

INSTRUCTIONS FOR USE

UREA AGAR

Ready-to-use tubes



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

1 - INTENDED USE

In vitro diagnostic device. For the differentiation of microorganisms on the basis of urease activity.

2 - COMPOSITION TYPICAL FORMULA*

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
Urea 40% solution	50 mL
Purified water	950 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Urea Agar Base is prepared according to Christensen's¹ modification of the Rustigian and Stuart² formula and to the formulation recommended by ISO 6579³ and FDA BAM⁴. The urease test is used to determine the ability of an organism to split urea, through the production of the urease enzyme. 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 8.2).^{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*), 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 6579³ 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's 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 - PHYSICAL CHARACTERISTICS

Medium appearance	pink-orange, limpid
Final pH at 20-25°C	6.8 ± 0.2

5 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Urea Agar	Ready-to-use tubes	552175	20 glass tubes with slanted medium, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, incubator and laboratory equipment as required, ancillary culture media and reagents for complete identification of the culture.

7 - SPECIMENS

Urea Agar Medium is not intended for primary isolation from clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

8 - 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 6579³: streak the agar slant surface and incubate at 34-38°C for up to 24 h. The positive reaction is often apparent after 2 h to 4 h.





9 - 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 hydrolysis⁵:

- 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.

10 - 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, the end user can perform its own Quality Control 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: 37°C for 6-48 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 ready-to use tubes of Urea Agar and of the raw material used for the production of prepared tubes, (dehydrated Urea Agar Base REF 402175, supplemented with Urea 40% Solution REF 42211601) 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 alkalisation 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 alkalisation 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Urea Agar Base is sterilized by autoclaving and the tubes are filled with the urea-supplemented medium under aseptic conditions; the tubes cannot be considered a "sterile product", but a product with controlled bio-contamination, within the limits of the defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.





- 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* diagnostics.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour).

15 - 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/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC
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 <i>In vitro</i> Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/03
Revision 2	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.

