**ETEST APPLICATION SHEET**

**SPECIMENS**
Cystic fibrosis, multiple drug-resistant organisms, critical specimens, critical infections, critical patients

**PROCEDURE**

- **Agar medium**
  See specific organism for appropriate agar media (e.g. MHA/aerobes, RPMI/fungi)

- **Inoculum**
  Suspension in saline (or broth) to 0.5 McFarland (or 1 McF). Dilute further 1:20, flood, remove excess fluid and dry the agar surface carefully.

- **Incubation**
  35°C (other) / ambient (other) / 24-48h (other) depending on the specific organism

- **Reading**
  For bactericidal drugs, read at complete inhibition of growth including microcolonies, hazes and isolated colonies. For bacteriostatic drugs, read at 80% inhibition when trailing is seen. When bactericidal is combined with bacteriostatic, read each agent according to their class.

**LITERATURE**

**DEFINITIONS**

- **MIC<sub>A</sub>**
  MIC of drug A alone

- **MIC<sub>B</sub>**
  MIC of drug B alone

- **MIC<sub>AB</sub>**
  MIC of A in the presence of drug B

- **MIC<sub>BA</sub>**
  MIC of B in the presence of drug A

**INTERPRETATION I**

- **Synergy**
  MIC of combination is ≥ 2 dilutions LOWER than MIC of the most active drug alone.
  e.g. MIC<sub>A</sub> = 8, MIC<sub>B</sub> = 16 (i.e. A= most active); MIC<sub>AB</sub> = 2. MIC of A is reduced from 8 to 2 in combination with B i.e. by 2 dilutions.

- **Antagonism**
  MIC of combination is ≥ 2 dilutions HIGHER than MIC of the most active drug alone.
  e.g. MIC<sub>A</sub> = 4, MIC<sub>B</sub> = 16 (i.e. A= most active); MIC<sub>AB</sub> = 16. MIC of A is increased from 4 to 16 in combination with B i.e. by 2 dilutions.

- **Indifference/additive**
  MIC of combination is within ± 1 dilution compared to the most active drug alone.
  e.g. MIC<sub>A</sub>= 1, MIC<sub>B</sub> = 2 (i.e. A= most active); MIC of A or B in combination = 1. Combination of A with B shows no change in MIC of A, the most active drug (Indifference)

**INTERPRETATION II**

These are arbitrary interpretive criteria for antibiotic combinations

<table>
<thead>
<tr>
<th>Fractional Inhibitory Concentration Index (FIC Index) calculations: FIC Index = MIC&lt;sub&gt;AB&lt;/sub&gt;/MIC&lt;sub&gt;A&lt;/sub&gt; + MIC&lt;sub&gt;BA&lt;/sub&gt;/MIC&lt;sub&gt;B&lt;/sub&gt; (see DEFINITIONS)</th>
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</thead>
<tbody>
<tr>
<td>Interpretation</td>
<td>FIC</td>
</tr>
<tr>
<td>Synergy</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Additive</td>
<td>&gt; 0.5 and ≤ 1.0</td>
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<tr>
<td>Indifference</td>
<td>&gt; 1 and ≤ 4.0</td>
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<tr>
<td>Antagonism</td>
<td>&gt; 4.0</td>
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</tbody>
</table>

**METHOD OVERVIEW**

**Fixed Ratio**

1. Perform standard MIC either in parallel with combination set-up or separately first.

2. Place strip A on the inoculated agar surface and leave for 1h at room temperature.

3. Mark the strip’s position on the back of the plate.

4. Remove strip A, clean with alcohol and save as a MIC reading scale.

5. Position strip B on top of the imprint of A e.g. ratio 1:1 (256/256), 1:8 (32/256).

6. Leave strip B on the agar plate and incubate.

7. Use the respective MIC strips/scales to read the MIC of each drug in the combination.

**MIC/MIC Ratio**

1. Perform standard MIC of drugs A and B prior to combination set-up.

2. Place strip A on the inoculated agar surface and leave for 1h at room temperature.

3. Mark the position of the strip and the MIC on the back of the plate.

4. Remove strip A, clean with alcohol and save as a MIC reading scale.

5. Place strip B on the imprint of A, vertically transposed so MIC<sub>A</sub> and MIC<sub>B</sub> overlap at the same position.

6. Leave strip B on the agar plate and incubate.

7. Use the respective MIC strips/scales to read by placing them in each gradient’s position.
### MIC/ MIC Ratio cont.

![Diagram showing the concept of superimposing gradients at different MIC positions.](image)

### RESULTS

<table>
<thead>
<tr>
<th></th>
<th>Single drugs</th>
<th>Combination (in MIC or fixed ratios)</th>
<th>Dilution difference</th>
<th>FIC index</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;A&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;B&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>Drug A</td>
<td>Drug B</td>
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<tr>
<td>Strain 1</td>
<td></td>
<td></td>
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<td>Strain 2</td>
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</table>

### REFERENCES

6. Etest references on file at AB BIODISK, please request via techsupport@abbiodisk.se