INSTRUCTIONS FOR USE

UREA BROTH BASE (STUART)
UREA 40% SOLUTION

Dehydrated culture medium and supplement

Urea Broth – from left:
uninoculated tube, *P.vulgaris*, *E.coli*

1 - INTENDED USE
In vitro diagnostic. Basal medium and urea supplement for the determination of urease enzyme as an aid for the differentiation of members of the *Enterobacteriaceae* family.

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

Yeast Extract	0.10 g
Potassium Dihydrogen Phosphate	9.10 g
Disodium Hydrogen Phosphate	9.50 g
Phenol Red	0.01 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 Broth Base, prepared according to the formulation of Rustigian and Stuart[1], is a medium for the determination of the urease enzyme (urea amidohydrolase), as an aid for the differentiation of members of the *Enterobacteriaceae*. The medium provides positive results for *Proteus* spp., most *Morganella* species and some strains of *Providencia stuartii*.[2] Urea Broth is mainly used to differentiate *Proteus* spp. (urease positive) from *Salmonella* spp. and *Shigella* spp. (urease negative) and other enterobacteria that hydrolyse urea slowly and are negative on this medium.
Yeast extract at low concentrations (0.01%) provides the essential elements required for the growth of *Proteus* strains that are highly urease-producing and use ammonium ions, produced by urea hydrolysis, as their sole source of nitrogen; enterobacteria that hydrolyse urea slowly can only rely on the limited concentration of yeast extract for growth and thus test negative for urea on this medium. In addition, the strong buffer system tends to reduce pH changes in the presence of low levels of ammonium ion production.[3]
Urea, added to the base medium, is hydrolysed by the microorganisms with the formation of ammonium ions and subsequent alkaline reaction that induces the purple-red turn of phenol red when the pH of the medium exceeds 8.1.[3]

4- DIRECTIONS FOR MEDIUM PREPARATION
Suspend 18.7 g in 950 mL of cold purified water. Heat to dissolve and 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 3-5 mL into sterile tubes.

5 - PHYSICAL CHARACTERISTICS
Urea Broth Base (Stuart)

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	orange, limpid
Final pH at 20-25 °C	6.8 ± 0.1
Urea 40% Solution	
Solution appearance	colourless, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Urea Broth Base (Stuart)	Dehydrated medium	4021802	500 g (26.7 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.

8 - SPECIMENS
Urea Broth, supplemented with urea solution, shall not be used for the direct inoculation of clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

9 - TEST PROCEDURE

Inoculate the broth heavily with 3 loopfuls (2 mm loop) from an 18-24 h pure culture obtained on TSI or another appropriate medium. Shake the tube gently to suspend the colonies. Incubate the tubes with loosened caps at 35-37°C in an incubator or water bath for 8-48 hours. Examine broths for colour change at 2, 4, 6, 18, 24, and 48 hours of incubation.

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.
The negative test is indicated by the unchanged colour of the medium.
Proteus spp. induce rapid alkalinization of the medium. *P. vulgaris* and *P.mirabilis* are positive after about 8 hours of incubation, *P.rettgeri* after about 12 hours; *M. morganii* may require up to 36 hours of incubation and in case of interpretation doubts compare the colour with a non-inoculated tube or incubate for further 24 hours.
However, within 48 hours of incubation these strains will develop a positive reaction.[2]
Bacteria with low and delayed urease activity (e.g., *Enterobacter*) will not test positive for urease due to the high buffering capacity of the medium.
Once the test has been recorded 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, 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: 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 Broth 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),* 3 delayed urease positive strains (*K.pneumomniae* ATCC 27736, *E.aerogenes* ATCC 13048 and *C.freundii* ATCC 8090) and 4 urease-negative strains (S.Typhimurium ATCC 14028, *S.flexnei* ATCC 12022, *E.coli* ATCC 25922 and *P.aeruginosa* ATCC 14027) are inoculated into the test tube medium. Colour change of the medium is observed after 6, 24, 48 hours of incubation at 35-37°C: only the 4 *Proteus* strains develop a positive reaction for urease.

13 - LIMITATIONS OF THE METHOD

- Urea Broth a highly buffered medium that requires large amounts of ammonia to raise the pH to 8.1 resulting in a colour change. Slowly and weakly urease-positive strains, due to the low concentration of yeast extract and a strong buffering system, appear as urease negative on Urea Broth (Stuart).[2,3]
- Purple-red turning occurs when the pH reaches 8.1; inoculation significantly affects the time required by the bacterial strain to develop these alkalinity values and thus a positive reaction.[3]
- The rate of urease reaction is also affected by the volume of liquid medium in the tube; Stuart et al.[1] report that with increasing volumes of 1.5 mL, 3 mL, 4.5 mL, 6 mL, for the same inoculum, the time of development of the positive reaction increases and that the minimum volume for the test is 1.5 ml.
- Urea Broth tubes are not suitable for quantitative evaluation of urea hydrolysis.
- 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. Apply Good Manufacturing Practice in the preparation process of tubed media.
- Dehydrated media and supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- All laboratory specimens should be considered infectious.
- The urea supplement is sterilized by membrane filtration.
- 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 supplement 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 10-30°C away 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 the validation of their shelf life, according to the storage method applied (temperature and packaging).

16 - REFERENCES

1. Rustigian R, Stuart A. Decomposition of urea by *Proteus*. Proc Soc Exp Biol Med. 1941; *47*:108-112
2. Public Health England. UK Standards for Microbiology Investigations. Urease test. TP 36, Issue n° 4, 04/2019
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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 1	Updated layout and content	2022/02
Revision 2	Removal of obsolete classification	2023/04

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

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