INSTRUCTIONS FOR USE

UREA AGAR BASE(CHRISTENSEN)
UREA 40% SOLUTION

Dehydrated culture medium and supplement

Urea Agar
From the left: *E.coli* urease -, *Proteus* sp. urease+

1 - INTENDED USE
In vitro diagnostic. Base medium and urea supplement for the differentiation of microorganisms on the basis of urease activity.

2 - COMPOSITION
UREA AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	1.000 g
Glucose	1.000 g
Sodium chloride	5.000 g
Potassium dihydrogen phosphate	2.000 g
Phenol red	0.012 g
Agar	12.000 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

UREA 40% SOLUTION (VIAL CONTENT)

	REF 42211601	REF 4240096
Urea	20 g	2 g
Purified water	50 mL	5 mL

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Urea Agar Base is prepared according to Christensen's[1] modification of the Rustigian and Stuart[2] formula and to the formulation recommended by ISO 6579[3] and FDA BAM[4]. The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. Two units of ammonia are formed with resulting alkalinity in the presence of the enzyme, and the increased pH is detected by a colour change of the pH indicator from yellow (pH 6.8) to bright pink (pH 8.1).[5,6] The addition of peptone and glucose and the reduction of the phosphate buffer concentration of the Christensen formulation allow the differentiation between rapid (1-6 hours) urease-positive organisms (*Proteae*) and delayed (24 hours-6 days) urease-positive bacteria (some *Klebsiella, Enterobacter* and *Citrobacter* strains) and bacteria other than the *Enterobacteriaceae* (e.g. some *Bordetella* and *Brucella* species).[5] This test can be used for differentiation between the yeasts, *Candida albicans* and *Cryptococcus neoformans*: a presumptive identification of *C. neoformans* is based on rapid urease production, whilst *C. albicans* does not produce urease.[6]
The urease test with Urea Agar is one of the tests recommended by ISO 6579[3] for the identification of *Salmonella* spp.
The peptone provides the essential elements for microbial growth; glucose is a source of energy and allows rapid microbial growth and eliminates possible false negative reactions; potassium dihydrogen phosphate at a concentration of 0.2%, lower than in Stuart formulation, allows to detect small amounts of alkali; sodium chloride maintains the osmotic balance of the medium and phenol red is a pH indicator.

4- DIRECTIONS FOR MEDIUM PREPARATION
Suspend 21 g in 950 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50°C and add, under aseptic conditions, 50 mL of Urea 40% Solution (REF 42211601). Mix well and dispense the complete medium in quantities of 10 mL into sterile tubes. Cool in slanted position (long slant/short butt).

5 - PHYSICAL CHARACTERISTICS
Urea Agar Base (Christensen)

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pink-orange, limpid
Final pH at 20-25 °C	6.8 ± 0.2
Urea 40% Solution	
Solution appearance	colourless, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Urea Agar Base (Christensen)	Dehydrated medium	4021752	500 g (23.8 L)
Urea 40% Solution	Liquid supplement	42211601 4240096	50 mL 10 x 5 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops, incubator and laboratory equipment as required, Erlenmeyer flasks, screw capped tubes, ancillary culture media and reagents for the identification of the colonies.

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8 - SPECIMENS

Urea Agar shall not be used for the direct inoculation of clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

9 - TEST PROCEDURE

Inoculate the slope heavily (from an 18-24 hours pure culture) over the entire surface by streaking the surface of the agar. Do not stab the butt; it serves as a colour control.
Incubate inoculated tube with loosened cap at 35-37°C and observe the colour change of the medium to red-violet after 2, 4, 6, 18, 24 hours and daily for a total incubation time of 6 days.
Method recommended by ISO 6579[3]: streak the agar slant surface and incubate at 37 °C for up to 24 h. The positive reaction is often apparent after 2 h to 4 h.

10 - READING AND INTERPRETATION

After incubation, observe the colour change of the medium. The positive test (urea hydrolysis) is indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt; any degree of pink is considered a positive reaction.[6]
The extent of colour indicates the rate of urea hydrolysis[5]:
- Strong positive: entire tube pink
- Positive: pink slant, no change in butt
- Weak positive: top of slant pink, remainder no change
- Negative results: no colour change in agar slant (e.g., *Salmonella* spp.)

Regarding the development time of the pink alkaline reaction, some microbial categories may be observed:
- Rapid positive: 1-6 hours for all positive *Proteae* microorganisms *(Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains).
- Delayed positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days).[5-6]
- For bacteria other than the *Enterobacteriaceae* (e.g *Bordetella* and *Brucella* species, yeasts) the inoculated slope should be further incubated for up 4-6 days before it is considered negative. [5,6]

Once the test has been registered as positive, discard the tubes without prolonging the incubation.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Urease positive control: *P.vulgaris* ATCC 9484
Urease negative control: *E.coli* ATCC 25922
Incubation: 35-37°C for 18-24 hours
ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Urea Agar Base supplemented with 50 mL/L of Urea 40% Solution, is tested for performances characteristics comparing the results with a previously approved Reference Batch. Pure colonies cultivated on Tryptic Soy Agar of 4 rapid urease positive strains (*P.mirabilis* ATCC 12453, *P.vulgaris* ATCC 9484, *P.rettgeri* ATCC 39944 and a clinical isolate of *P.morganii),* 2 delayed urease positive strains (*K.pneumoniae* ATCC 27736 and *C.freundii* ATCC 43864) and 2 urease negative strains (*E.coli* ATCC 25922, S.Typhimurium ATCC 14028*,)* are inoculated by streaking on the slope surface. After incubation at 35-37°C for 2-6 and 24-48 hours aerobically, the colour change to pink is observed and recorded. All strains show a reactivity according to the specifications.

13 - LIMITATIONS OF THE METHOD

- The urea test is based on the alkalinisation of the culture medium and is therefore not specific for the urease enzyme. The utilisation of peptones, especially on the slope, for example by *P.aeruginosa*, may raise the pH to alkalinity, resulting in false positive reactions. To eliminate possible false positive, run a control test using the same strain and the test medium without urea.[5]
- Urease positive *Proteus* spp. cause a rapid alkalinisation of the medium. For the results to be valid for the detection of *Proteae*, the results must be read within the first 2-6 hours interval of incubation. *C.freundii* and *K.pneumoniae* convert Urea Agar within 24-48 hours. This medium detects rapid urease activity only of urease positive *Proteae*.[5]
- Do not inoculate Urea Agar slopes with cultures obtained from liquid media.
- Prolonged incubations could give rise to false positive results due to urea autolysis; when a long incubation is expected, incubate also an un-inoculated tube to verify the occurrence of urea autolysis.
- Even if the microbial colonies are differentiated on the basis of urea hydrolysis, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The culture medium and the supplement are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions.
- Dehydrated media and supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE

Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.

- Apply Good Manufacturing Practice in the preparation process of tubed media.
- The urea supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium: upon receipt, store at +10°C /+30°C from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps.

UREA SUPPLEMENT: UPON RECEIPT store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the bottle has been opened the solution should be used immediately. Before use, examine the product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of their shelf life, according to the storage method applied (temperature and packaging).

16 - REFERENCES

1. Christensen WB. J Bact 1946; 52:461-466
2. Stuart CA, Von Stratum E, Rustigian R. J Bact 1945; 48:437
3. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: *Salmonella*. Rev 07/2020
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985..
6. Public Health England. UK Standards for Microbiology Investigations. Urease test. TP 36, Issue n° 4, 04/2019.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/09
Revision 3	Modifications of “precautions and warnings, “storage conditions and shelf life”	2022/02
Revision 4	Removal of obsolete classification, clarification on inoculation and reading methods	2023/04

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