



Urea Agar Base, Christensen

M112I

Intended Use:

Recommended for the detection of urease production, particularly by members of the genus *Proteus*. The composition and performance criteria are in accordance with ISO 6579-1 :2017.

Composition**

As per ISO 6579-1:2017	Urea Agar Base, Christensen	M112I
Ingredients	g / L Ingredients	g / L
Peptone	1.000 Peptone	1.000
Glucose	1.000 Dextrose (Glucose)	1.000
Sodium chloride	5.000 Sodium chloride	5.000
Potassium dihydrogen phosphate	2.000 Potassium dihydrogen phosphate	2.000
Phenol red	0.012 Phenol red	0.012
Agar	9.0-18.0 Agar	15.000
Urea 40%	50ml U40 Supplement (5 ml per vial) (FD048)	50ml
Final pH (at 25°C)	6.8±0.2 Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.01 grams in 950 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 45-50°C and aseptically add 50 ml of sterile U40 Supplement (5 ml per vial) (FD048) and mix well. Dispense into sterile tubes and allow to set in a slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Principle And Interpretation

Urea Agar was described by Christensen (1,2) which detected urease activity by all rapidly urease-positive *Proteus* organisms and also by other members of *Enterobacteriaceae* (1) that exhibited a delayed urease reaction (3). This is accomplished by

- adding glucose to the medium
- decreasing the peptone concentration, and
- decreasing the buffering system, as a less buffered medium detects even smaller amount of alkali (4).

ISO Committee has recommended Urea Agar Base, Christensen (M112I), with one phosphate, instead of two phosphates for detection of rapid urease activity (5).

Heavy inoculum of growth is inoculated on the surface of the slants. On incubation urea is utilized to form ammonia, which makes the medium alkaline, showing a pink-red colour by the change in the phenol red indicator. Prolonged incubation may cause alkaline reaction in the medium. Check using medium without urea as the negative control.

Peptone is the source of nitrogen and carbon, long chain amino acids, vitamins and other essential nutrients. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium of the medium whereas phosphates serve to buffer the medium. Urea is hydrolyzed to liberate ammonia. Phenol red indicator detects the alkalinity generated by visible colour change from orange to pink.

Type of specimen

Food and water samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7,8). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

- 1.Prolonged incubation may cause alkaline reaction in the medium.
- 2.Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (2).
- 3.The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive reaction.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellowish orange coloured clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 2.4% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed on addition of 40% U40 Supplement (5 ml per vial) (FD048) after an incubation at 35-37° C for 18-24 hours.

Organism	Growth	Urease
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	negative reaction no change
<i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	negative reaction no change
<i>Proteus mirabilis</i> ATCC 25933	luxuriant	positive reaction, cerise colour
<i>Proteus hauseri</i> ATCC 13315	luxuriant	positive reaction, cerise colour
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	luxuriant	negative reaction no change

Key : *Corresponding WDCM numbers.

Formerly known as *Enterobacter aerogenes*

Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10 - 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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5. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* sp, ISO 6579-1:2017.
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- 9.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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- 11.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2024

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