

	<b>Stanton Territorial Hospital</b> P.O. Box 10, 550 Byrne Road YELLOWKNIFE NT X1A 2N1	<b>Document Name:</b> Gram Stain Job Aid  <b>Distribution:</b> Microbiology Microscopy Manual	<b>Document Number:</b> MIC20120  <b>Date Issued:</b> September 19, 2022
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## **Performing the gram stain:**

<b>1</b>	<ul style="list-style-type: none"> <li>• Prepare a slide of the sample and allow to dry</li> </ul>
<b>2</b>	<ul style="list-style-type: none"> <li>• Place slide on the slide warmer in the BSC until dry</li> </ul>
<b>3</b>	<ul style="list-style-type: none"> <li>• Fix smear with methanol for 1 minute</li> <li>• Leave for 1 minute and decant</li> <li>• Allow the slide to air dry again</li> </ul>
<b>4</b>	<ul style="list-style-type: none"> <li>• Flood smear with crystal violet</li> <li>• Leave for 1 minute and decant</li> <li>• Rinse slide gently with running tap water</li> </ul>
<b>5</b>	<ul style="list-style-type: none"> <li>• Flood the smear with Gram's iodine</li> <li>• Leave for 1 minute and decant</li> <li>• Rinse slide gently with running tap water</li> </ul>
<b>6</b>	<ul style="list-style-type: none"> <li>• Decolorize by letting the decolorizing reagent flow over the smear</li> <li>• Rinse slide gently with running tap water</li> </ul>
<b>7</b>	<ul style="list-style-type: none"> <li>• Flood smear with safranin</li> <li>• Leave for 1 minute and decant</li> <li>• Rinse slide gently with running tap water</li> </ul>
<b>8</b>	<ul style="list-style-type: none"> <li>• Place slides in a slide holder and allow to drip dry</li> <li>• Stained slides may be placed in the O<sub>2</sub> incubator</li> </ul>

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