INTENDED USE

For the demonstration of Gram positive and Gram negative bacteria in tissues sections.
This method utilizes 2% alcohol rather than acetone to decolourize, and Twort’s stain as a counterstain leading the nuclei of Gram negative organisms to take up the neutral red component, and the background cytoplasm and collagen to take up the fast green component.

KIT PRESENTATION

Reagents

- Gram’s Crystal Violet 500mL
- Gram’s Iodine 500mL
- Twort’s Stain 500mL

Also required but not supplied:

- 2% acetic acid in alcohol
- 100% Ethyl Alcohol (Dehydrate)
- Xylene (Clear)

Do not use beyond the expiry date printed on the reagent labels.

TEST PROTOCOL

1. Dewax and rehydrate paraffin sections. (Remove mercury deposits if necessary.)
2. Stain with Crystal Violet for approximately 3 minutes
3. Rinse in gently running tap water.
4. Treat with Gram’s Iodine for 3 minutes.
5. Rinse with tap water and blot dry, dry in a warm place.
6. Differentiate with 2% acetic acid alcohol, section should appear light brown or straw coloured.
7. Rinse with water
8. Stain with Twort’s for 5 minutes.
9. Wash in distilled water.
10. Immerse slides in 2% acetic acid alcohol for about 5 seconds with agitation
11. Dehydrate and clear
12. Mount.

13. Examine under the microscope, using the oil immersion lens.

RESULTS

Placenta infected with E. coli and Streptococcus stained with Gram Tworts stain.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stain</th>
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</thead>
<tbody>
<tr>
<td>Gram Negative</td>
<td>pink / red</td>
</tr>
<tr>
<td>Gram Positive</td>
<td>blue / black</td>
</tr>
<tr>
<td>Nuclei</td>
<td>red</td>
</tr>
<tr>
<td>Cytoplasm, Collagen</td>
<td>green</td>
</tr>
<tr>
<td>Elastic fibres</td>
<td>black</td>
</tr>
</tbody>
</table>

PROCEDURAL NOTES

The preferred fixative for tissue sections is neutral buffered formalin.
Sections should be in paraffin embedded.
In step 10, rinse until no more red leaves the section.

REFERENCES

2. Twort F. W. (1924) J. State Med. 32 pg 351