

SOP-P075

Etest Strips_Antimicrobial Susceptibility Testing

Objective: A technique for determining the MIC (Minimum Inhibitory Concentration) Value of a given antibiotic in µg/ml that will inhibit the growth of a particular bacterium under defined experimental conditions.

Procedure:

Note: Be sure you have labeled your dish with the following information: Bacteria Type, Date, your Initials.

1. Remove one colony from your petri dish or test tube using a disposable inoculating Loop.(VWR cat#90001-100 BD Difco Blue 10ul) Not touching the sides of the tube, put the colony in the tube containing 4mls of sterile PBS. Swish back and forth and up and down until the colony is off the loop. Place the cap back on tightly and gently shake the tube against your hand until the colony is mixed well with the broth.
2. Soak a sterile cotton swab in the inoculum suspension and remove excess fluid by pressing it against the inside wall of the test tube. Remove more fluid when streaking a 90mm plate and less for a 150mm plate. Carefully streak the entire agar surface three times, rotating the plate 60 degrees each time to evenly distribute the inoculum.
3. Allow excess moisture to be absorbed for approximately 15 to 20 minutes so that **the surface is completely dry before applying the Etest gradient strips.**

Handling the Etest Strips:

4. Do not use if the Etest package has been damaged.
5. If stored in the refrigerator, allow the original package or storage container to reach room temperature before opening. Packages stored at room temperature can be used immediately.
6. When handling Etest strips manually, grip **only** the handle of the strip area labeled E. **Do Not Touch** the surface of the strip with the antibiotic gradient which is the opposite side of the MIC scale. Etest strips can be applied to the inoculated agar surface with forceps or a manual applicator.
7. Check that the inoculated agar surface is completely dry before applying Etest gradient strips. Open the package and handle the Etest strips as described in step #6. A template can be used to optimally position Etest strips in an equidistant pattern on an agar plate.
8. Be sure that the whole strip is in complete contact with the agar surface. **Do Not** place the strip upside down as no inhibition ellipse will form since the antibiotic will not diffuse across the non-porous plastic strip. If air pockets are seen under the strip, remove them by pressing gently on the strip (without moving it) with the applicator tip or forceps, working from the low concentration upwards. Small bubbles will not affect results.
9. **Once applied, the strip cannot be moved because of instantaneous release of antibiotic into the agar.**
10. In a 37° incubator, place the agar plates in an inverted position (lid down) in stacks no higher than 5. Let the plates incubate for the recommended period before reading, especially for delayed expression of resistance and slow growing and fastidious organisms.

Created by: Cheryl Holdman

Verified by: _____ Date: 8/30/2011 _____

Print Name

Sign Name