A7 AGAR

Culture, enumeration and identification of urogenital mycoplasmas

8 tests (REF 00090)

CP 0257-EN-2019-09

For *in vitro* diagnostic use only, for professional use only

Single use tests.

CE

I - INTENDED USE

The A7 Agar enables the culture, semi-quantitative enumeration and the morphological identification of *Ureaplasma urealyticum* (U.u.) / *Ureaplasma parvum* and *Mycoplasma hominis* (M.h.) from endocervical, urethral, urinary and sperm samples, as well as gastric secretions and other samples likely to contain urogenital mycoplasma infection.

2 - INTRODUCTION

The urogenital mycoplasmas, *U. urealyticum* and *M. hominis*, are known pathogens (3). They can be present as commensal organisms in the lower genital tract. A quantitative determination of infection is therefore useful. *U. urealyticum* is responsible for male genital infection. *M. hominis* proliferates during vaginosis and can spread to the upper genital tract. The urogenital mycoplasmas are also responsible for extragenital infections.

3 - PRINCIPLE

The mycoplasmas are relatively fragile organisms that will only multiply in the presence of numerous growthfactors. They are facultative anaerobes, demanding in their requirement for sterols. They metabolise sugars or arginine (*Mycoplasma hominis*) or urea (*Ureaplasma urealyticum*) in order to provide for their energy requirements.

The A7 Agar is a modified Shepard medium (4), containing serum, peptones, yeast extract and a mixture of vitamins. It is lacking in sugar, but contains urea and arginine as a source of energy. The agar is selective due to the addition of antibiotic and antifungal drugs, thereby inhibiting the development of Gram-positive and Gram-negative bacteria, and fungi. The inclusion of magnesium sulphate results in the *Ureaplasma urealyticum* colonies having a black coloration in the presence of urea (4). On agar media, the mycoplasma colonies are small and should be identified with the aid of a microscope.

It is recommended that as a complement to the identification of mycoplasmas, a solid media method should be combined with the use of a liquid media method (3). This can be achieved by using the MYCOSCREEN or MYCOFAST liquid methods in combination with the A7 Agar method.

4 - REAGENTS

Products: 8 Agar plates

Description

A7 AGAR : 55mm ready-to-use agar plates, individually wrapped in a cellophane packet.

Composition of the agar

Mycoplasma broth (25 g/L), yeast extract (9.4 g/L), foal serum (15%), urea (1.15 g/L), arginine (0.4 g/L), calcium chloride (0.3 g/L), manganese sulphate (0.1 g/L), vitamin supplement, antibiotics, antifungal and agar (14 g/L).

5 - PRECAUTIONS

- The agar plates in this kit are intended solely for *in vitro* use and must be handled by authorised personnel
- Patient samples and inoculated agar plates are potentially infectious and must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
- The agar plates contain raw materials of animal origin and must be handled with caution.
- Do not use the agar plates after the expiry date or do not use agar plates that have been contaminated or that have been poorly conserved before use.

6 - SAMPLE COLLECTION AND HANDLING

6.1 Sample collection

- Endocervical / Vaginal Samples: Use only a Dacron® or rayon swab or cytobrush (if using a 2 mL or 3 mL UMMt), or use the swab provided with the AMIES transport medium or the universal transport medium for viruses, chlamydia, mycoplasma and ureaplasma (if using a UMMt AMIES 2.6mL).

The cervix should be carefully cleaned with a swab to remove secretions before collecting the sample with a new swab. As Mycoplasmas adhere strongly to mucous cells, the mucous lining should be scraped well to obtain a rich specimen (1).

- Urethral sample collection: Clean the meatus and swab or scrape the area to obtain cells.

- Sperm, urine, other liquids: Collect sperm, the first micturition or other liquid samples in a sterile vial.

6.2 Sample transport

UMMt 2 or 3 mL

Inoculate a vial of UMMt medium with the swab sample, or if using a liquid sample transfer 200 µL or 300 µL of the liquid sample in 2mL or 3mL UMMt. Once seeded the UMMt medium can be conserved for up to 20 hours at room temperature (18 to 25 °C), or for up to 56 hours at 2 to 8 °C. For a storage during 3 days at -20 °C, first add two drops of "MYCOPLASMA Stabilizer"

AMIES medium or universal medium for viruses, chlamydia, mycoplasma and ureaplasma

Refer to the manufacturer's operating instructions

UMMt AMIES medium

Seed 300 µL of the AMIES transport medium or universal medium for viruses, chlamydiae, mycoplasma and ureaplasma into a vial of UMMt AMIES medium.

Once inoculated, the UMMt AMIES medium (2.6mL) can be stored at room temperature (18-25 °C) for 20 hours, or at 2-8 °C for 56 hours.

For storage for 3 days at -20 ° C, add 2 drops of "MYCOPLASMA Stabilizer" beforehand.

7 - PREPARATION AND STORAGE OF REAGENTS

The agar plates are ready-to-use. Stored in their original packaging the reagents are stable until the expiry date shown on the packet.

Do not expose the agar plates to large variations in temperature.

8 - MATERIALS REQUIRED BUT NOT PROVIDED

- Sampling equipment (Dacron® swab, cytobrush, AMIES transport medium or universal transport medium for viruses, chlamydia, mycoplasma and ureaplasma, sterile bottle for the collection of liquid specimens), Pipettes
- MYCOPLASMA Stabilizer (REF 00064)
- UMMt medium (REF 00835; 00061; 00083) for swab samples or samples in AMIES transport medium or universal transport medium for viruses, chlamydia, mycoplasma and ureaplasma
- Incubator calibrated at 35 to 37 °C, facilities for reproducing an anaerobic environment, optical microscope (x10 objective), waste container for contaminated waste

9 - METHOD

9.1 Inoculation

- Swab samples: Inoculate a vial of UMMt medium with the swab sample and mix well. Place 3 drops, each of about 30 µL (or a single transfer of 100 µL) of the seeded UMMt medium directly in the centre of an agar plate.

- Swab samples associated with their AMIES transport medium or universal transport medium for viruses, chlamydia, mycoplasma and ureaplasma: Homogenize the transport vial and transfer 300 µL into a UMMt AMIES medium. Place 3 drops, each of about 30 µL (or a single transfer of 100 µL) of inoculated and homogenized UMMT AMIES medium directly in the centre of an agar plate.

- Liquid samples: Mix the sample and place 3 drops, each of about 30 µL (or a single transfer of 100 µL) directly in the centre of an agar plate. If the sample must be transported, inoculate a 2 mL vial of UMMt medium with 200 µL of the liquid sample, or a 3 mL vial of UMMt with 300 µL of liquid (§6.2).

9.2 Incubation of the agar plate

Allow the agar plate to dry for at least 30 minutes at room temperature.

Incubate the agar plate for 48 hours at 35 to 37 °C under anaerobic conditions.

10 - READING AND INTERPRETATION

The colonies should be identified with the aid of an optical microscope (x10 objective), ensuring that the agar plate is read upside down.

10.1 Morphological identification

Ureaplasma urealyticum: Appearance of a brown-black precipitate (variable size, "sea urchin shaped").The colonies are small.

Mycoplasma hominis: "fried egg shaped" appearance. The colonies are larger than those of *U. urealyticum*.

10.2 Enumeration

Enumeration is performed upon an upside down agar plate with the aid of an optical microscope (x10 objective). The number of colonies per field of view is recorded according to the average of the number of colonies in 10 microscopic fields:

Less than 1 colony / field: $<10^3$ CFU/mL
1 to 5 colonies / field: approx. 10^4 CFU /mL
5 to 10 colonies / field: approx. 10^5 CFU /mL
10 to 20 colonies / field: approx. 10^6 CFU /mL
> 20 colonies / field: > 10^6 CFU /mL

CFU: Colony Forming Units

11 - QUALITY CONTROL

Quality control can be carried out from a lyophilized reference strain (*Ureaplasma urealyticum* ATCC 27618) adjusted to 10^4 CFU/mL. Inoculate an A7 Agar plate and proceed as indicated in this leaflet (§9 et §10). Expected Result: Presence of 1 to 5 "sea urchin shaped" colonies (*U. urealyticum*).

12 - CAUSES OF ERROR

- Sampling with unsuitable swabs or transport media.
- Direct inoculation of the agar plate with the swab sample.
- Incubation under aerobic conditions.
- Not respecting the recommended incubation temperature.

13 - LIMITS OF THE METHOD

- Samples containing a low mycoplasma load ($<10^3$ CFU/mL).
- The solid A7 Agar method should be combined with a liquid method.
- As for all microorganism detection methods, the quality of the sample can influence the test results. A negative test does not therefore necessarily indicate the absence of infection.

14 - PERFORMANCE

The performance of the A7 Agar culture method was evaluated by comparison with the MYCOFAST liquid method (2). The study was carried out in a hospital on 544 clinical samples (266 sperm samples, 155 endocervical samples, 82 placentas, 19 urethral samples and 22 miscellaneous samples). For 475 samples (88%), of which 140 were positive and 335 were negative, the results were concordant. The discordances concerned 69 samples, of which 8 were positive only with the A7 Agar (1,5%), 48 were positive only with the MYCOFAST method (8,8%) and 13 were contaminated (2,4%). Certain mycoplasma strains originating from contaminated samples could be detected with the A7 Agar.

15 - WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and the current regulations for this kind of product in the country of use.

16 - BIBLIOGRAPHY

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The changes from the previous version are highlighted in grey.

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