

Instruction for Use for Diatabs™

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Diatabs™ **For bacterial identification**

Manufacturer

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Intended use

To be used in qualitative procedures to detect *in vitro* microbial properties for the identification of microorganisms. The identification tests are available as individual tablets.

Principles of the procedure

Diatabs can be divided into two groups: Diatabs to be placed on agar plates, and Diatabs to be dissolved in liquid. Most of the Diatabs provide rapid tests using chromogenic enzymatic reactions and modified conventional tests. Diatabs placed on agar plates detect resistance mechanisms, natural susceptibility patterns or growth factor requirements. Following incubation, the plates are examined and the zone diameters of inhibition around the tablet are measured and compared with the zone interpretation table (sensitive/resistant; positive/negative) for the individual tablets. Diatabs used in liquid are based upon enzymatic properties of the microorganisms detected by various indicator systems.¹

More information about Diatabs for bacterial identification may be obtained from User's Guide Diatabs² at www.rosco.dk.

Storage instructions

- 1) On receipt, check the temperature symbol on the label. Diatabs with a 2 °C to 8 °C symbol should be stored in a refrigerator, and Diatabs with a 25 °C as an upper temperature symbol on the label should be stored at room temperature.
- 2) If Diatabs are stored in the refrigerator, allow the vials to reach room temperature before opening, i.e. 30 - 60 minutes, in order to avoid condensation forming on the tablets.
- 3) Keep Diatabs in vials well protected from direct light and avoid high humidity. Keep, if any, the humidity absorbing material (a desiccant capsule) in the vial.

The expiry date on the vials applies only to vials with lids stored at the correct temperature.

Reagents

Diatabs are 6 or 9 mm tablets furnished in vials each containing 15, 25, or 50 tablets, or in cartridges with 50 tablets. The 9 mm tablets are print-coded on both sides with a unique 5-character code. The 6 mm tablets used on agar are colour-coded and the Diatabs to be dissolved in liquid have no code. The user should keep track of the contents of the test tube when using more than one Diatabs.

Precautions

Follow the directions for use. None of the tablets are intended for susceptibility testing. Diatabs performance depends not only on the specific Diatabs but also on use of proper inoculum, incubation time and interpretation of zone diameter or colour change. Only properly trained technicians should use the products. Use a pair of tweezers to pick up the Diatabs or use a dispenser (9 mm tablets in cartridges).

Precautions should be taken against microbiological hazards by properly sterilizing specimens, containers, media, and test tubes after use. Diatabs, test tube and agar plates with potential pathogens are disposed of as microbiological waste.

Specimen

The specimen should be collected and handled following recommended guidelines. The specimen should be a pure culture fully typical of the species to be identified.

Procedure

Materials provided: Diatabs as labelled on the container.

Materials required but not provided: Culture media, test tubes, reagents, quality control organisms and laboratory equipment necessary to perform identification e.g. inoculating loops, swabs, pipettes and collection containers.

I. Diatabs placed on agar

The susceptibility of microorganisms isolated from clinical samples against antimicrobials and other agents may be useful in the identification. It is possible to characterize and distinguish microorganisms by measuring the size of the zone diameter after culturing with a standardized inoculum applied to a specific agar medium.

I.1. Inoculum Preparation

Suspend several morphologically similar colonies from an 18-24 h cultured agar plate (non-selective) into 4-5 ml 0.9 % NaCl solution to obtain turbidity comparable to 0.5 McFarland standard.

I.2. Inoculation

Within 15 minutes, dip a sterile cotton swab into the adjusted suspension and remove inoculum from the swab by exerting firm pressure on the inside of the tube. Within 15 minutes, swabs are used to inoculate the agar specified in Table 1. Inoculate the dried surface of the appropriate agar plate by streaking the swab over the entire surface. Allow the surface to dry for 3-5 minutes or maximum 15 minutes before applying Diatabs to the agar surface.

I.3. Incubation and reading of plates

Within 15 minutes, place the agar side up and incubate plates depending on species and according to Table 1 (aerobic, anaerobic, or in 5-10% CO₂). Examine the plates after overnight incubation, if nothing else is specified in Table 1.

The diameters of the zones of complete inhibition are measured as determined by gross visual inspection. Zones are measured to the nearest whole millimetre.

RESULTS:

Compare recorded zone diameter with those in Table 1. Results for a specific specimen may be reported as Sensitive (S) or Resistant (R) according to the interpretation zones. Use Table 1 to make a preliminary identification of the bacterial strain.

For RESIST-ID DIATABS the results to be reported are dependent on the differences between individual tablets. Use **Table 3** to interpret the results.

II. Diatabs used in liquid

II.1. Inoculum preparation

The organism to be tested should be 18-24 hours old and in pure culture. Slow-growing isolates may be tested using 48-hour old culture.

Prepare a heavy suspension (at least McFarland 4 standard) of the test organism in 0.25 ml saline in a tube. A battery of Diatabs may be inoculated with a single inoculum.

II.2. Inoculation

Add one Diatabs to the tube. Some Diatabs additionally require 3 drops of sterile paraffin oil added to the tube (specified in Table 2).

II.3. Incubation and reading of tubes

Seal the tube and incubate at 35-37 °C for 4 hours, overnight or as specified in Table 2.

After incubation observe for colour development. For some Diatabs a reagent has to be added before reading the colour and some Diatabs may demonstrate two reactions.

After interpretation of colour development, add a reagent and read the new colour (specified in Table 2).

RESULTS:

Diatabs in liquid are scored by colour reactions. Table 2 gives colour reactions for negative and positive strains. Record the test score in an appropriate report form.

III. Diatabs with a different procedure

Oxidase

Lay a thick filter paper in an empty petri dish and place the Diatabs on it. Add one drop of saline on top of tablet, wait 60 seconds and add another drop of saline on the top. When the filter paper is wet, smear the colony onto the wet filter paper approximately 3-8 mm from the edge of the tablet using a plastic loop. Reading after 2 minutes. More than one isolate can be tested using the same Diatabs and filter paper.

Factor X, V, and X+V

Tablets with growth factors, hemin (X-Factor) and NAD (V-Factor) or both factors are used for the differentiation of the *Haemophilus* spp. All three tablets are placed onto the agar, Factor X and Factor V at a distance of 2 cm from each other and factor X+V further away from these. Use only medium free of the two growth factors (e.g. TSA agar).

Incubate overnight at 35-37 °C for 18-24 hours, and observe for growth or no growth in the vicinity of a tablet. If the organism requires Factor X alone, it will grow only in the vicinity of the X and X + V factor tablets; if it requires Factor V alone, it will grow only in the vicinity of the V and the X + V factor tablets; if both X and V factors are required, it will grow only in the vicinity of the X + V factor tablets.¹

INTERNAL QUALITY CONTROL

Quality control procedure using ATCC strains or known positive or negative strains should be used to monitor the performance of the tablets. Quality control should be performed in accordance with established laboratory quality control procedures. Negative/positive colour reactions, sensitive/resistant strains or positive/negative resistance mechanism are given in the Tables and indicate correct performance of the entire procedure. If aberrant quality results are noted, the results should not be reported.

LIMITATIONS OF METHOD

1. Pure cultures of microorganisms should be used, since a mixed microbial population will result in aberrant results.
2. Use of Diatabs for the identification of microorganisms and interpretation of results require a technician trained in general microbiological methods and who should judiciously make use of knowledge, experience, specimen information, and other pertinent procedures before reporting identity of the isolate.
3. Results from additional tests may affect the final identification result.
4. The tablets are not intended for use in susceptibility testing procedures.
5. The accuracy of the Diatabs is based upon known *in vitro* microbial properties for specific clinically important bacterial species ¹. If atypical or inconsistent results are encountered, repeat testing is recommended. An unexpected result should be considered for reporting and isolates could be sent to reference laboratories for further testing.

REFERENCES

- 1) Murray P.R. et al. 2003. Manual of Clinical Microbiology, 8th ed., ASM, Washington DC.
- 2) Diatabs User's Guide 5th ed. 2000. www.rosco.dk