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Rapid Inoculum Standardization System: a Novel Device for Standardization of Inocula in Antimicrobial Susceptibility Testing

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A rapid inoculum standardization system for antimicrobial susceptibility testing without incubation or the conventional turbidity adjustment has been developed. The rapid inoculum standardization system consists of a plastic rod with crosshatched grooves on one end and a specific nutrient medium in a vial. The crosshatched grooves are designed to pick up and release a known number of viable microorganisms. In use, the end of the rod is touched to five colonies 1 to 2 mm in diameter from a primary agar plate, thus filling the groves with bacteria. The rod is placed into the vial, and the bacteria are suspended in the medium by agitation with a Vortex Genie Mixer. The resulting suspension contains 5×10^7 to 5×10^8 CFU/ml for most gram-negative bacilli and gram-positive cocci. Microorganisms such as streptococci that have colonies less than 1 mm in diameter require as many as 10 colonies for an adequate inoculum suspension. Ninety-five commonly encountered bacterial isolates were tested in triplicate by agar plate counts. The resulting overall geometric mean of the agar plate counts was 1.52×10^8 CFU/ml for the species tested. We have found that the rapid inoculum standardization system provides a consistent and reproducible method for the standardization of inoculum for antimicrobial susceptibility testing without the incubation period and turbidity adjustment.

The procedure for single-disk agar diffusion antimicrobial susceptibility testing has been carefully standardized (2, 10, 12). The method of Bauer et al. (8) is the most widely recognized testing procedure for determining bacterial susceptibility to antimicrobial agents. The method of Barry et al. (4, 6) has been recognized as an acceptable alternative for testing common rapidly growing bacterial pathogens (10, 12). The accuracy and reproducibility of these bacterial susceptibility tests depends on the use of defined materials and methods (2, 8). One of the important requirements is the control of the bacterial population of the inoculum within defined limits (1). The Bauer-Kirby method requires a manual adjustment of the inoculum density to a 0.5 McFarland turbidity standard. The original Bauer-Kirby method should have an inoculum derived from a broth culture in log phase (8). It has been demonstrated that a suspension of growth from a primary culture plate less than 24 h old also provides an acceptable inoculum for the Bauer-Kirby test (7, 9, 10).

The present report describes a rapid alternative method for obtaining the standardized inoculum needed for an antimicrobial susceptibility test. The rapid inoculum standardization system described here provides an inoculum without incubation or the conventional turbidity adjustment. A plastic rod has been developed to pick up and release a known number of bacteria from a primary culture plate 24 h old or less. The rod is placed into a vial of specific nutrient medium (4), and the vial is vortexed to make a homogeneous bacterial suspension. The resulting inoculum suspension contains 5×10^7 to 5×10^8 CFU/ml for most gram-negative bacilli and gram-positive cocci and can be used immediately. This range is functionally equivalent to the 0.5 McFarland standard (3, 11). Agar plate counts were performed on 95 selected isolates. These isolates demonstrated a wide variety of growth characteristics and morphologies comparable to those found in clinical laboratory work.

The rapid inoculum standardization method provides a consistent and reproducible method for disk antimicrobial susceptibility tests without incubation and without the turbidity adjustment for standardization of the inoculum.

MATERIALS AND METHODS

Bacterial strains. The test microorganisms were strains from the Centers for Disease Control, Atlanta, Ga., supplied by C. Thornsberry and C. N. Baker.

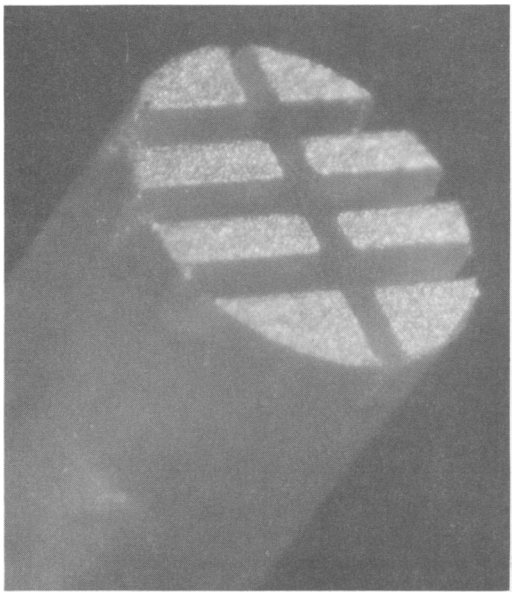

FIG. 1. End of the RISS inoculation rod. Magnification, ×450.

These organisms were maintained frozen in defibrinated sheep blood. The 95 isolates included 22 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 12 *Proteus mirabilis*, 1 *Proteus vulgaris*, 2 *Proteus rettgeri*, 2 *Proteus morganii*, 3 *Providencia stuartii*, 1 *Providencia alcalifaciens*, 3 *Serratia marcescens*, 1 *Enterobacter cloacae*, 1 *Enterobacter hafniae*, 2 *Pseudomonas aeruginosa*, 8 *Staphylococcus aureus*, 2 *Staphylococcus epidermidis*, and 20 *Streptococcus faecalis*. In addition, four isolates of *Escherichia coli* ATCC 25922, three of *Staphylococcus aureus* ATCC 25923, and two of *Pseudomonas aeruginosa* ATCC 27853 were included for quality control purposes. This group of isolates was selected to include a variety of growth characteristics and morphologies.

The organisms were removed from the freezer and thawed. The gram-negative isolates were subcultured onto MacConkey agar plates, and the gram-positive isolates were subcultured onto tryptic soy agar plates supplemented with 5% sheep blood (Diagnostic, Inc., St. Paul, Minn.) for the first subculture. The isolates were subcultured again before use. The inocula were prepared from the second subcultures.

Inoculum. The rapid inoculum standardization system (RISS) consists of a capped vial containing the inoculation rod and 0.8 ml of one of two broth media (4) in a crushable glass ampoule. These media were used only as suspending diluents. Five colonies 1 to 2 mm in diameter were picked from a primary subculture plate less than 24 h old with the inoculation rod (Fig. 1) and were placed into the vial. The ampoule was crushed, and the unit was vortexed for 10 s to release the bacteria from the grooves of the rod and to obtain a homogeneous suspension. For colonies of the *Streptococcus* species, approximately 0.5 to 1 mm in diameter, 10 colonies were picked. The suspension was used immediately without incubation or turbidity adjustment.

Colony counts were performed on each inoculum by using tryptic soy agar pour plates. All isolates were tested in triplicate. A 10-μl portion of each inoculum was diluted in 30 ml of sterile distilled water. A 10-μl portion of each dilution was added to the pour plate. The pour plates were incubated overnight for 24 h at 35°C and were counted with a 3M brand automatic colony counter, model 620 (Minnesota Mining & Manufacturing Co., St. Paul, Minn.). The geometric means and 95% confidence limits were determined.

Inoculation rod. A comparison was made between the number of colonies touched with the inoculation rod and the counts of viable bacteria obtained. Colony counts were done as previously described. The quality control organisms previously listed were used for this phase of the study. Also included was an isolate of *K. pneumoniae* 21, which is a very mucoid organism. The inoculation rod is shown in Fig. 1.

RESULTS

The colony counts of the number of viable organisms for the quality control bacteria are presented in Fig. 2. The means and ranges are presented and are within the target range of 5×10^7 to 5×10^8 CFU/ml. The colony counts for the test isolates are presented in Fig. 3. An overall geometric mean of 1.52×10^8 CFU/ml was found. The geometric means and 95% confidence limits are also presented in Fig. 3. The geometric means of the genera of bacteria tested were within the target range. The variety of colony morphologies tested does not adversely affect the ability of the device to obtain an

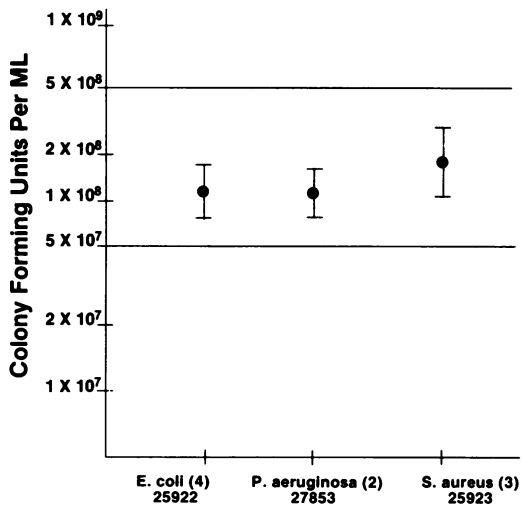

FIG. 2. Viable plate count ranges and means for the quality control organisms. Numbers in parentheses show the numbers of isolates tested in triplicate for each organism.

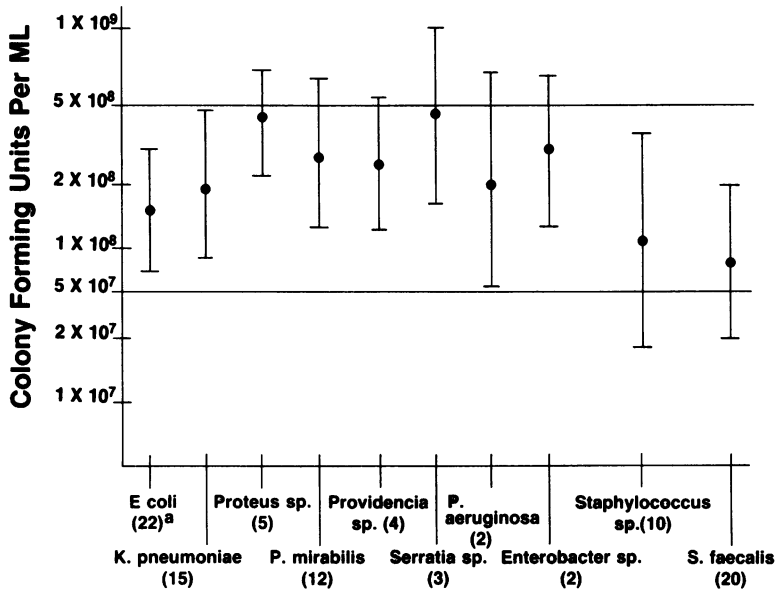

FIG. 3. Geometric means and 95% confidence limits for the test isolates. Numbers in parentheses show numbers of isolates tested in triplicate for each organism.

inoculum that is functionally equivalent to a 0.5 McFarland standard.

Preliminary disk susceptibility tests made on control organisms with the RISS were compared with the standards of the National Committee for Clinical Laboratory Standards (10). No major differences were found (data not shown). An expanded clinical evaluation of the use of overnight cultures and the 3M RISS will be published by C. Baker, C. Thornsberry, and R. Hawkinson. Their clinical evaluation provides substantial data on the performance of the RISS for disk and microdilution susceptibility tests.

Figures 4 through 7 show the relationship

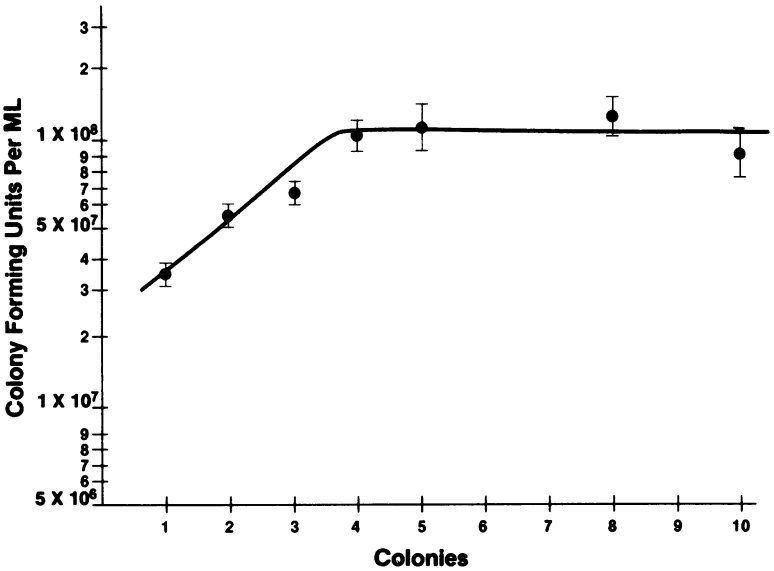

FIG. 4. Range and mean of viable *P. aeruginosa* ATCC 27853 obtained with the inoculation rod versus the number of colonies picked.

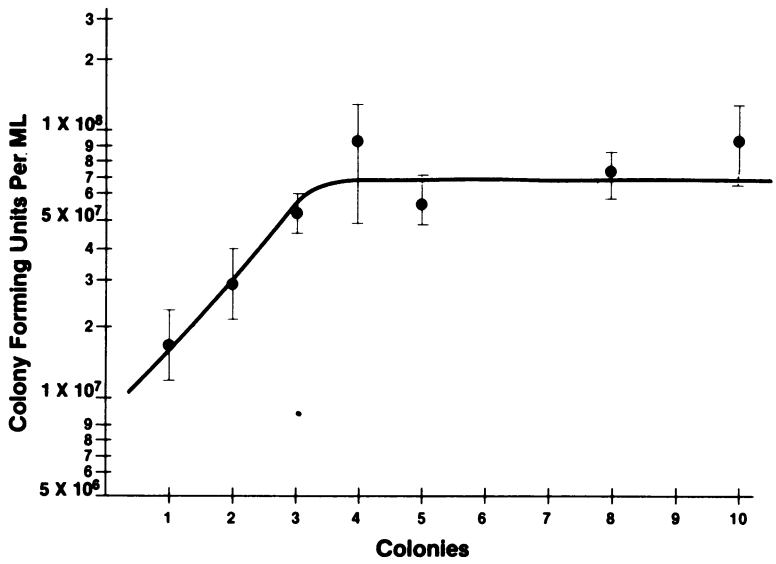

FIG. 5. Range and mean of viable *E. coli* ATCC 25922 obtained with the inoculation rod versus the number of colonies picked.

between the number of colonies picked and the viable colony count obtained. The data for *P. aeruginosa* (Fig. 4) indicate that with four to five colonies approximately 10^8 CFU/ml are obtained. *E. coli* (Fig. 5) shows a leveling off with four to five colonies at approximately 7×10^7 CFU/ml. The data for *S. aureus* (Fig. 6) indicate that two colonies will fill the rod to approximately 10^8 CFU/ml. Figure 7 shows that five colonies of *K. pneumoniae* will fill the rod at approximately 10^8 CFU/ml. The functioning of the inoculation rod demonstrates that *S. aureus* may fill up the rod with two colonies and not overfill when five or more colonies are picked. The same is true for *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. Once the rod end is filled, no overfilling was found when more colonies were touched. Therefore, five colonies 1 to 2 mm in diameter were selected to fill the rod end. In instances of very small colonies, e.g., *S. faecalis*

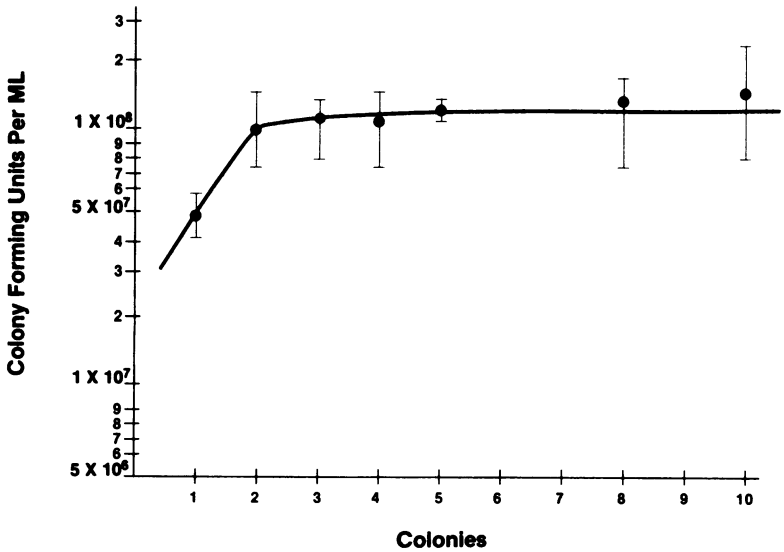

FIG. 6. Range and mean of viable *S. aureus* ATCC 25923 obtained with the inoculation rod versus the number of colonies picked.

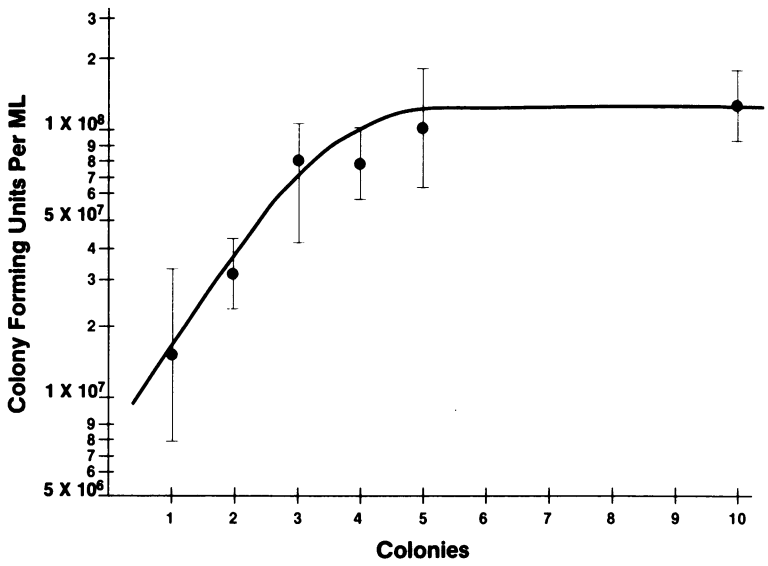

FIG. 7. Range and mean of viable *K. pneumonia* 21 obtained with the inoculation rod versus the number of colonies picked.

(Fig. 3), 10 colonies were selected, yielding approximately 10^8 CFU/ml.

DISCUSSION

The Bauer-Kirby method for single-disk agar diffusion susceptibility testing requires a manual adjustment of the inoculum density to a 0.5 McFarland standard, which is rather subjective and time consuming (4–6). This paper presents an alternate method for the inoculum preparation for the disk susceptibility tests. The RISS achieves an inoculum functionally equivalent to the 0.5 McFarland standard ± 0.5 log range. The results indicate that viable suspensions of bacteria can be made with the inoculation rod and used immediately without incubation or turbidity adjustment. The distinct advantage of this technique is the elimination of the incubation period and of the manual turbidity adjustment. This gives more flexibility to the work flow in the laboratory. The preliminary disk susceptibility results with the few quality control organisms tested indicated similar zone ranges for the RISS and the system of the National Committee for Clinical Laboratory Standards. Bacterial size and colony morphology do not adversely affect the accuracy and reproducibility of the inoculation rod method. No overfilling was found once the grooves of the inoculation rod were filled with microorganisms. We have shown that with the RISS direct suspensions of the inoculum can be prepared and used immediately as long as the number of viable organisms is standardized as reported previously by Barry (7). The RISS offers a convenient and simple inoculum preparation method, consistent and reproducible without incubation or turbidity adjustment, for standardization of the inoculum for antimicrobial susceptibility tests.

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