**Gram Stain Slide Preparation Procedure**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Purpose** Gram stain is the principal stain used for microscopic examination of bacteria. With this staining procedure, bacteria can be grouped into Gram-positive and

Gram-negative types by their reactions to the stain. Gram-positive bacteria stain

bluish-purple and Gram-negative stain pink.

When the bacteria are stained with primary stain crystal violet and fixed by Iodine (mordant), some of the bacteria can retain the primary stain and some are decolorized by acetone/alcohol. The cell walls of Gram-positive bacteria have a thick layer of protein-sugar complex called peptidoglycan which retains the crystal violet stain and cannot take up the safranin counterstain, giving it a bluish-purple color. Likewise, for Gram-negative bacteria, their cell wall also takes up the crystal violet, but their thin peptidoglycan layer is unable to retain the crystal violet upon decolorization with acetone/alcohol and gets counterstained by safranin leading to a pink appearance.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Scope** Phlebotomists, Medical Laboratory Technicians, and CLS may prepare and stain

smears. Only CLS will read and report Gram-stained smear results.

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Policy** • Positive Gram Stain from CSF and other sterile body fluids are considered

critical values and must be called to a licensed caregiver or provider

responsible for the care pf the patient and documented appropriately in the

laboratory information system (LIS) according to critical value reporting

policy.

• CSF specimens are prioritized as STAT and processed immediately upon

Receipt.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Specimen** The specimens may consist of a variety of clinical material. The following

**sources** processes are applicable to Sterile, Non-Sterile and Anaerobic Cultures.

1. For smears of pus, aspirations, or similar exudates, spread the sample to a

thin film over a large area (1.5 X 3.0 cm) on the slide by using a sterile loop

or applicator stick.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Specimen •** If in ESwab, vortex for 5 sec~~s~~ and place 1-2 drops of the fluid onto the

**sources,**  slide then spread the sample using sterile loop.

continued • **DO NOT** use the swab from the ESwab to spread the sample.

1. For specimens received in a syringe, transfer to a sterile tube and vortex if

appropriate.

1. Extremely thick or purulent specimens may be diluted in a drop of sterile

saline on the slide for easier preparation.

* If in ESwab, vortex 5 sec and place 1-2 drops of the fluid onto the

slide, then spread the sample using a sterile loop.

1. When Eswabs ~~(ESwab only)~~ are used to prepare the smear, remove 1-2

drops of transport fluid and place onto the microscope slide and allow to

air dry or place in 55°C heating plate.

1. For specimens where only one swab, if not an ESwab, is submitted,

inoculate the culture first, then use the swab last to prepare the smear. At

this point, the swab can no longer be used due to prospective

contamination from the slide. The swab should be discarded.

1. Prepare smears from swabs, if not an ESwab, containing dried material or small amounts of the specimen by emulsification in a drop of normalsterilesaline on the slide.
2. For smears of specimens such as sputum, select any flecks or mucus or blood-tinged particles present since they are more likely to yield infectious agents.
3. For **sterile sites** such as CSF and body fluids (amniotic fluid, aorta, aortic valves,bone, aspirates, brain, bursa, corneas, liver aspirates, etc.), specimens should be submitted in sterile leak-proof containers.

ESwab are also acceptable, but not preferred and should be discouraged.

1. For sterile body fluids, best practice is to prepare a Gram smear with a cytocentrifuge or centrifuge specimens according to local practices. Place 1-2 drops of the sediment onto a microscope slide and allow to air dry or place in 55°C heating plate.
2. Tissue mass such as bone, liver, lymph nodes, lung, spleen, should be submitted to the Regional Reference Laboratory (RRL) in leak-proof containers with minimal sterile saline (enough to keep it moist). ESwab may be used as a last resort where the provider pulls the swab off the cap and discards it, then places the tissue into the liquid. All biopsies and tissue sections will be processed at the regional laboratory (ground up, plated, and processed).

Gram Stain order can be canceled that is pending on the local list. Since RRL will process the tissue mass upon receipt and report the Gram stain results under the sterile site orderable, the appropriate cancel reason to use is “Order Transferred-current sample.”

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Specimen NOTE:** If the Gram Stain order is for a tissue specimen, but the swab **sources,** specimen received is a swabbed tissue and not an actual tissue mass,

continued these specimens should be processed at the medical center.

1. ESwab may be used for **non-sterile sites** (e.g., axilla, drainages, milk,

nipple discharge, rectal abscess, semen, urethra, wound abscess) **only if**

**it’s not possible to obtain the actual specimen.** Smears for Gram stain

are generally processed by the Regional Reference Laboratories (RRL).

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Speci**men Follow protocol for specimen rejection on specimen identification integrity.

**rejection \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Materials** The following materials and supplies are needed:

**and supplies**

**•** Clean glass slides

**•** Cotton swabs (Sterile)

• Inoculating loop, 10uL

• Saline (Sterile)

• Sterile pipettes

• Vortex

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Equipment**

• Cytocentrifuge (if available)

• Bacti-incinerator or heating Plate

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Safety or** • Standard precautions should be used when handling all specimens,

**Special** reagents, and controls.

**Safety •** Appropriate personal protective equipment should be used when testing

**Precautions** patient samples or performing scheduled maintenance.

• Personal Protective Equipment (PPE) includes gloves, laboratory coat,

and eye protection while handling any blood/body fluids.

• Always wash your hands before and after handling any biological

materials.

• Dispose of sharps according to policy and procedures.

• Dispose of reagent waste according to local policy and procedures.

• Refer to local Hazardous Waste Disposal Policy.

• **Note:** A staining rack is preferred to collect all the used stain for hazardous

disposal during manual Gram Stain procedure.

***Refer to the safety manual for general safety requirements***

***\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_***

**Quality**  Quality Control (QC) slides are ordered from AlphaTec Intelligent Diagnostics

**Control** or equivalent for Quality control use.

|  |  |
| --- | --- |
| QC Organism | Acceptable Result |
| *Staphylococcus aureus* ATCC 25923 | Purple = Gram Positive |
| *Escherichia coli* ATCC 25922 | Pink = Gram Negative |

Frequency, troubleshooting, and documentation of QC testing of Gram Stain

reagents.

1. Performed once each day of use by designated staff and with each new

Lot and or shipment of reagents.

1. If QC fails, do not report patient results. Check stain expirations and repeat

QC. Notify a manager if QC continues to fail.

1. For automated instruments, check volumes for the individual reagents being

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

**Quality Control,**

Continued dispensed. Perform preventive maintenance procedure if volumes are

incorrect. Check nozzle patterns for acceptability and if not, perform preventive maintenance to clean them. Repeat QC Gram stain. Notify a manager if QC continues to fail.

1. The results of the control smear must be read by a second and/ or third CLS as the case maybe. QC must be acceptable before patient smears are

reported.

1. QC must be documented each day of use on the Gram Stain QC logs.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**ESwab and** A**.** To prepare Gram stain smears of ESwabs specimens, as recommended by

**Urine** the manufacturer (applicable to Sterile, Non-Sterile and Anaerobic

**Specimen** Cultures)

**sources**

|  |  |
| --- | --- |
| Step | Action |
| 1 | Take a clean glass microscopic slide, place it on a flat surface and inscribe an area using a grease pencil to identify the location of the specimen inoculum. |
| 2 | Gently mix (vortex) the ESwab tube containing the swab sample for five (5) seconds to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid Amies transport medium. |
| 3 | Unscrew the ESwab cap and using a sterile pipet, transfer 1-2 drops of liquid Amies transport medium to the inscribed area on the glass slide. |
| 4 | Allow the smear to air dry or place the slide on an electric slide heater or 55°C heating plate for 10 mins. |
| 6 | Follow the laboratory procedure for performing the Gram stain. |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

B. Urine Specimens:

|  |  |
| --- | --- |
| Step | Action |
| 1 | Urine specimens should be discouraged from having a Gram stain performed due to poor sensitivity.  If performed, do not centrifuge urine specimens. Mix specimen well. |
| 2 | Use a sterile pipet to transfer one drop to a slide. Do not spread the drop out. Allow the drop to dry. |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Slide** Follow the steps below in preparing slides for Gram Staining.

**Preparation**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Label the frosted end of the slide with the following information to identify the specimen or culture. This may include (medical center dependent as determined by local practices):   * Patient last name, first name * Medical Record number and/ or last 6 digits of accession # * Date smear prepared * Specimen source |
| 2 | Make smears on clean, new 1” X 3” glass slides as per local practices.   * For fluid cultures, use a cytocentrifuge to prepare smears.   If a cytocentrifuge is not available, prepare smears from transport medium (eg. ESwab) by taking a loopful (10 µl minimum) of the medium and spreading it on a glass slide or directly from the sediment of centrifuged fluid. |
| 3 | Allow the smears to air-dry or place on 55°C heating plate for about 10 minutes. Methanol fix air-dried slides for 1 minute (up to 5 min as necessary) . |
| 4 | Gram stain slide by following Gram Staining procedure. |

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

*Continued on next page*

**Gram Stain Slide Preparation Procedure,** continued

*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

**NOTE:** Phlebotomist needs to prepare two slides, stain one and keep the

other slide on the extra tray for future reference. Affix the corresponding patient label on the tracking binder.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Controlled** The following controlled documents support this policy.

**Documents**

|  |
| --- |
| **References** |
| SCPMG-PPP-0530 Gram Stain Slide Preparation |
| SBC-PPP-0210 Gram Stain for Microbiological Specimens |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Authors •** Carolina C. Fletcher, MHA, CLS, BSMT

• Imelda Young, CLS