided

KM0016 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

| Product Code      | Product Format                        |
|-------------------|---------------------------------------|
| KM0016-500G-500   | 1 x 500g Dehydrated Culture Media Tub |
| KM0016-5KG-5000   | 1 x 5kg Dehydrated Culture Media Tub  |
| KM0016-10KG-10000 | 1 x 10kg Dehydrated Culture Media Tub |

## 8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.*, autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

## 9. Specimens

KM0016 GC Agar is suitable for the testing of the following specimens <sup>(4-8)</sup>:

- Clinical samples: nasopharyngeal, throat, eye, urethral and cervical swabs, pus, cerebrospinal fluid and blood samples.

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 chosen plated media for isolation of target organism to come to room temperature.

Process the clinical specimen as soon as possible after it has been received to avoid loss of gonococci viability and the increase in contaminants.

### 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 5 - 10% CO<sub>2</sub> for 18 - 48 hours <sup>(3)</sup>.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform MALDI-TOF MS <sup>(3)</sup>, further biochemical serological, or molecular tests to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

## 11. Quality Control

| Organism                           | Incubation                                                   | Result (Specificity)  |
|------------------------------------|--------------------------------------------------------------|-----------------------|
|                                    | <u>GC Agar with VCAT Selective Supplement</u>                |                       |
| <i>N. gonorrhoeae</i> (NCTC 8375)  | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Growth: Grey colonies |
| <i>N. gonorrhoeae</i> (NCTC 12700) | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Growth: Grey colonies |
| <i>N. gonorrhoeae</i> (ATCC 43070) | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Growth: Grey colonies |
| <i>P. mirabilis</i> (NCTC 10975)   | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Inhibited             |
| <i>C. albicans</i> (NCPF 3179)     | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Inhibited             |
| <i>S. epidermidis</i> (NCTC 13360) | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Inhibited             |
| <i>C. albicans</i> (ATCC 60193)    | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Inhibited             |
| <i>N. sicca</i> (ATCC 9913)        | 37 ± 1°C in 5% CO <sub>2</sub> at high humidity for 48 hours | Inhibited             |

(ATCC is a trademark of the American Type Culture Collection)

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

## 12. Performance

To fully verify KM0016 GC Agar performance, dehydrated culture media samples were used to prepare GC Agar with VCAT and 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<sup>4</sup>-10<sup>5</sup> cfu for the non-target organisms then incubated at 37 ± 1°C in 5% CO<sub>2</sub> at high humidity for 48 hours. All samples of prepared media showed expected recovery and

morphology of the required test organisms: *Neisseria gonorrhoeae* (NCTC 8375), *Neisseria gonorrhoeae* (NCTC 12700) and *Neisseria gonorrhoeae* (ATCC 43070), and no recovery of the non-target test organisms: *Proteus mirabilis* (NCTC 10975), *Candida albicans* (NCPF 3179), *Staphylococcus epidermidis* (NCTC 13360), *Candida albicans* (ATCC 60193) and *Neisseria sicca* (ATCC 9913). Therefore, it can be concluded that KM0016 GC Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

### 13. Limitations of the Media

- *N. gonorrhoeae* grows poorly on blood agar, so a non-selective GC agar with lysed or chocolatised horse blood should be used instead<sup>(3)</sup>.
- Depending on the plated media manufactured, and due to natural variation, some strains may grow poorly on the medium.
- Additional biochemical, serological, or molecular tests should be performed on sub-culture isolates to confirm identity.

### 14. Precautions and Warnings

This product is considered non-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 KM0016 Material Safety Data Sheet.

### 15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

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. Public Health England (2015) Identification of *Neisseria* species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 6(3). London: Standards Unit, Microbiology Services.
4. Public Health England (2014) Screening for *Neisseria meningitidis*. UK Standards for Microbiology Investigations. Bacteriology, B 51(2). London: Standards Unit, Microbiology Services
5. Public Health England (2018) Investigation of pus and exudates. UK Standards for Microbiology Investigations. Bacteriology, B 14(6.2). London: Standards Unit, Microbiology Services.
6. Public Health England (2017) Investigation of bacterial eye infections. UK Standards for Microbiology Investigations. Bacteriology, B 2(6.1). London: Standards Unit, Microbiology Services.
7. Public Health England (2017) Investigation of genital tract and associated specimens. UK Standards for Microbiology Investigations. Bacteriology, B 28(4.6). London: Standards Unit, Microbiology Services.
8. Public Health England (2015) Investigation of throat related specimens. UK Standards for Microbiology Investigations. Bacteriology, B 9(9). London: Standards Unit, Microbiology Services.
9. Clinical and Laboratory Standards Institute (2004) CLSI M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media. 3rd Edition. Wayne, PA: CLSI.

### Version History\*

- 001 03/10/2022 - New document created.
- 002 14/04/2023 - Revisions document to bring more into line with current guidelines.
- 003 13/03/2025 - Update based on new formatting and device file.

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

TABLE OF APPLICABLE SYMBOLS

|                                                                                                             |                                                                                                                       |                                                                                                                               |                                                                                                                 |                                                                                                            |
|-------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
|  REF<br>Catalogue number |  LOT<br>Batch code                 |  IVD<br>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 |