Instructions for Use

REF - KM0173 **Urea Broth Base**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. Urea Broth Base when supplemented with Urea 40% Solution for the preparation of Christensen's medium[1], is intended to be used to detect urease activity in microorganisms.

2. Composition*

Ingredient	g/L
Peptone	1.0
Glucose	1.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Sodium chloride	5.0
Phenol red	0.004

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

A modification of Christensen's [1] medium by Rustigian and Stuart [2]. This medium is used to detect rapid urease activity of *Proteus* species, although it can be used to detect urease activity of other *Enterobacteriaceae* including urease producing *Salmonella* species and *Shigella* species. Unlike Christensen's medium when used for the latter purpose, it is not necessary to increase the incubation time.

KM0173 is recommended by the UK Standards for Microbiology Investigations [3 4], the Standing Committee of Analysts [5 6] and the Bacteriological Analytical Manual [7 8 9] and is tested in accordance with ISO 11133:2014 [10].

4. Principle

The peptone supplies the required nitrogen, carbon, and vitamins. Glucose is a fermentable carbohydrate. Disodium phosphate and potassium dihydrogen phosphate are buffers and sodium chloride maintain osmotic balance. Phenol red is a pH indicator. When organisms utilise urea, ammonia is formed which turns the medium alkaline. The indicator, phenol red, changes the medium colour from pale-yellow to pink-red in an alkaline environment, indicating positive urease activity.

5. Preparation Instructions

Suspend 0.9g of dehydrated culture medium in 95ml of 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 and aseptically add 5ml of sterile 40% urea solution.

Gently homogenise the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Pale orange liquid
pH	N/A	6.8 ± 0.2

7. Materials Provided

KM0173 Urea Broth Base 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
KM0173-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0173-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0173-10KG-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

KM0173 Urea Broth Base can be used with the following specimens:

- Clinical Specimens: Gastric biopsy [4], or pure colonies isolated from, urine, stools, or wound infections to check the urease activity of the isolated microorganisms.
- Water Specimens: Pure colonies isolated from drinking water, ground/river/sea water, sewage and effluents to check the urease activity of the isolated microorganisms.
- Food Specimens: Pure colonies isolated from foodstuffs and environmental samples to check the urease activity of the isolated microorganisms.

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

Clinical Specimens

- Process the clinical specimen as required by local guidelines.

Isolated Colonies [3]

- Inoculate the medium directly with isolated colonies from a pure culture and mix gently to suspend the colonies.
- Incubate the bottles at 35°C for 24-48 hours. Examine broths at 8, 12, 24 and 48 hours of incubation to observe any change in colour.

Gastric Biopsy [4]

- Inoculate the medium directly with isolated colonies from a pure culture and mix gently to suspend the colonies.
- Incubate the bottles at ambient temperatures for up to 24 hours. Examine broths hourly up to 6 hours and again at 24 hours incubation to observe any change in colour.

Water Specimens [5 6]

- Process the water specimen as required by local guidelines.
- Inoculate the medium directly with isolated colonies from a pure culture and mix gently to suspend the colonies.
- Incubate the bottles at 37 ± 1°C for 18-24 hours (Microorganisms such as *Yersinia* spp. may require a lower incubation temperature of 30 ± 1°C for up to 72 hours).

Food Specimens [7 8 9]

- Process the food specimen as required by local guidelines.
- Inoculate the medium directly with isolated colonies from a pure culture and mix gently to suspend the colonies.
- Incubate the bottles at 35°C for 24 ± 2 hours (the reaction may appear as early as 2-4 hours).

After incubation, examine broths for colour change from orange to pink, indicating positive urease activity. Biochemical and, if indicated, serological testing using pure cultures may be necessary for complete identification. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
E. coli (NCTC 12241)	37 ± 1°C for 4-6 hours	Growth: No colour change
P. mirabilis (NCTC 10975)	37 ± 1°C for 4-6 hours	Growth: Medium turns pink

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 KM0173 Urea Broth Base performance, dehydrated culture media samples were used to prepare Urea Broth and tested to assess turbidity and colour change. Prepared samples were inoculated and incubated at 37 ± 1°C for 4-6 hours. All samples of prepared media showed expected turbidity and colour change with the required test organisms: *Escherichia coli* (NCTC 12241) and *Proteus mirabilis* (NCTC 10975). Therefore, it can be concluded that KM0173 Urea Broth Base, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Do not heat or reheat the medium as urea decomposes very easily.
- The high buffering system in this medium mask urease activity in organisms that are delayed positive. If in doubt as to a result, compare with an uninoculated tube or incubate for an additional 24 hours.
- This medium is recommended for the detection of urease activity in all *Proteus* spp., *Providencia rettgeri* and urease-positive *Providencia stuartii*. *Morganella morganii* slowly hydrolyses urea and may require approximately a 36-hour incubation for a strong urease-positive reaction to occur.
- Variations in the size of the inoculum can affect the time required to reach positive (alkaline, pH 8.1) results.
- Due to natural variation, some strains may grow poorly in this medium.

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 KM0173 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. Christensen, W. (1946) Urea Decomposition as a Means of Differentiating *Proteus* and Paracolon Cultures from Each Other and from *Salmonella* and *Shigella* Types. J. Bacteriol., 52(4): 461-466.

2. Rustigian, R. and Stuart, C. (1941) Decomposition of urea by *Proteus*. Proc. Soc. exp. Biol. Med. 47(1): 108-112.

3. Public Health England (2025) Urease test. UK Standards for Microbiology Investigations. Bacteriology – Test Procedures, TP 36(4.1). London: Standards Unit, National Infection Service.

4. Public Health England (2025) Investigation of infectious causes of dyspepsia. UK Standards for Microbiology Investigations. Bacteriology, B 55(7.1). London: Standards Unit, Microbiology Services.

5. 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.

6. 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.

7. Feng, P., Weagent, S.D. and Jinneman, K. (1998) BAM Chapter 4A: Diarrheagenic *Escherichia coli*. Bacteriological Analytical Manual, 8th Edition, Rev. 2020. Silver Spring, MD.

8. Andrews, W.H., Wang, H., Jacobson, A., Ge B., Zhang, G. and Hammack, T. (1998) BAM Chapter 5: *Salmonella*. Bacteriological Analytical Manual, 8th Edition, Rev. 2023. Silver Spring, MD.

9. Andrews, W.H. and Jacobson, A. (1998) BAM Chapter 6: *Shigella*. Bacteriological Analytical Manual, 8th Edition, Rev. 2023. Silver Spring, MD.

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.

Version History*

001 29/05/2023 - New document created

002 29/04/2026 - Added reference to SMI B55, and associated specimens and methodologies

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