Modified Method for Direct Standardization of Bacterial Inoculum for use with the Disc Diffusion Susceptibility Test

SUMMARY AND EXPLANATION

The Kirby-Bauer procedure is used for the determination of antimicrobial susceptibility based on the agar gel disc diffusion principle and measurement of the zone(s) of inhibition. Mueller Hinton agar is the medium of choice and a liquefied inoculum is required for uniform coverage of the plate and proper interpretation. The Kirby Bauer method is published as a consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS) and is periodically reviewed and updated.

The inoculum concentration expressed as CFU/ml has been shown to be an important factor in obtaining valid results with the Kirby-Bauer procedure. The classical Kirby-Bauer procedure employed a bacterial suspension prepared from live cultures with an approximate concentration of 1 X 10^8 CFU/ml. Preparation required incubation in broth for 1-2 hours to achieve the desired inoculum concentration, visually equivalent to the 0.5 McFarland turbidity standard. Several studies have subsequently shown that direct standardization of the inoculum, i.e., without incubation, is an acceptable alternative for routine testing purposes. The direct standardization method should only be used for rapidly growing bacteria such as Enterobacteriacae, Staphylococcus spp., Pseudomonas aeruginosa, Acinetobacter spp., enterococci, and some non-enterococcal streptococci.

PRODUCT DESCRIPTION

This modification of the direct standardization procedure utilizes individual plastic tubes each containing 1 ml. of sterile saline and sealed with a leak proof cap. Also included are disposable plastic loops for harvesting colonies and sterile cotton swabs for distributing the inoculum over the surface of the Mueller Hinton plate. When used as directed this method should achieve a concentration of 1.0^8 to 1.5^8 CFU/ml.

PROCEDURE

1. For each organism to be tested, remove one tube from the package, loosen the cap, and place upright in a rack.
2. Remove one inoculation loop from the package using aseptic technique to avoid contaminating the remaining loops.
3. For colonies of the size typical of Enterobacteriacae, touch the loop to (2) representative colonies. (See notes below.)
4. Remove the cap from the upright saline tube, immerse the loop, and agitate to release the specimen into the saline.
5. Cap the tube and mix well by gently inverting several times.
6. Remove the cap and insert a sterile swab into the suspension allowing it to saturate.
7. Express excess fluid from the swab by rotating and pressing the swab firmly against the specimen container.
8. Streak the saturated swab over the entire surface of the Mueller Hinton plate 3 times rotating the plate ¼ turn after each streak.
9. Allow the plate to dry for 3-5 minutes but no longer than 15 minutes before applying the antibiotic sensitivity discs.
10. Incubate the inoculated plate at 37° for 24 hours and measure all resulting zones of inhibition. Record results.

Note: For small colonies (0.5-1.0 mm diameter), touch 5 colonies with the loop. For pinpoint colonies, continue to incubate the plate until colonies reach a minimum size of 0.5 mm. If the colonies fail to reach that diameter, an alternative method may be required.

TECHNICAL NOTES AND PRECAUTIONS:

1. This product is for veterinary use only.
2. Store tubes at room temperature.
3. If tube or cap is cracked, saline appears cloudy, or evidence of leakage has occurred, do not use that tube.
4. Colonies should be harvested from fresh culture plates (≤ 24 h) unless as noted above.
5. Observe aseptic techniques and established precautions against microbiological hazards throughout the procedure.
6. Following use, all materials must be safely disposed of in accordance with your local requirements for biohazard disposal.
7. Use of a 0.5 McFarland standard to assess turbidity will enhance precision.

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