Gram & AF stains

Staining: Differential Stains

Gram stain

Acid-fast stain

Gram negative

Gram positive

Outer membrane

Peptidoglycan

Plasma membrane

Gram Stain

<table>
<thead>
<tr>
<th></th>
<th>Gram negative</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>before staining</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>primary stain, mordant</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>decolorizer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>counterstain</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
When staining an unknown, add controls!

Known Gram +  ?  Known Gram -

Acid-fast Stain

*mycobacteria*

Acid-fast Bacterial Cell Wall
Gram & AF stains

Mycobacteria colonies

Mycobacterium tuberculosis

http://www.textbookofbacteriology.net/tuberculosis.html

Acid-fast technique

<table>
<thead>
<tr>
<th>acid-fast species</th>
<th>non acid-fast species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. carbolfuschin</td>
<td></td>
</tr>
<tr>
<td>2. acid-alcohol</td>
<td></td>
</tr>
<tr>
<td>3. methylene blue</td>
<td></td>
</tr>
</tbody>
</table>

Gram stain protocol

1. Score with a labeled needle.
2. Score the smear with a labeled needle.
3. Score the smear with a labeled needle.
4. Score the smear with a labeled needle.
5. Score the smear with a labeled needle.
6. Score the smear with a labeled needle.
7. Score the smear with a labeled needle.