SIR MYCOPLASMA 62781
10 TESTS

ANTIBIOGRAM FOR UROGENITAL MYCOPLASMA
1- CLINICAL USE
SIR MYCOPLASMA is a reagent for the antibiogram analysis of urogenital mycoplasma: *Ureaplasma* spp (*Ureaplasma urealyticum, Ureaplasma parvum*) and *Mycoplasma hominis* (Mh). At present, the differential identification of *Ureaplasma urealyticum* and *U. parvum* is difficult to achieve.
SIR MYCOPLASMA consists of a microplate containing 8 antibiotics active against these microorganisms and commonly used for the treating of genital infections.
The emergence of resistant strains, particularly to cyclines and macrolides (first–line treatment antibiotics) (10, 11, 18), renders antibiogram analysis absolutely necessary in order to avoid therapeutic failures.
The use of a standard inoculum makes this technique highly concordant with the method of reference (9).

2- PRINCIPLE
SIR MYCOPLASMA is a liquid–medium antibiogram where each antibiotic is present at two different concentrations (doxycycline, tetracycline, azithromycin, josamycin, erythromycin, ofloxacin) or a single concentration (clindamycin, pristinamycin).
The test is based on metabolic inhibition.
The growth of mycoplasma is objectively measured by their metabolic activity: hydrolysis of urea in U9 broth by *Ureaplasma* spp, and hydrolysis of arginine in arginine broth by Mh, with release of ammonia, which makes the medium turn alkaline and the phenol red pH indicator in the medium turns from yellow to red.
If the microorganism is sensitive to the tested antibiotic, its metabolism is inhibited and the medium remains yellow.
If the microorganism is resistant, it is able to grow and the medium turns red.

3- PRESENTATION
SIR MYCOPLASMA comprises:
- 10 SIR microplates (the diagram of the 16 wells is reproduced on the label, thus allowing identification of the sample).
- 10 adhesive cover seals.
- 1 package insert.
Each microplate is individually wrapped in aluminium with a cover seal and a desiccating sachet.

4- STORAGE
When stored at + 2 – 8°C, SIR MYCOPLASMA can be used until the expiration date printed on the packaging.
5- **EQUIPMENT REQUIRED (NOT SUPPLIED)**

- U9 broth: code 62762 (pack of 10 ampoules of lyophilised U9 broth Q.S.P. 2 ml)
- Arginine broth: code 62763 (pack of 10 ampoules of lyophilised arginine broth Q.S.P. 2 ml)
- MYCOPLASMA DUO (box of 20 tests – code 62740)
- Sterile distilled water
- 2 ml, 200 µl, 100 µl, 20 µl pipettes
- Incubator at 37°C
- Contaminated waste disposal container

6- **MODE OF OPERATION**

**A) THE SIR MICROPLATE**

The SIR microplate is a microstrip comprising two rows of 8 wells in which the various antibiotics are coated. The antibiotics are rehydrated when the inoculum is aliquoted in the wells to obtain concentrations that are approximate to the critical concentrations, thus allowing analysis of the sensitivity profile of the strains tested and classification as sensitive, intermediate or resistant for each antibiotic.

**Growth control in the absence of antibiotic**

- **Doxycycline** (4 mg/L and 8 mg/L)
- **Tetracycline** (4 mg/L and 8 mg/L)
- **Azithromycin** (2 mg/L and 4 mg/L)
- **Josamycin** (2 mg/L and 8 mg/L)
- **Erythromycin** (1 mg/L and 4 mg/L)
- **Clindamycin** (2 mg/L)
- **Pristinamycin** (2 mg/L)
- **Ofloxacin** (1 mg/L and 4 mg/L)

**B) CHOICE OF ANTIBIOTICS**

The choice of antibiotics to be tested was made based on therapeutic prescriptions advised for neonatal and genital mycoplasma infections (17). There is to date no critical concentration specific for mycoplasma; as a result, the critical concentrations chosen in the SIR MYCOPLASMA microarray are based on those published in a number of scientific journals and/or recommended by medical societies such as the CA–SFM (Antibiogram Committee – French Microbiological Society). The choice of azithromycin concentrations is based on an evaluation carried out using clinical strains (19).
Cyclines tested: tetracycline and doxycycline (at concentrations of 4 and 8 mg/L).

The Mh and Ureaplasma spp. strains described as being resistant to tetracyclines are detected unambiguously since it has been published that the Minimum Inhibitory Concentrations (MICs) were $\geq$ 8 mg/L as opposed to $\leq$ 2 mg/L for sensitive strains (15, 18). For this class of antibiotics, there are low-level resistances that require an incubation time of 48 hours (19). The frequency rate of resistance to cyclines among strains varies according to the country and the level of exposure to antibiotics (18).

Macrolides tested: erythromycin (at concentrations of 1 and 4 mg/L), azithromycin (at concentrations of 2 and 4 mg/L), josamycin (at concentrations of 2 and 8 mg/L).

Mh strains are naturally resistant to erythromycin and azithromycin. Josamycin remains active. Consequently, the results obtained with these antibiotics should confirm the identification of the strain previously obtained. The frequency of acquired resistance to macrolides among Mh and Ureaplasma spp strains isolated in the clinic is not known but probably is very low (18).

Lincosamide tested: clindamycin (at a concentration of 2 mg/L). Ureaplasma spp strains are naturally resistant to this antibiotic (18).

Streptogramine tested: pristinamycin (at a concentration of 2 mg/L). Mycoplasma strains are highly sensitive to this antibiotic.

Fluoroquinolone tested: ofloxacin (at concentrations of 1 and 4 mg/L). This molecule is active on Mh and Ureaplasma spp. The resistance frequency is not well known but has been estimated at less than 1% for Ureaplasma spp. (6, 18).

C) CARRYING OUT THE ANTIBIОGRAM

The antibiogram can be achieved starting from the content of well X of MYCOPLASMA DUO (code 62740).

For duration and temperature of biological sample storage, please refer to recommendations currently in use (13).

1) Standardisation of inoculum

In order to seed the antibiogram with an inoculum containing $10^3$ to $10^5$ CCU/ml (CCU = Colour Changing Units), it is necessary to carry out a pre-culture of the medium seeded with the sample.

Pre-culture (carried out following the protocols described below) leads to growth of the mycoplasma to a maximum titre of $10^6$ to $10^7$ CCU/ml. A 1/100 dilution of such a pre-culture in U9 or arginine broth, depending on the species isolated, produces a standard inoculum.
Using MYCOPLASMA DUO

When the antibiogram is carried out using MYCOPLASMA DUO, the content of well X at 24 or 48 hours of incubation for Mh, and 24 hours for Ureaplasma spp, corresponds to the pre–culture; thereafter, the content of the well needs to be diluted in U 9 or arginine broth following the protocol described below in order to obtain the standard inoculum.

1. For *Mycoplasma hominis*
Starting from well X (at 24 or 48 h of incubation), make a 1/100 dilution in arginine broth (20 µl of the content of well X in 2 ml of arginine broth).

2. For *Ureaplasma* spp
At 24 h of incubation: make a 1/100 dilution of the content of well X in U9 broth (20 µl of the content of well X in 2ml of U9 broth).

At 48 h of incubation: in this case, it is not possible to use the content from well X because there is a risk that Ureaplasma might have undergone autolysis. Make a 1/10 dilution of the Transport suspension medium stored at +4°C (200 µl in 2 ml of U9 broth).

Starting from mycoplasma–enriched culture broth

The urogenital sample can be placed in culture medium specific for mycoplasma (15). If one is waiting for identification and numeration results, the antibiogram can be carried out later and the medium stored at +4°C (cf. the product insert of the culture medium being used). Thereafter, incubation of the culture medium at 37 °C for 16 hours is recommended in order to obtain a mycoplasma–enriched inoculum.

The usual titre obtained is $10^6$–$10^7$ CCU/ml. According to the published recommendations (15), the realization of the antibiogram suggests using a standard inoculum. Thus, 20 µL of the enriched culture medium are diluted in 2 ml of U9 or arginine broth (1/100 dilution), depending upon the identification of the mycoplasma strain.

2) Aliquoting of the standard inoculum

100 µl of standard inoculum (1/100 dilution of the pre–culture, in U9 or arginine broth) is aliquoted in each well of the SIR microplate. Cover the microplate with sealing film and incubate at 37°C for 48 hours.
3. **TRANSPORT SUSPENSION MEDIUM**
   - 200 µl
   - 2 ml U9 Broth
   - 20 µl Arginine Broth

OR

2. **MYCOPLASMA-ENRICHED CULTURE MEDIUM**
   - 20 µl
   - 2 ml U9 Broth
   - 20 µl Arginine Broth

OR

1. **MYCOPLASMA DUO**
   - Incubation after only 24h
   - 20 µl
   - 2 ml U9 Broth
   - 20 µl Arginine Broth

SIR MYCOPLASMA

M. hominis Antibiogram

Ureaplasma spp Antibiogram
D) SAMPLES CONTAINING *Ureaplasma* spp and Mh

In this case, seed 2 SIR microplates: one with the standard inoculum obtained by diluting in U₉ broth (*Ureaplasma* spp strain antibiogram), and the other with the standard inoculum obtained by diluting in arginine broth (Mh strain antibiogram).

7- INTERPRETATION OF RESULTS

The antibiogram can be read as soon as the growth control wells have turned from yellow to red.

A reading is taken at 24 h and another at 48 h. The 48–h reading allows the detection of low–level resistances to cyclines (19) and confirmation of results when the starting inoculum turns out to be low (10³ CCU/ml) or when the colour change was doubtful after 24 hours.

| 2 yellow wells: | Absence of growth
| Sensitive strain |
| 2 red wells: | Growth in the presence of antibiotic
| Resistant strain |
| The well with a low concentration of antibiotic: red
The well with a high concentration of antibiotic: yellow | Intermediate strain |

For clindamycin and pristinamycin (a single concentration), only 2 results are possible: Sensitive or Resistant.

**Example:**

- yellow/yellow: S Sensitive strain
- red/yellow: I Intermediate strain
- red/red: R Resistant strain

**Particular case:** if an orange colour is observed. One may observe the beginning of a change from yellow to red, resulting in an orange colour in the low–concentration wells. This must be interpreted as positive and a clear–cut colour change can be obtained after 48 hours.

8- QUALITY CONTROL OF MANUFACTURER

All products manufactured and marketed by the Bio–Rad Company are monitored by a quality assurance system from reception of the raw materials through to marketing of the finished products.
Every batch of finished product is subject to quality control and will only be marketed if it complies with acceptance criteria. The documents on the production and control of each batch are kept for reference.

9- INTERNAL QUALITY CONTROL

Quality control can be carried out using a strain from a lyophilised collection (*Ureaplasma parvum* ATCC 700970). Resuspend the lyophilised strain in 1 ml of freshly reconstituted U9 medium (reference 62762). Make a 1/10 dilution in freshly reconstituted U9 medium.

Incubate the seeded U9 medium at 37°C for 18 hours under aerobic atmosphere.

Starting from this Ureaplasma–enriched U9 medium, make a 1/100 dilution (take a 20 µl aliquot of medium and add to 2 ml of reconstituted U9 broth).

Seeding of the SIR MYCOPLASMA microarray is carried out following the usual protocol, i.e. by aliquoting 100 µl in each well. Cover the microarray with sealing film and incubate at 37°C for 48 hours. The microarray can be read if the growth control wells have turned from yellow to red.

*The expected profile is as follows:*

<table>
<thead>
<tr>
<th>Growth control</th>
<th>Growth control</th>
<th>DO 4–8 mg/L</th>
<th>TE 4–8 mg/L</th>
<th>JM 2–8 mg/L</th>
<th>AZM 2–4 mg/L</th>
<th>E 1–4 mg/L</th>
<th>CM 2 mg/L</th>
<th>PT 2 mg/L</th>
<th>OFX 1–4 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S/I</td>
<td>R</td>
<td>S</td>
<td>S/I</td>
<td></td>
</tr>
</tbody>
</table>

10- PERFORMANCES

The choice of antibiotics tested using SIR MYCOPLASMA has evolved according to the therapeutic treatments, SIR MYCOPLASMA antibiogram has been re–evaluated in view of the modified treatments.

In the case of the two evaluations described below, the reference method used was that of Minimal Inhibitory Concentrations in liquid medium (MIC) (6, 15).

The first evaluation (without azithromycin wells) was carried out on 80 strains including 60 strains of *Ureaplasma* spp and 20 strains of *Mycoplasma hominis* (9).

With *Ureaplasma* spp strains:

For tetracyclines, concordance percentages varied from 90 % [79.5–96.2 %] a to 95 % [86.1–99 %] a depending on the antibiotic.
For macrolides, concordance percentages varied from 92 % [81.6–97.3 %] to 100 % [95.1–100 %] depending on the antibiotic.

For clindamycin, the concordance percentage was 85 % [73.4–92.9 %].

For pristinamycin, the concordance percentage was 100 % [95.1–100 %].

For fluoroquinolones, the concordance percentage was 93 % [83.8–98.2 %].

With *Mycoplasma hominis* strains:

The concordance percentage is 85 % [62.1–96.8 %] for tetracycline and 100 % [86.1–100 %] for all other antibiotics.

Total concordance for all the antibiotics mentioned previously is 94.6 % [92.4–96.4 %] with 2.6 % major discordances. No strain was found sensitive with the SIR MYCOPLASMA microarray when it was resistant with the reference method (no very major discordance).

A second evaluation was carried out following replacement of minocyclin by azithromycin at concentrations of 2 and 4 mg/L.

A comparative study was made between the method for determining MICs in liquid medium and the SIR MYCOPLASMA microarray for azithromycin wells. The strains tested are reference strains and wild–type clinical strains. Each inoculum was standardised and confirmed to be within acceptable and recommended limits, i.e. 10^4 and 10^5 CCU/ml (15). The results of the two methods were interpreted as Sensitive (S), Intermediate (I) or Resistant (R) for the various microarray concentrations.

For 15 mycoplasma reference strains (13 ATCC *Ureaplasma* spp strains and 2 ATCC *Mycoplasma hominis* strains), the concordance percentage for the S or R clinical classification was 100 % [81.9–100 %]. All *Ureaplasma* spp strains had a sensitive profile and the two *Mycoplasma hominis* strains had a resistant profile, which corresponds to the natural resistance of this species.

For 15 clinical isolates of *Ureaplasma* spp, the concordance was 100 % [81.9–100 %] between the clinical classification afforded by the SIR MYCOPLASMA microarray and that obtained with reference MICs.

*: [CI 95%] = 95% confidence interval.

11- LIMITS OF THE TEST

If the medium turns to red and becomes cloudy, this indicates growth of bacteria other than mycoplasma (the growth of the latter does not alter the clarity of the medium). This phenomenon can be observed in arginine and U9 culture broth if they have been incubated at 37 °C. The presence of other bacteria may be confirmed on chocolate agar.
The final interpretation of results, as for any biological result interpretation, cannot be made on the basis of a single test and needs to be based on clinical data and biochemical, cytological and immunological results.

If the inoculum used for seeding of the SIR MYCOPLASMA microarray is low (<$10^3$ CCU/ml), the colour change in the wells may be unreliable. Should the result appear abnormal, repeating the standard inoculum and antibiogram using a SIR MYCOPLASMA microarray is recommended.

If, for a given antibiotic, the well with the highest concentration is red and that with the lowest concentration remains yellow, the result cannot be used. It is recommended that another antibiogram be carried out starting from a new standard inoculum.

Macrolides, especially erythromycin, are known to be pH sensitive (14). Indeed, colour change in the low–concentration well may be observed, thus leading to an intermediate result owing to a pH effect rather than antibiotic activity.

This colour change phenomenon may also be observed with azithromycin wells.

12- BIBLIOGRAPHY


(US) - CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)
(F) - Marquage CE (Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro)
(E) - Marcado CE (Directiva europea 98/79/CE sobre productos sanitarios de diagnóstico en vitro)
(I) - Marchiatura CE (Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici in vitro)
(D) - CE Konformitätskennzeichnung (Europäische Richtlinie 98/79/EG über In-vitro-Diagnostika)
(P) - Marcação CE (Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico in vitro)
(S) - CE-mærkning (Europa direktiv 98/79/EF om medicintekniska produkter lär i vitro-diagnostik)
(DK) - CE-mærkningen (Europa direktiv 98/79/EF om medicinsk udstyr til i vitro-diagnostik)
(GR) - Χαρακτηριστικό CE (Ευρωπαϊκή οδηγία 98/79/CE περί in vitro διαγνωστικών εργαλείων)
(PL) - CE oznaczenie (Dyrektywa unijna 98/79/CE dotycząca produktów medycznych do badań in vitro)
(LT) - CE ženklas (Eurų sąsajos direktyva 98/79/EE dėl in vitro diagnostikos medicinos prietaisų)
(H) - CE jelzés (98/79/CE Európai irányelv az in vitro orvosi diagnosztikai eszközökkről)
(EST) - CE märgistus (Euroopa direktiv 98/79/EE in vitro diagnostikakomenditsemineadmete kohta)
(SK) - CE označenie o zhode (Európska direktíva 98/79/CE o in vitro diagnostické zdravotnické postupy)
(CZ) - CE značka (Evropská direktiva 98/79/CE o diagnostických zdravotnických prostředcích in vitro)

(US) - For in vitro diagnostic use
(F) - Pour diagnostic in vitro
(E) - Para diagnóstico in vitro
(I) - Per uso diagnostico in vitro
(D) - In-vitro-Diagnostikum
(P) - Para uso em diagnóstico in vitro
(S) - In vitro diagnostik
(DK) - I in vitro diagnose
(GR) - Για in vitro διαγνωστική χρήση
(PL) - Do stosowania in vitro
(LT) - in vitro diagnostikai
(H) - Csak in vitro diagnosztikai alkalmazásra
(EST) - In vitro diagnostiliseks kasutamiseks
(SK) - Na diagnostiku in vitro
(CZ) - Pro diagnostiku in vitro

(US) - Manufacturer
(F) - Fabricant
(E) - Fabricante
(I) - Produtore
(D) - Hersteller
(P) - Fabricante
(S) - Tillverkare av
(DK) - Fremstillet af
(GR) - Κατασκευαστής
(PL) - Producent
(LT) - Gamintojas
(H) - Gyártó
(EST) - Tootja
(SK) - Výrobca
(CZ) - Výrobce

(US) - Catalogue number
(F) - Référence catalogue
(E) - Número de catálogo
(I) - Numero di catalogo
(D) - Bestellnummer
(P) - Número de catálogo
(S) - Katalognummer
(DK) - Katalognummer
(GR) - Αριθμός κατάλογου
(PL) - Numer katalogu
(LT) - Katalogo numeris
(H) - Cikkszám
(EST) - Katalooginumber
(SK) - Katalógové číslo
(CZ) - Katalógové číslo

(US) - Authorised Representative
(F) - Représentant agréé
(E) - Representante autorizado
(I) - Distributore autorizzato
(D) - Bevollmächtigter
(P) - Representante Autorizado
(S) - Auktoriserad representant
(DK) - Autoriseret repræsentant
(GR) - Εκπροσώπησης εκπροσώπων
(PL) - Upoważniony Przedstawiciel
(LT) - Igaliotasis atstovas
(H) - Meghatalmazott Képviselő
(EST) - Voltatud esindaja
(SK) - Autorizovaný zástupca
(CZ) - Zpříslušněný zástupce

(US) - Expiry date DD/MM/YYYY
(F) - Date de peremption JJ/MM/AAAA
(E) - Estable hasta DD/MM/AAAA
(I) - Da utilizzare prima del GG/MM/AAAA
(D) - Verwendbar bis TT/MM/JJJJ
(P) - Data de expiração DD/MM/AAAA
(S) - Uitgangsdatum Dag/Mānad/Ār
(DK) - Anvendes før DD/MM/AAAA
(GR) - Ημερομηνία λήψης DD/MM/YYYY
(PL) - Data ważności DD/MM/YYYY
(LT) - Galioja iki DD/MM/YYYY
(H) - Szavatossági idő NN/HH/EEEE
(EST) - Aegumistähtest PP/ KK/AAAA
(SK) - Používateľné do DD/MM/RRRRR
(CZ) - Datum expirace DD/MM/RRRRR
(US) - The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.

(F) - Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.

(E) - Los otros idiomas que se requieren para la conformidad de la Directiva Europea pueden ser obtenida en su oficina local Bio-Rad.

(I) - Le altre lingue che sono richieste in conformità con le Direttive Europee possono essere ottenute dal locale agente Bio-Rad.

(D) - Die anderen Sprachen, die in Übereinstimmung mit der europäischen IVD Direktive benötigt werden, erhalten Sie über Ihre lokale Bio-Rad Niederlassung.

(P) - As restantes línguas, obrigatórias em conformidade com a Directiva Europeia, podem ser obtidas através da subsidiária Bio-Rad mais próxima de si.

(S) - Övriga språk som krävs i enlighet med EG-direktivet kan erhållas från din lokala Bio-Rad-representant.

(DK) - De øvrige sprog som kræves i henhold til EU direktiv kan fås ved henvendelse til den lokale Bio-Rad leverandør.

(GR) - Τις υπολογισμένες λεπτότητες που απαιτούνται για συμμορφώσεις στην ευρωπαϊκή οδηγία μπορείτε να τις προμηθεύεστε από τον τοπικό σας αριθμό της Bio-Rad.

(PL) - Tłumaczenie w innych językach które są wymagane w Dyrektywie Unijnej może być otrzymane od lokalnego przedstawiciela firmy Bio-Rad.

(LT) - Vertimas, reikalingus pagal Europos sąsajusios direktyvos reikalavimus, j kitas kalbas galite gauti iš vietinio Bio-Rad atstovo.

(H) - A leírás az Európai Irányelv által előírt egyéb nyelvenek hozzáférhető a Bio-Rad helyi kirendeltségeinél.

(EST) - Teised vastavalt Euroopa Direktiivile nõutavad keeled on saadaval kohaliku Bio-Rad-edasimüüja kliest.

(SK) - Ostatné jazykové verzie, ktoré sú vyžadované v zhode s Európskou direktívou, možno obdržať od vášho lokálneho zástupcu Bio-Rad.

(CZ) - Další jazykové verze vyžadované ve shodě s evropskou direktivou jsou k dispozici u lokálního zastoupení firmy Bio-Rad.