BK Antibiogram

357-0301

DEFINITION

Every pure strain (i.e. that has never been in contact with an antibiotic) of tubercle bacillus spontaneously includes a certain proportion of resistant bacilli:

- These may be selected by an inappropriate treatment, particularly a monotherapy. Bacilli within the patient then develop resistance to the antibiotic used: this is **acquired resistance**.
- Patients with resistant bacilli may contaminate healthy subjects, who will subsequently present tuberculosis to these same bacilli, whence the term **primary resistance**.

These two types of resistance - acquired and primary - of tubercle bacilli have been observed *in vivo* by clinicians and *in vitro* by bacteriologists.

The role of the antibiogram is to identify antibiotic(s) to which the bacillus is resistant and thus establish methods of treatment.

PRINCIPLE

By measuring resistance by the proportions method (1), the various media contained in this pack make it possible to determine the susceptibility of a strain of Koch's bacillus to the principal anti-bacterial:

- Izoniazid (INH): 4 concentrations (0.1; 0.2; 1 and 10 µg/ml)
- Streptomycin: 1 concentration (4 µg/ml)
- Rifampicin: 1 concentration (40 µg/ml)
- Ethambutol: 1 concentration (2 µg/ml)

The pack contains control tubes and media impregnated with antibiotics.

PRESENTATION

Packs

- 30 screw-capped tubes **code 357-0301**

STORAGE

- + 2°C to 8°C in a cool dry place.
- Expiration date and batch number are shown on the package.

1. DIRECT METHOD

Whenever a pathological specimen contains at least 1 BAAR* for 10 microscopic fields on a smear colored by the Ziehl-Neelsen method, or 1 BAAR per microscopic field on a smear colored by auramine, it is possible to carry out a direct antibiogram in the following manner:

- Using the bacilli-rich pellet resulting from centrifugation of the pathological specimen, make a stock suspension by adding 3 ml of sterile distilled water.

- Based on this preparation, considered a 1/10 dilution, make up 2 successive dilutions as in the table below: each dilution is inoculated at the rate of 0.2 ml per tube.

Microscope	Dilutions for inoculation
Less than 1 BAAR/field	undiluted and 10^{-2}
1 to 10 BAAR/field	10^{-1} and 10^{-3} dilutions
More than 10 BAAR/field	10^{-2} and 10^{-4} dilutions

* BAAR Bacillus Resistant to Acid and Alcohol

2. INDIRECT METHOD

Preparation of bacillary suspension

- Using a platinum inoculating loop, collect about 5 mg of culture. The specimen is placed in a sterile ball containing about 30 glass marbles of 3 - 5 mm diameter.
- Shake the ball and its dry contents for 20 to 30 seconds.
- Add 0.1 ml of sterile distilled water.
- Shake for 10-15 seconds.
- Add 5 ml of sterile distilled water.
- Shake for 10-15 seconds.

Calibration

Using sterile distilled water, adjust the opacity of the bacillary suspension obtained.

Inoculation

- Using this suspension, prepare base 10 dilutions, from 10^{-1} to 10^{-5}.
- Inoculate the series of tubes with the 10^{-1}, 10^{-3} and 10^{-5} dilutions.

Incubation

In both cases, whether direct or indirect method: after 3 or 4 days incubation at 37°C, the tubes are sealed hermetically, either by a screw top or by means of a plastic cap.

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READING AND INTERPRETATION

A reading can be taken from the 21st day, by counting colonies appearing in the different tubes, in order to deduce the proportion of resistant bacilli for the tested strain.

A strain is said to be **resistant** when the number of resistant bacilli it contains reaches or exceeds a certain ceiling percentage, generally fixed at 1%.

Dilution	Control tubes	Control PZA	RAMP* 40µg	SM 4µg	EMB 2µg
	Number of colonies per tube				
10^{-1}					
10^{-3}					
10^{-5}					
% of total bacillary population					

Conclusion: sensitive or resistant

Dilution	INH			PZA
	0.1µg	0.2µg	1µg	200µg
	Number of colonies per tube			
10^{-1} 10^{-3} 10^{-5}				
% of total bacillary population				

Conclusion: sensitive or resistant

* Tuberculous mycobacteria are also sensitive to Rifampicin and Rifamycin. Atypical mycobacteria present variable sensitivity with regard to these two antibiotics, e.g. *M. kansaii*, unlike tubercle bacilli, is resistant to Rifamycin S.V. which is incorporated, due to its better stability, instead of Rifampicine. In the case of this mycobacterium it will therefore be necessary to measure its sensitivity to Rifampicin on a medium prepared extemporaneously.

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

- **GROSSET J., MEYER L. (1980):** Mycobacteries atypiques et mycobactérioses. Encycl. Méd. Chirur., Paris, Mal. Infect. 8038 C_{10}, **7**.
- **CANETTI G., RIST N., GROSSET J. (1961):** Mesure de la sensibilité du bacille tuberculeux aux drogues anti-bacillaires par la méthode des proportions. Rev. Tubercul. Pneumol., **27**: 217.

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