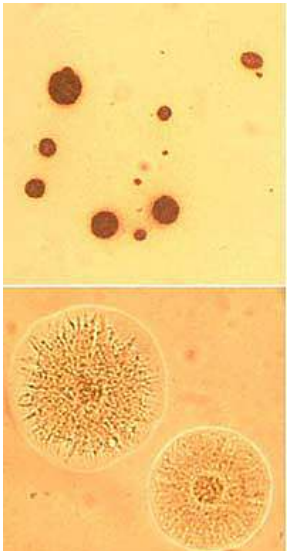
INSTRUCTIONS FOR USE

UREAPLASMA DIFFERENTIAL AGAR A7

Ready-to-use plates



From top to bottom:
reactive colonies of *Ureaplasma urealyticum* and large un-reactive *Mycoplasma hominis* colonies.
(Edis Medco)

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the cultivation and isolation of *Ureaplasma urealyticum* and other members of the genus *Ureaplasma* from clinical specimens and for their differentiation from *Mycoplasma* spp.

2- COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	17.0	g
Soy peptone	3.0	g
Sodium chloride	5.0	g
Dipotassium hydrogen phosphate	2.5	g
Glucose	2.5	g
Manganese sulphate	0.2	g
Phenol red	0.002	g
Agar	14	g
Purified water	825	mL
Horse serum	200	mL
L-cysteine HCl 4% solution	2.5	mL
Biovitex ^	5	mL
Yeast extract 25% solution	10	mL
Urea 10% solution	10	mL
Antibiotic mix	17.5	mL

^ For Biovitex formulation see REF 42185011
* The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Following puberty, *Ureaplasma* spp., and *M.hominis* can be isolated from the lower genital tract in many healthy sexually active adults, but there is evidence that these organisms play etiological roles in some genital tract diseases.[1]
Ureaplasma Differential Agar A7, prepared according to a modification of the formulation described by Shepard and Lunceford[2,3], is used for the cultivation and isolation of *Ureaplasma urealyticum* and other species of the genus *Ureaplasma,* especially from urogenital samples and for their differentiation from *Mycoplasma hominis.*
Peptones, glucose, yeast extract, and horse serum supply the nutrients necessary for the growth of mollicutes. The addition of L-cysteine and Biovitex, a mixture of amino acids and vitamins, enhance the growth of *Ureaplasma* and Mycoplasma species. Urea, manganese sulphate and phenol red constitute the indicator and differential systems of the medium. Ammonia produced by *U.urealyticum*, by means of the urease enzyme, is detected by manganese indicator that produces a stable reaction product (manganese dioxide) which develops in and on the surface of individual colonies. Moreover, the alkaline pH induces the colour change of the phenol red pH indicator: as a consequence, the medium surrounding the *Ureaplasma* colonies changes to red. *Mycoplasma hominis* does not hydrolyse urea, fail to change the medium pH and grows with large colourless colonies. The antibiotic mixture inhibits most Gram-negative and Gram-positive bacteria, which may be present in the samples.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	Amber, limpid
Final pH at 20-25°C	6.1 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Ureaplasma Differential Agar A7	Ready-to-use plates	542181	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, jars and reagents for incubation in anaerobic / CO_2 conditions, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Ureaplasma Differential Agar A7 is used for the bacteriological processing of clinical samples such as urines, vaginal and urethral specimens.[2,3,] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1]

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Centrifuge the urine specimen and inoculate 0,03 mL drops of sediment. Several specimens per plate may be used.
The urethral and vaginal exudates must be inoculated directly by smearing on a small area of the agar surface.
Allow inocula to be absorbed into the medium before incubation.

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

Incubate inoculated plates in an atmosphere of air supplemented with 5-10% CO_2 or in anaerobic environment of 95% N_2 plus 5% CO_2; anaerobic jars with catalyst and disposable generator envelops are adequate if dedicated incubator is not available.[1]
Incubate at 35-37°C for 2-4 days. In certain cases, it may be necessary to prolong incubation.[1]

9 - READING AND INTERPRETATION

After incubation, observe the plates macroscopically and under a microscope with direct light at low magnification (100 X), mainly at the edges of the inoculated area; record each specific morphological and chromatic characteristic of the colonies.
U.urealyticum is urease positive, grows with dark-golden or brown colonies with the typical “sea urchin” morphology. The area around the colonies turns to light red.
Mycoplasma spp. usually demonstrated a fried-egg appearance, are un-reactive and fail to produce the manganese dioxide, since they are urease negative.
Plates with characteristic growths should be subjected to confirmatory testing with appropriate techniques.

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.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
U.parvum ATCC 27813	35-37°C / up to 6 days / AN	brown colonies
M.hominis ATCC 15488	35-37°C / up to 6 days / CO_2	fried-egg colourless colonies
E.faecalis ATCC 29212	35-37°C / up to 6 days / CO_2	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection;

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to use plates of Ureaplasma Differential Agar A7 is tested for productivity, specificity and selectivity.
Productivity and specificity are tested by semi-quantitative ecometric technique, by incubating the plates at 35-37°C for up to 6 days, inoculated with the following target strains: *U.parvum* ATCC 27813 (anaerobic incubation) and *M.hominis* ATCC 15488 (CO_2 enriched atmosphere). *U.parvum* typical colonies appear microscopically brown due to manganese dioxide formation, whereas *M.hominis* colonies demonstrate a fried-egg appearance without the development of deep brown manganese product.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of non-target strains**:** *E.faecalis* ATCC 29212, *P.aeruginosa* ATCC 9027, *C.albicans* ATCC 18804. The growth of non-target strains is completely inhibited.

12 - LIMITATIONS OF THE METHOD

- Uniform circular “fried egg” appearance of *M.hominis* colonies is more characteristic of laboratory which is not always seen in primary clinical isolated strains. [3]
- Larger numbers of *U.urealyticum* are generally isolated in primary agar cultures from urethral exudates than from a urine specimen from the same urethritis patient.[3]
- A specific transport medium for *U.urealyticum* should be used for the transport of the specimens.[3]
- Unusual growth of *U.urealyticum* on A7 such as growth in association with colonies of other species of mycoplasmas, and growth associated with single or grouped urethral epithelial cells, urethral threads, and exudates are not artifacts.[3]
- If the medium is cut during inoculation of a clinical specimen, often *Ureaplasm*a organisms are entrapped and subsequent urease activity produces intense streaks of manganese reaction product in the agar.[2]
- If dark brown manganese accretion colonies are examined under low power by indirect transmitted or oblique light, colonies appear white.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, 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.
- This culture medium is 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 the product doesn’t contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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 plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.

- 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 plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Waites KB, Bébéar C. Mycoplasma and Ureaplasma. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Shepard MC, Lunceford, CD. Differential agar medium (A7) for identification of Ureaplasma urealyticum (human T mycoplasmas) in primary cultures of clinical material. J Clin Microbiol 1976; 3(6): 613-625
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	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/01
Revision 2	Removal of obsolete classification	2023/03

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

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