Disk diffusion susceptibility testing (Bauer-Kirby method)

For staphylococci, Enterobacteriaceae, Pseudomonas aeruginosa, enterococci, and other organisms that grow well on Mueller-Hinton agar in air at 35°C

1. Touch 4-5 isolated colonies of the SAME type with a loop or swab to pick up tiny amount from each one. Pure culture required.

2. Rub colonies on side of 5 ml saline tube to make very thin smooth suspension.
   - Draw lines on card

3. Check using lined card & add more organisms or dilute the suspension to match McFarland 0.5 turbidity.

4. Dip new swab into suspension & squeeze out excess liquid on sides of tube.

5. Rub swab over same Mueller-Hinton plate total surface 3 times in 3 different directions to create smooth layer of inoculum.

6. Within 15 minutes, add the antibiotic disks.

7. Tap each disk or tap plate upside down to be sure disks do not fall off.

8. Incubate 16-18 hours in air at 35°C.

9. Measure in mm diameter of zone of inhibition across center of disks using ruler or calipers.
   - No zone = 6 mm

10. Use CLSI tables to interpret zone size results for each organism & drug combination.

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# Special susceptibility testing Gram +’s (agar diffusion methods)

**# 1 MRSA/MRSE**

*Staphylococcus aureus & S. lugdunensis*
- Use 30 mcg Cefoxitin disk to screen for MRSA
- Read after 24 hours incubation at 35°C
- Cefoxitin zone ≤ 21 mm report as Oxacillin-resistant (Not Cefoxitin)

For all other Coag Neg Staph: Cefox
- ≥ 25 mm = S
- ≤ 24 mm = R

**# 2 Clinda resistant staphylococci**

*Staphylococcus aureus & S. lugdunensis*
- Use Erythromycin and Clindamycin disks 15-26 mm apart to detect inducible clindamycin resistance

**# 3 VISA or VRSA**

*Staphylococcus aureus*
- Use Vanco screen plate & Vanco disk
- Growth on Vanco plate & no zone = VRSA
- Growth on Vanco plate & zone ≥ 7 mm = possible VISA/VRSA
- Etest or send isolate to reference lab

- BHl plate with 6 µg/ml Vanco
- Inoculate with swab from 0.5 McFarland suspension

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Detection of MRSA

*Staphylococcus aureus* & *S. lugdunensis*  
(oxacillin heteroresistance and inducibility)

- Do not trust automated systems (Vitek, MicroScan)
- Use 1 µg Ox AND 30 µg Cefoxitin disk to screen for MRSA
- Read after 24 hrs incubation at 35°C
- Cefoxitin results ≤ 21 mm OR Ox results ≤ 10 mm report as Oxacillin-resistant (Do not report Cefoxitin)
**#2 Special susceptibility testing S. pneumo (Etest) & ESBLs**

Use CLSI tables to interpret results for the zones of inhibition for each combination of organism and antibiotic.

**# 4 Pen-resistant S. pneumoniae**

*Streptococcus pneumoniae*
- Use Mueller-Hinton + 5% sheep blood agar
- Incubate in CO₂ instead of air
- Report actual Minimum Inhibitory Concentration
  - ≤ 2 = Suscept for IV or IM dose
  - ≤ 0.06 = Suscept for meningitis and oral dose
- Oxacillin disk can predict suscepct only (≥20 mm)

**# 5 Extended-spectrum beta-lactamases**

*Enterobacteriaceae*
- Presence determined by use of zone size thresholds
- Disk approximation tests for ESBLs no longer recommended

<table>
<thead>
<tr>
<th>CLSI M100-S20 (2010)</th>
<th>S</th>
<th>Int</th>
<th>R</th>
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<tbody>
<tr>
<td>Cefazolin</td>
<td>≥ 23</td>
<td>20-22</td>
<td>≤ 19</td>
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<tr>
<td>Cefotaxime</td>
<td>≥ 26</td>
<td>23-25</td>
<td>≤ 22</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>≥ 25</td>
<td>22-24</td>
<td>≤ 21</td>
</tr>
<tr>
<td>Ceftriaxone</td>
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<td>20-22</td>
<td>≤ 19</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥ 21</td>
<td>18-20</td>
<td>≤ 17</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥ 21</td>
<td>18-20</td>
<td>≤ 17</td>
</tr>
</tbody>
</table>

Report both Pen & Cefotaxime or Ceftriaxone MICs for S. pneumo from CSF

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Phenotypic testing of Enterobacteriaceae still necessary for some drugs

**Drugs for which ESBL testing is still necessary**

- Cefamandole
- Cefoperazone
- Cefonicid
- Moxalactam

Place disks ~15 mm from central Amox/Clav disk

Extension of zone of inhibition toward the Amox/Clav disk in the center indicates presence of ESBL (keyhole test)
#3 Special susceptibility testing ESBL’s

Still need this method for Cefamandole, Cefoperazone, Cefonicid, Moxalactam

Extended-spectrum beta-lactamases  2nd method

Use direct McF 0.5 E. coli ATCC 25922 suspension to inoculate plates

E. coli, Klebsiella, Proteus, other GNRs

- Use 4 disks per organism
- >5 mm larger zone size with disk + Clavulanate than same drug without Clavulanate = ESBL

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Use CLSI tables to interpret results for the zones of inhibition for each combination of organism and antibiotic.

Always use direct suspension to inoculate plates.

Enterobacteriaceae
• Presence determined by use of zone size thresholds
• Modified Hodge test no longer recommended

<table>
<thead>
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<th>S</th>
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<tr>
<td>Imipenem</td>
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</tr>
<tr>
<td>Doripenem</td>
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<td>≤19</td>
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</tbody>
</table>

Modified Hodge Test no longer necessary.