

Bacterial Antibiogram

DEFINITION

Bacterial Antibigrams is used for determination of susceptibility to antibacterials.

The method used diffusion in Agar Medium, i.e.:

- solid media distributed in Petri dishes (round or square),
- disks impregnated with different antibacterial drugs corresponding to the pharmaceutical specialities available to the clinic.

PRESENTATION

Media

See corresponding Technical Sheet(s)

Disks impregnated with antibacterials

Pack of 4 cartridges of 50 disks.

The list of these disks, with presentation codes, is available in our catalogue.

METHODOLOGY

Media used

- **Mueller-Hinton** **code 356-4884**
Agar at normal tonicity
- **Mueller-Hinton** **code 356-3825**
Blood agar (for *Streptococci*)
- **Mueller-Hinton** **code 355-6136**
Salt agar (for resistant, heterogeneous strains of b-lactamine *staphylococci*)

NB:

- Agar in the Petri dishes is 4 mm thick.

- Dishes filled with agar should be dried for 30 minutes at 37°C before use.

Inoculum

Only an inoculum satisfying both qualitative and quantitative criteria enables a valid appreciation of the sensitivity of a bacterial strain.

• Qualitative criteria

The inoculum must be prepared with a strain obtained from a pure, 18-hour culture in a liquid medium.

a) If the product to be analyzed is not polymicrobial, this culture can be carried out directly in a liquid medium.

b) Conversely, if it is a product contaminated by several bacteria, it is essential to carry out selective isolation on agar beforehand, then to proceed as follows:

- Prepare a haemolysis tube ($\varnothing = 13$ mm)

containing 1 ml of broth,

- Collect 1-10 strictly identical colonies of test bacteria from the agar and prepare a suspension in such a way as to obtain an opacity corresponding to an 18-hour culture.

• Quantitative criteria

The bacterial suspension obtained must then be diluted in such a way as to obtain confluent colonies on the agar for the study of sensitivity to antibacterial.

Proposed dilutions

<i>Enterobacteria, Pseudomonas</i>	1/300
<i>Staphylococci, Enterococci</i>	1/100
<i>Streptococci, Haemophilus</i>	1/10

These dilutions can be prepared with the aid of calibrated inoculating loops (a loop with a diameter of 1.5 to 2 mm delivers 1/50 ml).

Inoculation

• Flood the whole surface of the agar with 3-5 ml of the bacterial suspension dilution, removing the excess by drawing up, inclining the dish in several directions.

- Dry the dishes for 15 minutes at 37°C.

Application of disks

Deposit the disks on the agar, 15 mm from the edge of the dish, pressing lightly to ensure contact with the agar. This procedure can be done using tweezers, but use of a Bio-Rad automatic distributor speeds up the operation. It is possible to place:

- 6 disks on a round dish with \varnothing 90 mm,
- 16 disks on a square dish with 120 mm sides.

NB: The number of disks per dish should be such that zones of inhibition do not overlap.

Pre-incubation and pre-diffusion

In order to obtain a pre-diffusion of antibiotics, it is best to leave the dishes for 30 minutes at room temperature before placing them in the incubator at 37°C.

Reading

For each antibiotic:

- Measure the diameter of the zone of inhibition - using a caliper rule or compasses - as close as possible to the surface of the agar.
- Relate this measurement to the

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corresponding scale of concordance, starting at the extreme right of the schema (concordance scale supplied with our document *Bacterial antibiogram*).

The dish can be applied directly to the concordance scale, but this method is open to errors of parallax.

Interpretation

a) Categories of strains

- A strain is “**sensitive**” (S) when it is affected by the antibiotic treatment delivered by systemic administration at normal dosage.
- A strain is “**intermediate**” (I) when it is affected by: topical treatment or increased doses by systemic route.
- A strain is “**resistant**” (R) when it does not respond to antibiotic therapy, whatever the conditions.

b) Minimal Inhibitory Concentration (MIC)

The scale in the document *Bacterial antibiogram*, following the column of measurements, gives the concordance between the diameter of the zone of inhibition and M.I.C., determined in mg/ml by the method of dilutions in liquid medium.

IMPORTANT

The test of the antibiotic susceptibility of certain bacteria (e.g. *Neisseria*, *Haemophilus*, anaerobic bacteria) must be carried out according certain particular procedures described in specialized works which we can make available to you if necessary.

There is a technical sheet on the BK antibiogram.

STORAGE

- Media: see *corresponding Technical Sheet(s)*
- Disks: +2-8°C in a dry place
- Expiration date and batch number are shown on the package.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

BIBLIOGRAPHY

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