Dignity Health Central Coast Service Area

**SUBJECT**: Gram Stain Procedure

**ORIGIN:** Clinical Laboratory/Microbiology

| **Document Category:** | | | |
| --- | --- | --- | --- |
| ☒ Policy | ☒Procedure | ☐Standardized Procedure | ☐Other: |

| **Applies to:** | | |
| --- | --- | --- |
| ☒ Santa Maria Campus,  Marian Regional Medical Center | ☒Arroyo Grande Campus,  Marian Regional Medical Center | ☒French Hospital Medical Center |
| ☐St. John’s Pleasant Valley Hospital | ☐St. John’s Regional Medical Center | |

**\*NOTE - STAT GRAM STAINS:**

Includes all gram stains ordered STAT, plus all neonatal specimens, fluids, CSF, and blood cultures. STAT gram stains are to be performed immediately upon receipt and the stained slides presented directly to a CLS for reading and reporting.

# purpose:

The gram stain is one of the most basic of all bacteriologic tests and allows bacteria to be grouped according to their staining reaction. The difference in staining reaction between gram-positive and negative bacteria is attributed to the distinctive chemical and physical structure of the cell walls of these organisms. This differential stain is usually described as being related to the higher lipid content of gram-negative bacterial cell walls. Some suggest it is instead due to a reaction with a magnesium ribonucleate protein complex. Crystal Violet is taken up equally well by both gram positive and negative bacterial cell walls. A Crystal Violet Iodine complex is formed in the cell wall of each type of bacteria upon addition of Iodine. When a Decolorizer is applied, lipids are extracted from the cell walls of gram negative bacteria. Lipid extraction causes an increase in cell wall permeability and results in the loss of the dye complex. The effect of Decolorizer on gram positive bacteria is dehydration. This decreases the cell wall permeability and increases the retention of the Crystal Violet Iodine complex. The result is gram positive bacteria appearing violet due to retention of the Crystal Violet Iodine complex and gram negative bacteria appearing pink-red due to the staining from the Safranin Counterstain.

# REAGENTS/SUPPLIES:

### Remel Gram Stain Control # R40142

### Glass slide

### Crystal Violet stain

### Iodine

### Decolorizer

### Safranin Counterstain

### Bibulous Paper

### Microscope

### Immersion Oil

# QUALITY CONTROL:

### Obtain a Remel Gram Stain Control and stain per procedure.

### Each new lot or shipment of gram stain kit or bottle of gram stain reagent is logged on the Reagent log sheet and quality results recorded before use.

* + 1. In Microbiology Result Entry, type GSQC in the entry field. Select “acceptable” or “unacceptable”

**Non-Microbiology CLS:**

A Gram stain quality control slide shall be stained and read with each batch of specimen gram stains. The quality control results will be documented in the computer for each gram stain.

**Microbiology CLS:**

A Gram stain quality control slide shall be stained daily and documented in Cerner for each specimen.

### Expected results:

| Reaction | Cerner Result |
| --- | --- |
| Gram positive cocci = purpleGram negative rods = pink to red | GSQC=Acceptable |

### Unacceptable results:

### If the quality control slide is unacceptable, the CLS will have the slides in question remade and stained.

# procedure:

1. Apply the test specimen to a clean glass slide in a manner that will yield a thin, uniform smear.
2. Fix smear to slide using one of the following fixation techniques:

* Place the slide on a heat block (56°C) for several minutes or air dry. Allow the slide to cool to room temperature before staining. NOTE: Do not overheat the slide. Excessive heating will cause atypical staining.
* Methanol fix the slide by flooding with absolute methanol (95%) for 1-2 minutes, then tilt the slide to drain off methanol and allow it to air dry.

1. Staining Procedure:

* Cover slide with Crystal Violet Reagent for one minute.
* Rinse the slide with deionized or tap water.
* Cover slide with Iodine Reagent for one minute.
* Gently rinse the slide with deionized, or tap water and allow it to drain.
* Tilt the slide and flood with a few drops of Decolorizer until no violet runs off. This will usually take 10 seconds or less. Do not over decolorize.
* Rinse slide gently with deionized or tap water.
* Cover slide with Safranin for one minute.
* Rinse slide gently with deionized or tap water.
* Allow the slide to drain and air dry, or gently dry with lintless bibulous paper or paper towel.
* Examine slide under oil immersion lens.

# reporting results:

1. Press F2 in the Entry field and select “Gram Stain Report”
2. Bacteria appearing violet to purple in color after staining are considered gram positive. Bacteria appearing pink to red in color are gram negative. Beyond reporting the microscopic morphology and gram staining characteristics of bacteria the presence of red blood cells, white blood cells, epithelial cells, mucus, yeast, hyphae, pseudohyphae, etc. should be noted and reported.

* GPC=Gram positive cocci
* GNR=Gram negative rods
* YST=yeast
* RBC=RBCs
* WBC=WBCs
* PMN=Polynuclear cells

1. All cells, mucus, etc. should be reported on a semi-quantitative basis as follows:

* White blood cells (WBC/PMN) **must be** reported on all specimens. If there are no WBCs seen, report as “No WBCs seen”.
* Grade cells and bacteria with the following guidelines, except sputum WBCs/Epithelial cells:
* 1p = <1 per oil immersion field
* 2p = 1 per oil immersion field
* 3p = 2-10 per oil immersion field
* 4p = >10 per oil immersion field

1. Epithelial cells and WBCs **must** be reported for all sputum specimens.

* For **Sputum specimens** use the following grading for WBCs, and Epithelial cells:

WBC: < 25, or >= 25/lpf

Epithelial cells: < 10, or >=10/lpf

* **Expectorated** sputum samples with < 25 WBC/lpf and >=10 epithelial cells/lpf and excessive oral contamination on the gram stain may have the culture canceled. The physician, PA, or patient’s nurse must be notified of the cancellation and to recollect, if indicated.

**\*\*\*\*No invasively collected respiratory cultures (cultures collected by respiratory therapy or by the doctor) will be canceled regardless of gram stain results**.

**\*\*\*\*All canceled cultures must be called to the caregiver for that patient**. **All canceled cultures will be documented on the cancellation log and in the computer**.

1. Gram Stain Review and Correlation:

Gram stains must be correlated with culture growth on the final report. Document in the final report any discrepancies between gram stain and growth.

Gram stains performed by general laboratory (not microbiology), must be reviewed by microbiology and documented as reviewed in the computer.

# LIMITATIONS:

* The gram stain provides preliminary identification only and is not a substitute for cultural studies of the specimen.
* Certain conditions are known to damage the cell wall, causing gram positive bacteria to falsely appear gram negative or gram variable. These include antibiotic treatment, cultures more than 48 hours old, inflammatory responses in the host, and autolytic enzymes (e.g., S. pneumoniae). Ideally, specimens should be collected before the patient begins antibiotic therapy. Gram stains should ideally be performed on colonies taken from culture media that do not contain antibiotics preferably on colonies that are 18-24 hours old.
* Errors in technique which can alter gram stain results include the following:
* Fixation with excessive heat alters morphology and makes organisms more susceptible to over decolorization.
* Insufficient exposure to iodine can prevent Crystal Violet from bonding firmly with the cell wall, thus making gram positive organisms more susceptible to over decolorization. To ensure reliable gram stain results, only fresh iodine should be used.
* Prolonged decolorization can cause gram positive bacteria to appear gram negative.
* Insufficient decolorization can make gram negative organisms falsely appear gram positive.
* Insufficient counterstaining can fail to stain gram negative bacteria and background material, whereas excessive counterstaining will leach the Crystal Violet Iodine complex from gram positive bacteria and stain them with safranin, thus making them falsely appear gram negative.
* Prolonged washing between any of the steps can cause over decolorization.

# references:

### Gram stain procedure package insert. Hardy Media. 2010.

### Balows, Albert et. al., Editors. Manual of Clinical Microbiology, 5th Edition. American Society for Microbiology, Washington, D.C., 1991