WCSB, Wageningen University	IBISBA-SOP-WU12
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Gram staining of bacteria

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Instruction

Gram staining of bacteria

1. Introduction / Purpose

Use this protocol to perform the Gram staining of bacterial cells.

Keywords: Gram - staining – gram staining

2. Equipment and chemicals

2.1. Equipment

- Light microscope
- Heating plate

2.2. Chemicals

- A Gram staining kit can be purchased at Sigma-Aldrich (77730). Alternatively, they sell the components separately as well.
- Crystal violet solution
- Iodine
- Decolorizer
- Safranin
- Demi water, preferably from the tap
- Sterile demi water

2.3 Other materials

- Fresh agar plate with bacterial colonies

Special consumables

- Glass staining tray
- Microscope slides
- Filter paper

3. Procedures

Slide preparation

With a sterile loop place a drop of sterile water on a microscope slide. Take a new sterile inoculation loop and pick a very small sample of a bacterial colony from the plate and gently stir into the drop of water on the slide to create an emulsion.

Heat fixing

Place the microscope slide with the smear side up on a heating plate until the smear is dry (a few minutes).

Gram stain procedure

- Put the microscope slide across a staining tray that stands on a piece of filter paper.
- Add drops of crystal violet to cover the smear and let stand for 1 minute.
- Hold the slide upside down and gently rinse with running tap demi water.
- Put the slide back across the staining tray and add drops of iodine to cover the smear and let stand for 1 minute.
- Hold the slide upside down and gently rinse with running tap demi water. The smear will appear as a purple circle on the slide.
- Put the slide back across the staining tray and tilt it slightly. Apply the decolorizer solution drop by drop until the decolorizer solution runs almost clear. Be careful not to over-decolorize.
- Immediately hold the slide upside down and gently rinse with running tap demi water.
- Put the slide back across the staining tray and add drops of safranin to counter-stain and let stand for 30 seconds.
- Hold the slide upside down and gently rinse with running tap demi water. Air dry the slide.
- View the smear using a light microscope under oil-immersion.

5. Remarks / troubleshooting

- Use young cultures on agar plates (24-36 hours) because old Gram-positive cells can have a thinner peptidoglycan layer and thus will give a false Gram-negative result.
- Take a tiny bit of cells (when you are able to see them, you will have too much).
- Be careful with the decolorizer, you don't want to decolorize Gram-positive cells.
- If you want to label your slides, use a sticker and a pencil. Using a marker directly on the glass slide won't work because it will wash off during the procedure.
- The filter paper is used to keep your table clean.
- The heating plate is hot, **be careful not to burn yourself.**

6. Biosafety

No biosafety issues are associated with this protocol.

7. Acknowledgements

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