APPENDIX

STAINS

1. GRAM'S STAIN

Crystal violet

Solution A

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Crystal</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol (95%)</td>
<td>- 20 ml</td>
</tr>
</tbody>
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Solution B

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Ammonium oxalate</td>
<td>- 0.8 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>- 80.0 ml</td>
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</tbody>
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Solution A and solution B should be mixed.

Gram's iodine (Lugol's iodine)

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<tbody>
<tr>
<td>Iodine</td>
<td>- 1.0 g</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>- 2.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>- 300 ml</td>
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</table>

Ethyl alcohol (95%)

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<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>- 0.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>- 5.0 ml</td>
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</tbody>
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Safranin

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Safranin</td>
<td>- 0.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>- 100 ml</td>
</tr>
</tbody>
</table>
2. Indole reagent – Kovac’s reagent

Para Dimethylamino benzaldehyde - 5.0 g
Conc. HCl - 25.0 ml
Amyl alcohol - 75.0 ml

3. Methyl red indicator

Methyl red - 0.1 g
95% ethanol - 300 ml

Methyl red was dissolved in ethanol and made up to 500 ml with distilled water.

4. VP test reagent (Barritt’s reagent)

Solution A

Alpha-naphthol - 5.0 g
Absolute alcohol - 100 ml

Solution B

Potassium hydroxide - 4.0 g
Distilled water - 1000 ml

Solution A and Solution B were mixed

5. Indole

Tryptone - 10.0 g
NaCl - 5.0 g
DL-tryptophan - 1.0 g
Distilled water - 1000 ml
pH - 7.0
6. MR-VP broth
   Peptone - 7.0 g
   Dextrose - 5.0 g
   Potassium phosphate - 5.0 g
   Distilled water - 1000 ml
   pH - 6.9

7. Phenol red broth
   Trypticase - 10.0 g
   Sodium chloride - 5.0 g
   Sucrose - 5.0 g
   Phenol red - 0.018 g
   Distilled water - 1000 ml

8. Simmon's citrate agar
   Magnesium sulphate - 0.2 g
   Ammonium dihydrogen phosphate - 10 g
   Dipotassium hydrogen phosphate - 1.0 g
   Sodium citrate - 2.0 g
   Sodium chloride - 5.0 g
   Bromothymol blue - 0.08 g
   Agar - 15.0 g
   Distilled water - 1000 ml
   pH - 6.9