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| **Purpose** | This document will define the procedure for Gram staining bacteriology specimens. The proper staining and reading of Gram stains is essential to physicians for their diagnosis and treatment of patients’ conditions, especially regarding whether or not to use antibiotics, and the choice of antibiotics. The qualities of the specimen, as well as the presence of infection, are among the many clues discernable from a well-prepared and well-read Gram stain. |

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| **Policy** | Pre made control slides of gram positive and gram negative organisms is provided by the Regional laboratory. |

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| Workplace Safety |  |

 | All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.* For standard precautions and safety practices in the laboratory; see **Safety Practices**, specifically, but not limited to, equipment safety, proper body mechanics, sharps exposure and proper use of personal protective equipment (PPE).
* For Universal Body Substance precautions, see **Universal Body Substance Precautions**, specifically, but not limited to, exposure to body fluids.
* For proper hand-washing, see **Hand washing Policy**, specifically, not limited to, proper hand-washing.
* For proper infection control, see **Infection Control**, specifically, but not limited to, proper use of gloves.
* For proper handling of regular and infectious waste, see **Handling of Regular and Infectious Waste**, specifically, but not limited to, proper disposal of regular and biohazardous waste.
* For proper cleaning of work area, see **Cleaning Work Areas**.
* For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.
* For proper storage and disposal of chemical hazardous waste, see **Storage & Disposal of Chemical Hazardous Waste**.

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| **Specimen** | Any suitable specimen from the body which requires microscope identification of Gram positive or Gram negative organisms by using the Gram stain procedure. This may include any body fluid, any specimens for wounds, tissue, abscesses, lesions, aspirates, and drainage specimens as the primary specimen.**Note:****If received for gram stain, the following types of samples are not suitable for gram stain and must be rejected; stool, blood, tissue, catheter tip, and blood culture.** |

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| Equipment and reagents | Equipment* Microscopic slides
* Staining rack
* Microscope
* Immersion oil
* Ceramic Heat Block

Reagents* Crystal Violet Solution
* Gram Iodine solution
* Acetone-Alcohol
* Safranin
* Water

Note: all reagents received pre-made and ready to use. Replace stain daily. |

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| Quality control | Commercially prepared controls slides of gram positive and gram negative organisms are used for controls. 1. Gram stain control slides are stained once per day by the day shift to check on the quality of the staining procedure, of materials, and also on the gram staining technique. Each control slide includes both gram positive and gram negative bacteria.
2. A Laboratory Assistant may stain the slide for the Technologist to read
3. All Quality control results are recorded on the gram stain Quality Control sheet in the Bacteriology Quality Control notebook.
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| Procedure | Follow the steps outlined below to Gram stain**Verify that QC is performed for the day and that QC results are acceptable prior to testing any patients.** |
| **Gram Stain: SMEAR PREPARATION** Proper smear preparation should produce a monolayer of organisms sufficiently dense for easy visualization but thin enough to reveal morphological characteristics. ***NOTE:*** 1. ***When using the same pipette or swab, always inoculate culture media first before preparing the smear.***
2. ***Use clean, new glass slides.***
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| Specimen type | Action |
| **Specimens received on swabs** (A separate swab should preferably be submitted) | 1. Label clean slide with patient information
2. Roll the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements.
3. Alternatively, when only one swab is received, place the swab in a small amount of saline and vortex. Squeeze the swab against the side of the tube, and use the swab to prepare a smear. Use the remaining suspension to inoculate culture media.
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| **Specimens not received on swabs: aspirates, exudates, sputa, stools** | 1. Label clean slide with patient information
2. If the specimen is received in a syringe, first transfer entire amount to a sterile tube; vortex specimen if appropriate.

*NOTE: For syringe with attached needles, accept sample, safely remove and discard needle. Notify manager about incident. Manager will address safety issue with the department concerned and issue a UOR.*1. Select purulent or blood-tinged portions by using a sterile applicator stick, pipette, or wire loop. Very thick or purulent specimens may be diluted in a drop of saline on the slide for easier smear preparation.
2. Spread the sample over a large area of the slide to form a thin film.
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| **Urine Specimen** | 1. Label clean slide with patient information
2. Do not centrifuge; mix specimen well.
3. Use a sterile Pasteur pipette to transfer 1 drop to a slide; do not spread the drop.
4. Allow the drop to dry.
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| **Dried material or very small amounts of clinical specimens** | 1. Label clean slide with patient information
2. Emulsify specimen in 0.5 ml of sterile saline; vortex if necessary.
3. Use a sterile Pasteur pipette to transfer 1 drop to a slide.
4. Use the pipette tip to spread the drop into an even thin film.
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| Procedure | continued |

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| **SMEAR PREPARATION,** continued |
| Specimen type | Action |
| **CSF and other body fluids requiring centrifugation including samples collected using e-swab** | A. Using the Cytospin smear preparation for gram stain **(preferred method)*** Label slide with patient information and load to a Single Cytofunnel.
* Mix sample well, using a sterile transfer pipette drop 2-6 drops of CSF or other body fluids in the disposable cytofunnel well. Place cap on the chamber
* Load prepared cytofunnel to Cytospin for process
* Cytospin set at 7 min. 700 rpm LOW acceleration
* For more information about cytospin procedure see procedure **LAMC-PPP-0310 Cytospin Smear Preparation.**

B. Centrifugation of samples using sterile tube.* Centrifuge sample using a sterile tube at 3000rpm 10 min.
* After centrifugation, use a sterile pipette to remove supernatant to a sterile tube, leaving approximately 0.5 ml as sediment.
* Vortex or forcefully aspirate the sediment in and out of a sterile pipette several times.
* Label clean slide with patient information
* Use the sterile pipette to transfer a small drop of the sediment to a clean slide.
* Do not spread the drop out; allow it to air dry.
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| **Biopsies and tissue sections** | 1. Transfer test site to SWL Micro Man ss.
2. Send out specimen to SWL Regional lab for gram stain testing.
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| Procedure | continued |

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| Gram Stain: STAINING |
| Step | Action |
|  | Allow the prepared smear to air dry or place smear on a ceramic heat block set at 40°C.  |
|  | Flood the slide with crystal violet solution. |
|  | After 2 minutes pour off the crystal violet and gently wash the slide with water |
|  | Flood the slide with iodine solution. |
|  | After 1 minute rinse off the iodine solution with water. |
|  | Decolorize with the acetone-alcohol solution for 30 seconds and wash immediately with water. |
|  | Flood with safranin solution for 30 seconds and rinse off with water |
|  | Blot the slide dry with a clean filter paper or a clean paper towel. |
|  | Examine the smear under oil immersion (100X) |
|  | Reviewing Sterile Specimens with Positive/Abnormal results for Gram Stain* Sterile specimens with positive/abnormal results for Gram stain should be reviewed by another/second CLS.
* The slide should be reviewed by scanning the entire slide, same procedure the first CLS examined the slide
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The waste stream from the staining process is drained to a 5 gallon flammable hazardous waste container. When the container is full, it is stored in a large flammable cabinet located at room 1818 for disposal. Replace with an empty 5 gallon flammable waste container available at the same large flammable cabinet in room 1818.

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| **Interpretation** | 1. Gram positive bacteria stain blue or purple against a red or pink background while gram negative bacteria stain red or pink against a red or pink background.
2. All bacterial organisms are reported as well as any other organisms / elements present. This also includes the presence of fungi, white blood cells, mucus and epithelial cells if present.
3. Shapes of bacteria may be cocci (round), rods (elongate), diplococci (kidney bean shaped pairs), and coccobacilli (rounded and elongated).
4. The report should reflect the degree (Many, Moderate, Few, None) of quantity of organisms, fungi, and white cells (polys) present. If organisms are in clumps this must be noted. Fungi may be reported as budding or with hyphae.
5. If organisms appear gram variable it may be due to poor staining properties, therefore, the controls should be rechecked for proper staining characteristics and then a new smear of the patient should be prepared before reporting as gram variable.
6. If there are no organisms present, it must be stated as such.
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| REPORTING RESULTS: |
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| Step | Action |
| **1** | Log onto CERNER. |
| **2** | On your CERNER Apps:* Click on Result Entry for Microbiology

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| **3** | At the Result Entry:* Enter the accession number of the patient to be resulted on the Accession Field
* Type FIN on the Entry Field (final report)
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| **4** | At the Final Report window. * Under the Response section, you will have cells to input the necessary display code for reporting.

* Press **F2** to access the **Response List of Values**
* Filter the by choosing **Report Response**
* Click on the necessary response code for reporting and press **OK**
* After inputting the codes for resulting

- Choose **Perform** to hold the result if it needs to be called to a provider. (Positive/Abnormal results for CSF and Body Fluid should be called) - Choose **Verify** to release the Final report. |
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 **5** Inputting a comment for Positive/Abnormal results for CSF and Body Fluid

* After choosing Perform, under **Microbiology Result Entry**
* click on the **Comment** button 
* Under the **Comment** window, choose the **Order Note** tab and

 press the **Edit** button

* Press **F2** to access the templates used for called results.
* Fill up the necessary information like date, time, and name of the

 person called.

* Close the comment window
* Double click on the final report to be verified
* Choose the **Verify button** to result out the Final Report

Grading to follow for reporting results

Enter all elements seen semi-quantitatively.

 Rare (2-5 / 100X)

 Few (5-10 / 100X)

 Moderate (10-15 / 100X)

 Many (>15 / 100X)

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| DISPLAY | DESCRIPTION |
| CORRWP | Test performed on wrong patient |
| CRPT | Corrected Report |
| EPIS | Epithelial cells seen |
| F | Few |
| FES | Fungal elements seen |
| FUN | Fungal elements present |
| GNC | Gran negative cocci |
| GRPB | Gram positive rods, bacillus-like |
| GVCB | Gram variable coccobacilli |
| Intra | Intracellular |
| KOHN | No fungal elements observed |
| KOHY | Yeast observed |
| M | Many |
| Mix | Mixed flora, no predominant organism |
| Mod | Moderate |
| NF | Normal flora isolated |
| NFES | No fungal elements seen |
| No | No |

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| DISPLAY | DESCRIPTION |
| Nohy | No hyphal elements seen |
| NOS | No organism seen |
| NSQC | No squamous cells |
| NWBC | No white blood cells seen |
| OCC | Occasional |
| PLEO | Pleomorphic |
| PMN | Polymorphonuclear leukocytes |
| PMNS | Polymorphonuclear neutrophils |
| R | Rare |
| RBC | Red Blood Cells |
| S | Sparse |
| SGL | In single |
| Squam | Squamous epithelial cells |
| Tet | In tetrads |
| WBC | White blood cells |
| WBCS | White blood cells seen |

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| Procedural notes | The Gram stain procedure consists of staining a fixed film with crystal violet solution containing ammonium oxalate, washing this off with water, and flooding with Gram’s iodine solution. The iodine solution is washed after a time and the film decolorized with acetone-alcohol, and counterstained with a dye of contrasting color, such as safranin. By this means, bacteria may be divided into two groups: those which retain the original crystal violet in spite of the decolorization and appear dark purple on a background of the counterstain and are considered as gram-positive bacteria. Those which are decolorized and are colored by the counterstain are considered as gram-negative. Certain bacteria are gram-variable, sometimes retaining the stain and sometimes not. But this group is not large enough to detract from the practical differential value of the stain. Sometimes, inaccuracies may occur due to either under or over decolorization of the slide during the acetone-alcohol phase of staining. But this situation is usually corrected by repetition of the stain. Certain organisms such as Gardnerella vaginalis may stain gram-variable (showing both gram negative and gram positive characteristics). |

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| Controlled Documents |

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| **Document Number** | **Document Name** |
| LAMC-PPP-0123 | Safety Practices |
| LAMC-PPP-0127 | Infection Control |
| LAMC-PPP-0128 | Universal Body Substance Precaution |
| LAMC-PPP-0129 | Handling of Regular and Infectious Waste |
| LAMC-PPP-0130 | Cleaning Work Areas |
| LAMC-PPP-0132 | Hand-washing Policy |
| LAMC-PPP-0134 | Storage and Disposal of Chemical Hazardous Waste |
| LAMC-PPP-0310 | Cytospin Smear Preparation using Thermo Scientific Cytospin |

See below the list of controlled documents |
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