



## Bacitracin Susceptibility Test Discs

DD015

Bacitracin Susceptibility Test Discs are used for the identification and differentiation of Group A streptococci (especially *S.pyogenes*) from other beta-haemolytic streptococci.

### Directions

**Pure Cultures :** Evenly inoculate the surface of Tryptose Blood Agar Base (M097) with pure culture of beta-haemolytic streptococci to be tested. Aseptically place a Bacitracin disc on the inoculated surface and incubate the inverted plate at 35-37°C for 18-24 hours in 10% CO<sub>2</sub>. Observe for the presence of zone of inhibition around the Bacitracin disc. A zone indicates that the Streptococcus is presumptively of Group A. If desired further confirmation can be obtained by serological grouping.

**Clinical Materials :** Incubate Tryptose Blood Agar Base(M097) plate with throat swab or other material. Spread the inoculum to obtain discrete colonies on some portion of the plate, so as to determine the species in mixed growth. Aseptically place a Bacitracin disc on the secondary area of inoculation and incubate the inverted plates for 18-24 hours at 35-37°C in 10% CO<sub>2</sub>. Examine for zones of inhibition. Bacitracin is inhibitory to many organisms except b-haemolytic streptococci, however the presence of a zone of inhibition does not essentially indicate Lancefield Group A streptococci. If the colonial morphology is carefully observed, it is possible to select presumptive Group A streptococci. By serological grouping, further confirmation can be obtained.

### Precautions

Use known Group A and non-Group A streptococci to determine the accuracy of the discs and inoculum.

### Principle And Interpretation

The growth of Group A beta-haemolytic streptococci on blood agar is inhibited by 0.04 units Bacitracin disc. Micrococci and streptococci are also inhibited by 0.04 units disc, while all coagulase-negative staphylococci are resistant (4).

Bacitracin susceptibility test discs are filter paper discs impregnated with 0.04 units of Bacitracin. Bacitracin discs can save considerable time, labour and materials if used as a screening test before serological grouping. Maxted showed that Group A streptococci were more sensitive to Bacitracin than beta-haemolytic strains of other groups (1). Hence he suggested that Bacitracin might be used as a rapid diagnostic agent for Group A streptococci.

Levinson and Frank(2) who employed Bacitracin impregnated filter paper discs for this purpose, observed that many sensitive beta-haemolytic streptococci were of Group A. Steamer et al compared Bacitracin disc, fluorescent antibody technique and Lancefield precipitin technique and found that the Bacitracin disc technique was most convenient for routine clinical laboratory (3). Bacitracin sensitivity test along with Furacin and Optochin tests are useful for distinguishing *Aerococcus viridans* and *S. milleri* from enterococci and *Streptococcus mitis* (2).

### Quality Control

#### Appearance

Filter paper discs of 6 mm diameter bearing letters "B" in continuous printing style.

#### Cultural response

Average diameter of zone of inhibition for *S.pyogenes* observed on Tryptose Blood Agar (M097) after an incubation at 35-37°C for 18-24 hours.

Organism	Zone of inhibition (mm)
<i>Streptococcus pyogenes</i>	15 -20 mm
ATCC 19615	

## Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

## Reference

1. Maxted W. R., 1953, J. Clin. Path., 6:234.
2. Levinson M. L. and Frank P.F., 1955, J. Bact., 69:234.
3. Streamer C.W et al, 1962, Am. J. Dis. Children, 104:157.
4. Guthof O., 1960, Ztschr. F hyg. U. Infektionskr., 146:425

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