BD BBL™ Prompt™ Inoculation System

For use with the Disc Diffusion Susceptibility Test

INTENDED USE

The BBL™ Prompt™ Inoculation System is used to prepare standardized suspensions of bacteria for the Bauer-Kirby disc diffusion antimicrobial susceptibility test procedure. The system may be used for rapidly growing bacteria such as Enterobacteriaceae, Staphylococcus spp., Pseudomonas aeruginosa, Acinetobacter spp., enterococci and some nonenterococcal streptococci. It also may be used for Haemophilus influenzae.

SUMMARY AND EXPLANATION

The Bauer Kirby procedure is a standardized method for the determination of antimicrobial susceptibility based on the agar gel disc diffusion principle.1-3 This method is published as a consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS) and is periodically reviewed and updated.4

The inoculum concentration has been shown to be an important factor in obtaining valid results with the Bauer Kirby procedure.2-5 the original Bauer- Kirby procedure employed inoculum prepared from log phase cultures. This requires incubation in broth for several hours to achieve the desired inoculum density, equivalent to the 0.5 McFarland turbidity standard, which produces approximately 1 x 108 CFU/mL.3-5 Several studies have subsequently shown that direct standardization of the inoculum, i.e., without incubation, is an acceptable alternative.5-7 The direct adjustment of inoculum preparation is the preferred method for fastidious organisms that may grow slowly in broth. The M2-T4 edition of the NCCLS disc diffusion standard contained a revised procedure for Haemophilus influenzae, in which a new medium, Haemophilus Test Medium (HTM), was employed.8 this procedure recommended matching the inoculum density to the 0.5 McFarland standard using a photometric device.

The BBL Prompt Inoculation System is a device that allows direct standardization of inoculum without adjustment of turbidity or preincubation.9-11 this method is considered acceptable for routine testing purposes.4 it has been shown that the Prompt system is also satisfactory for preparing inoculum for H. influenzae in the Bauer-Kirby procedure.12

PRINCIPLES OF THE PROCEDURE

The Prompt Inoculation System wand is touched to several bacterial colonies on a primary isolation plate and is placed in the tube of saline provided with the system. The bacteria are suspended in the saline by agitation with a vortex mixer. When the Prompt Inoculation System is used in this manner, an inoculum

Containing approximately 1.5 x 108 colony forming units per mL (CFU/mL) can be expected for most bacteria. This is equivalent to the inoculum density that is achieved by matching the turbidity to that of a 0.5 McFarland standard.4

Agreement of susceptibility results is greater than 95% when compared to the standardized method.11 The Prompt inoculum should be used within 6 h after preparation for most bacteria. For H. influenzae, the inoculum should be used within 3 h. The Prompt Inoculation System facilitates inoculation by eliminating:

1) The incubation period and 2) the need for manual adjustment of inoculum density.

PRODUCT DESCRIPTION

The Prompt Inoculation System consists of an inoculation wand and a tube of saline. The wand is a polypropylene rod attached to a stopper. At the tip of the wand are cross-hatched grooves designed to hold a specific number of bacteria. The cell suspending solution, consisting of 1 mL of sterile saline, is provided in a plastic tube with a snap off cap.

Warnings and Precautions:

For in- vitro Diagnostic Use.

1. Colonies must be selected from FRESH culture plates (≤ 24 h).
2. DO NOT FLAME the plastic inoculation wand. It will melt.
3. To avoid contamination, always keep fingers above the ridge on the inoculation wand.
4. Keep wands covered when not in use.
5. Do not use if colonies are very small (< 0.5 mm in diameter).

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After inoculation, the Prompt inoculation tube and wand and other contaminated materials should be sterilized by autoclaving.

Storage Instructions: Prompt units should be stored at room temperature, (2 – 27°C).

Product Deterioration: Prompt inoculation tubes should not be used if the tube or cap is cracked or if the saline is cloudy.

SPECIMEN COLLECTION AND TRANSPORT

Specimens received in the laboratory should be treated in the usual manner for the preparation of a primary culture plate.

PROCEDURE

Material Provided: 62 Prompt inoculation wands and 60 Prompt inoculation tubes.

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: The Prompt Inoculation System may be used for the preparation of inocula in disc diffusion procedures.

A. Preparation of Bacterial Suspension

1. Remove the required number of Prompt inoculation tubes from the box and place in a test tube rack.
2. Remove an inoculation wand from the box.

3. Holding the wand tip perpendicular to the agar surface, touch five isolated colonies greater than 1 mm in diameter. (As a reference, the tip of the wand is 2 mm in diameter.) Do not penetrate the agar. Do not scrape or drag the tip across the colonies.
NOTE: If the colonies are small (0.5 mm to 1 mm in diameter), touch ten instead of five. For very small, pinpoint colonies, continue incubation of the primary plate until they reach a diameter of approximately 0.5 mm to 1 mm. If the colony diameter is not likely to reach 0.5 mm (e.g., some streptococci), an alternative method for inoculum preparation should be used.
4. While holding the inoculation wand with one hand, remove a Prompt
Inoculation tube from the rack.
5. Bend the cap of the tube sideways until it snaps off.
6. Place the inoculation wand into the tube and press down with a twisting motion to assure a tight seal.
7. Vortex the tube vigorously for ten s to release the bacteria from the wand tip. If the organism is not released from the wand, let the solution sit for
5 min and vortex again.
8. Repeat steps 2 – 7 for all tubes.
9. The bacterial suspension should be used within 6 h of preparation. If not used immediately after preparation, shake vigorously to resuspend the bacteria just prior to use. For H. influenzae, use within 3 h of preparation.
B. Inoculation of Mueller Hinton Agar or HTM Agar Plate
1. Remove the inoculation wand from the tube. Discard in an appropriate container to be sterilized.
2. Dip a sterile cotton swab into the bacterial suspension. To remove excess liquid, rotate the swab several times with a firm pressure on the inside wall of the tube above the fluid level.
3. Streak the plate using conventional techniques and proceed with the susceptibility test.4
User Quality Control: The Prompt Inoculation System is used as part of a multicomponent diagnostic test. When employed for the NCCLS inoculum preparation procedure, it should produce the results given in the NCCLS standard for the control cultures.4
Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

RESULTS
When used according to directions, the Prompt Inoculation System will provide the proper inoculum for the Bauer-Kirby test for most clinical isolates. The susceptibility test results obtained with the Prompt Inoculation System show an overall 97.0% agreement with the standard Bauer-Kirby procedure.

LIMITATIONS OF THE PROCEDURE
1. The Prompt Inoculation System should not be used when the colony size is less than 0.5 mm in diameter. Examples of organisms that may not meet the
0.5 mm size requirement are Streptococcus spp. other than enterococci,
S. bovis and S. agalactiae (group B).
Picking colonies which are too small will result in underinoculation and may cause a resistant organism to appear to be susceptible. An alternate method of inoculum preparation should be used for colonies less than 0.5 mm in diameter.
2. Some mucoid organisms such as "stringy" Klebsiella or Pseudomonas may not adhere to the wand when attempting to pick up the colony. This will be visually apparent. An alternate method of inoculum preparation should be used for such organisms.

SPECIFIC PERFORMANCE CHARACTERISTICS
Three hundred (300) susceptibility results obtained from inocula prepared by the Prompt system technique were compared to the results obtained from inocula prepared according to NCCLS standard procedure.11 The relative frequencies of genera tested were Escherichia (16.7%), Klebsiella (10%), Proteus (14.7%), Providencia (8%), Morganella (4%), Enterobacter (8.7%), Serratia (4.7%), Pseudomonas (6.7%), Staphylococcus (12.7%), enterococci (8.7%) and other group D and group B Streptococcus (5.3%). The interpretive agreement
Between disc diffusion susceptibility tests inoculated according to the NCCLS standard procedure and the Prompt Inoculation System was 97.0% for 3,578 drug bacteria combinations. When held for 6 h, significant differences were noted in zone diameters; however, overall interpreted agreement was 97.9% for the 1187 drug bacteria combinations tested. The overall mean for 742 gram- negative and gram-positive bacterial counts was 1.85 x 108 CFU with a 95% confidence interval of 4.66 x 107 to 7.36 x 108 CFU.11 In another study with 14 strains of H. influenzae, the mean inoculum density of freshly prepared suspensions was 1.5 ± 0.1 x 108 CFU/mL. After 3 h at room temperature, the density was 0.7 ± 0.2 x 108 CFU/mL.13 In this study, the overall agreement when compared with a photometric device was 98.4%, and with the visual McFarland standard method, 97.9.13

AVAILABILITY
BBL™ Prompt™ Inoculation System, 60 Tubes and 62 Inoculation Wands.
Cat. No. 226306

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