

Instructions for Use

REF - KM0141

Nutrient Agar

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0141 Nutrient Agar is a basic general-purpose medium suitable for use in the cultivation of the less fastidious organisms.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Meat extract	3.0
Peptones	5.0
Sodium chloride	5.0
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Nutrient agar was first described by the American Public Health Association (APHA) in 1917 ⁽¹⁾. It is used to check the purity of subcultures from isolation plates prior to biochemical or serological tests. It is particularly useful for the cultivation of the less fastidious organisms, particularly those that do not require the addition of blood or other enrichment. It is one of the most important and commonly used non-selective media for the routine cultivation of microorganisms. When used to prepare agar slopes or agar butts, the medium can be used to maintain control organisms. Nutrient Agar is an ideal medium for the demonstration and teaching purposes as it allows more prolonged survival of cultures at ambient temperature without the risk of overgrowth that might occur with more nutritious mediums.

KM0141 is recommended by the UK Standards for Microbiology Investigations ^(2 3), the International Organization for Standardization ^(4 5 6 7) and the Standing Committee of Analysts ^(8 9 10 11) and is tested in accordance with ISO 11133:2014 ⁽¹²⁾.

4. Principle

Nutrient agar is made with various nutrients which allow the growth of a wide variety of microorganisms that do not usually require specific nutrients or supplements. The peptone is the source of nitrogen or protein that acts as a source of amino acids for the bacteria. Beef extract is the primary source of carbon which is essential for the formation of carbohydrates in the bacteria. It also contains other components like some vitamins, different trace minerals, organic compounds, and salts, which further enhance the growth of different organisms. Sodium chloride is added to the medium to maintain the osmotic equilibrium of the medium and prevent the change in pH of the medium during growth. Agar is the solidifying agent that provides a stable surface for the organism to grow on, which allows for the observation of colony morphology and enumeration of the organism.

5. Preparation Instructions

Suspend 28.0g/L of dried culture media in deionised/purified water and allow to soak whilst mixing for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Straw firm gel
pH	N/A	7.4 ± 0.2

7. Materials Provided

KM0141 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
KM0141-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0141-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0141-10KG-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

KM0141 Nutrient Agar is suitable for the testing of the following specimens:

- Clinical: isolation and maintenance media, X&V testing for *Haemophilus* differentiation.
- Food and Environmental: products for human consumption, animal feed and environmental samples
- Water: isolation and maintenance media for water, sewage and effluents.

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 to come to room temperature.

Clinical Specimens^(2 3)

- Process the clinical specimen as required.
- Inoculate an isolated pure culture directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate in recommended temperature and conditions depending on sample type and suspected target microorganism (see QC table below)

For full method of X&V testing, see SMI TP 38⁽³⁾.

- Process the clinical specimen as required.
- Emulsify the test organism in a diluent such as 0.85% saline (E&O BM0380) or distilled water (E&O BM0655).
- Dip a sterile cotton swab into the inoculum suspension and spread evenly over the entire surface.
- After the agar surface is dry, place X, V and XV discs onto the agar surface (ensuring the discs are minimum 1cm apart to reduce the chances of false results).
- Incubate the plates at $36 \pm 1^\circ\text{C}$ in 5% CO_2 for 18-24 hours.

Food/Water/Environmental Specimens⁽⁴⁻¹¹⁾

- Process the food specimen as required by local guidelines.
- Inoculate an isolated pure culture directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate in recommended temperature and conditions depending on sample type and suspected target microorganism (see QC table below)

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>B. subtilis</i> (NCTC 10400)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Opaque/Grey colonies
<i>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies
<i>P. aeruginosa</i> (NCTC 12903)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Cream colonies
<i>H. influenzae</i> (NCTC 11931)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Growth round X+V disc, grey colonies
<i>H. parainfluenza</i> (NCTC 7857)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Growth round V and X+V discs, grey colonies
<i>C. albicans</i> (NCPF 3179)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies
<i>A. brasiliensis</i> (NCPF 2275)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies with black centre, reverse side is pale yellow with hyphae
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Opaque/Grey colonies
<i>S. typhimurium</i> (NCTC 12023)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Opaque/Grey colonies
<i>Y. enterocolitica</i> (NCTC 12982)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Cream colonies

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 local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0141 Nutrient Agar performance, dehydrated culture media samples were used to prepare Nutrient Agar and tested to assess colony morphology and recovery level (where an acceptable range is $\geq 70\%$ and $\leq 120\%$) compared to a non-selective reference medium, as well X and V factor testing. Prepared samples were inoculated with 30-150cfu and incubated at $37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Bacillus subtilis* (NCTC 10400), *Staphylococcus aureus* (NCTC 12981), *Pseudomonas aeruginosa* (NCTC 12903), *Haemophilus influenzae* (NCTC 11931), *Haemophilus parainfluenza* (NCTC 7857), *Candida albicans* (NCPF 3179), *Aspergillus brasiliensis* (NCPF 2275), *Escherichia coli* (NCTC 12241), *Salmonella typhimurium* (NCTC 12023) and

Yersinia enterocolitica (NCTC 12982). Therefore, it can be concluded that KM0141 Nutrient Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Different organisms might differ in their growth requirement and may show variable growth patterns on the medium. This makes the medium quite unreliable during isolation.
- Because nutrient media supports the growth of many microorganisms, chances of contamination are quite high during isolation.
- It cannot be used as a selective medium for the cultivation of fastidious organisms that have specific nutrient requirements.
- Nutrient agar mostly only allows the isolation of bacteria but not microorganisms like yeasts and fungi.
- In some cases, some microorganisms might demonstrate similar colony morphologies on nutrient agar which makes it difficult to distinguish them without microscopic examination.

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 KM0141 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. American Public Health Association (1917) Standard Methods of Water Analysis, 3rd ed. Washington DC: APHA.
2. Public Health England (2021) Introduction to the preliminary identification of medically important bacteria and fungi from culture. UK Standards for Microbiology Investigations. Identification, ID 1(3). London: Standards Unit, National Infection Service.
3. Public Health England (2019) X and V factor test. UK Standards for Microbiology Investigations. Bacteriology – Test Procedures, TP 38(4). London: Standards Unit, National Infection Service.
4. International Organization for Standardization (2017) 6579-1:2017 Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. Geneva, ISO.
5. International Organization for Standardization (2017) 21528-1:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 1: Detection of *Enterobacteriaceae*. Geneva, ISO.
6. International Organization for Standardization (2023) 16654:2001/Amd.2:2023 Microbiology of food and animal feeding stuff – Horizontal method for the detection *Escherichia coli* O157. Geneva, ISO.
7. International Organization for Standardization (2017) 10273:2017 Microbiology of the food chain – Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Geneva, ISO.
8. The Microbiology of Drinking Water (2016) Part 4 – Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7). Standing Committee of Analysts, Environment Agency, Nottingham, UK.
9. The Microbiology of Drinking Water (2012) Part 5 – Methods for the isolation and enumeration of enterococci. Standing Committee of Analysts, Environment Agency, Nottingham, UK.
10. The Microbiology of Drinking Water (2006) Part 9 – Methods for the isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube-most probable number techniques. Standing Committee of Analysts, Environment Agency, Nottingham, UK.
11. The Microbiology of Drinking Water (2002) Part 10 – Methods for the isolation of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment. Standing Committee of Analysts, Environment Agency, Nottingham, UK.
12. 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.

Version History*

001 10/10/23 - New Document Created

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



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TABLE OF APPLICABLE SYMBOLS

 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