

**Document Title: III. GRAM STAINED SMEARS**

Author	Effective Date	Supersedes Procedure #
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## CHANGE CONTROL FORM

**Document Title: III. GRAM STAINED SMEARS**

**Document Number: SH.CP.MC.BAC.0003.000 Version: from 6 To 7**

Submitted By: Debra Jesien Date: 10/18/2013

1. Check one:

- New procedure     New process     New form     New flow chart   
Revised procedure     Revised process     Revised form     Revised flow chart   
New job aid     New labels   
Revised job aid     Revised label

Brief description of change:

The following additions have been made:

- Targeted gram stain TURN-AROUND TIMES for each specimen type.
- Instructions for performing gram stain on Sterile Fluids rec'd in BactAlert bottles
- Pictorial diagram and instructions for recording microscope coordinates
- Guidelines to be used by 2<sup>nd</sup> reader of Gram Stains
- Procedure to be followed for ADD ON Gram Stains
- More complete explanation of how to proceed with problem Gram Stains.
- For Positive CSF Gram Stains: Print 2 instant reports – 1 goes to Steriles bench, the other to Bacteriology supervisor  
–dc seen on CSF Gram Stain must be phoned to MCHD as well as physician.  
Refer to "Microbiology Master Reporting Chart"

The following deletion has been made:

It is no longer necessary to have Gram stains from pus reviewed by a 2<sup>nd</sup> reader unless the pus is from an internal organ (liver abscess, brain, kidney, etc...)

- S. O. P. validation needed? (Circle one) NO YES If yes, attach validation sheet
- Process validation needed? (Circle one) NO YES If yes, attach process validation documentation
- Associated procedure and other documents (list those that need to be written or revised): \_\_\_\_\_
- Table of Content (TOC) update needed? (Circle one) NO YES If yes, attach Updated TOC

Author: Paula Migneault Date: \_\_\_\_\_  
Revised by: Debra Jesien Date: 10/18/13  
Approved by: Tonight J. Hardy, PhD Date: 10/21/2013

## GRAM STAINED SMEARS

### Principle:

The gram stain is used to classify bacteria based on color, size, form and cellular morphology. Bacteria stain either gram positive or gram negative based on differences in cell wall composition. Gram positive bacteria retain crystal violet and appear purple due to a thick peptidoglycan layer that resists decolorization by alcohol. Gram negative bacteria are damaged by the alcohol decolorizer so that crystal violet-iodine complexes leak out and are replaced by safranin counterstain, giving them a red appearance.

### TARGETED TURN-AROUND TIMES:

CSF and specimens that have been ordered as "STAT" .....	≤1 hour
Tissues/Surgical and other Sterile Fluids.....	≤2 hours
Sputum/Tracheal Aspirates.....	≤2 hours
All others.....	≤4 hours

### I. Occasion for gram-stained smears

#### A. Gram stains are routinely performed on the following specimen types:

- 1) Sterile body fluids
- 2) Tissues and surgical specimens
- 3) Sputum, BAL, Bronchial washes, Bronchial brushes, Tracheal aspirates, Transtracheal Aspirates etc...
- 4) Sinus
- 5) Abscesses from any internal organ (brain, liver, lung, etc...)
- 6) Breast abscesses
- 7) Any quantity of pus
- 8) Superficial wounds and drainages
- 9) Cul-de-sac if fluid is received

#### B. Gram stains are performed by request on the following specimens types:

- 1) Urine
- 2) Urethra
- 3) Bartholin cyst
- 4) Semen

#### C. Gram stains are not performed on the following specimen types:

- 1) Stool, rectal abscesses, rectal or anal swabs
- 2) Oral, mouth or throat specimens
- 3) Nasal and nasopharyngeal swabs
- 4) Skin and scalp
- 5) Sterility cultures such as: Donor cornea, bone bank, water, media, etc...
- 6) Catheter or Shunt tips
- 7) Implements such as screws, electrodes, mesh, metal plates, etc....  
TNP using the following cancellation comment:

"inappropriate specimen for test requested"  
(choice |XIAS on cancellation keypad)

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- D. **Routine gram stains are not performed on Vaginal or cervical swabs (unless requesting Nugent score).** TNP using the following cancellation comment:

“Gram stain not performed due to poor correlation with culture.”  
(choice |XBCX on cancellation keypad)

- E. **Sterile fluids submitted in BactAlert bottles**

- 1) **GS is automatically ordered in the LIS whenever fluid in bottles is received and FBTL is ordered.**
- 2) **If no additional fluid other than that in the bottles is received the gram stain cannot be performed. TNP using the following cancellation comment:**

“Specimen received in Bactalert bottles”.  
(choice |XBGS on cancellation keypad)

- F. **Culture positive platelets**

- 1) GS is ordered in LIS along with FBTL
- 2) From GS key pad - choose “?fluid in bottles |>SORG “
- 3) Report descriptively

Example:

gram positive cocci in clusters with quantity. Do not add resembles Staphylococci

- 4) From AER or ANA Bottle keypad - select appropriate plate media

- G. **Request for ADD-ON Urine Gram Stains**

- 1) **If culture has just been set up, has not been looked at yet, or has only a “Prelim” status, honor the gram stain request.**
- 2) **If the culture has an “Interim” or “Final” status in which a potential pathogen (and susceptibility testing, if appropriate) has been reported, do not honor the request. The culture information can be used to determine treatment options. TNP the gram stain using the following cancellation comment:**

“Refer to culture which is already in progress”.  
(choice |XCIP on cancellation keypad)

## II. Slide preparation techniques

**Note: Slides prepared from specimens with AFB precaution must be air dried in the Biosafety cabinet and exposed to UV light for a full 10 minutes before being removed from the cabinet. Slides are then heat fixed at 85°C for 15 minutes prior to staining.**

- A. **CSF, Urine and non-viscous fluids:**

- 1) Place one drop of the fluid in the center of a clean glass microscope slide.
- 2) Air dry.

- B. **Abscesses, Pus, Joint fluid, Pleural fluid and viscous or bloody fluids:**

- 1) Obtain a clean microscope slide and place one drop of the specimen at the end of the slide near the patient label.
- 2) Use the side of a sterile stick to gently spread the specimen to the other end of the slide, distributing it

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- evenly. Leave any excess specimen at the end of the slide farthest away from the patient label.
- 3) For extremely thick specimens it may be necessary to make a second slide diluted with 1 drop of sterile saline.
  - 4) Air dry.

### C. Respiratory specimens

- 1) Using a sterile swab select most purulent portion of material.
- 2) Gently roll swab across a clean glass microscope slide, distributing it evenly.
- 3) Air dry. Heat fix slide at 85°C before staining.

### D. Specimens received on swabs

- 1) Gently roll swab across a clean glass microscope slide.
- 2) Air dry.

### E. Tissue samples

- 1) Grind specimen in sterile saline according to specimen processing guidelines (refer to Set-up Procedure Manual)
- 2) Obtain a clean microscope slide and place one drop of the ground specimen at the end of the slide near the patient label.
- 3) Use the side of a sterile stick to gently spread the specimen to the other end of the slide, distributing it evenly. Leave any excess specimen at the end of the slide farthest away from the patient label.
- 4) For extremely thick specimens it may be necessary to make a second slide diluted with 1 drop of sterile saline.
- 5) Air dry.

## III. Procedure for performing a Gram stain<sup>1</sup>

- A. Heat-fix the inoculated slide.
- B. Flood slide with Crystal Violet (10 - 30 seconds).
- C. Rinse with tap water, shaking off excess water.
- D. Flood slide with Gram's Iodine (20 - 60 seconds).
- E. Rinse with tap water, shaking off excess water.
- F. Holding the slide at an angle, carefully flood with Gram's decolorizer, and rinse immediately with tap water. Thick slides may require prolonged decolorization.
- G. Shake off excess water.
- H. Flood slide with Safranin (30 seconds).
- I. Rinse with tap water, shaking off excess water.
- J. Air dry or gently blot dry the slide before examining microscopically.

## IV. Distribution

### A. Day shift

Monday – Friday there is usually a person assigned to read all Gram-stained smears. **When there is no assigned reader or the workload is heavy, scheduled technologists from all areas should assist with the reading of smears.** The technologist at each station is ultimately responsible for reading smears that belong to his/her station.

### B. Other shifts

When personnel qualified to read Gram stains are present, "sterile" specimen smears, and sputum and/or tracheal aspirate smears must be read as soon as possible; other slides as time permits.

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### V. Review of slides

A. Smears from the following specimens need not be routinely reviewed by a second reader:

- Sputum, Tracheal aspirates, Bronchial Washings, ETT and Deep Tracheal Lavage
- Urine
- Fluids/tissues/pus/exudates with  $\leq 10$  PMNs/LPF and no organisms seen (NOS)
- Exudates - excluding abscesses from internal organs (Liver, Brain, Kidney, etc.)

B. Slides from the following specimen types should always be independently (unbiased) reviewed by a second person, if possible, before being reported. This person may be a senior technologist, a supervisor, or another qualified staff member.

- Smears from fluids/tissues with  $>10$  PMNs/LPF
- Cytospin preparations
- Brain abscesses, abscesses from internal organs
- Urogenital smears
- "positive" Sterile fluid/tissue gram stains which require a call to a healthcare provider
- **Any slide that the initial reader is unsure of or would like to be examined by a 2<sup>nd</sup> reader**

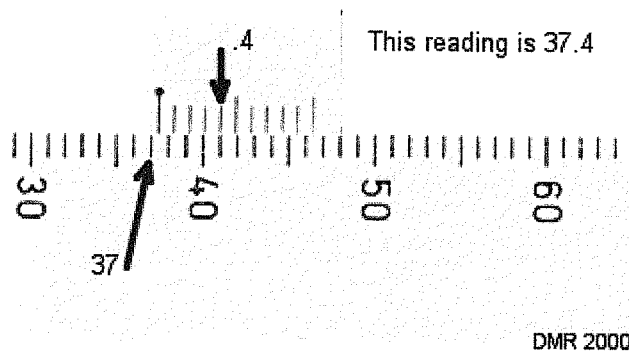
C. Any gram stained smear with a questionable interpretation or an unusual organism must be reviewed. These findings should be reviewed by a Tech V, supervisor, or director. Record coordinates, on the gram stain worksheet and as hidden comments in the LIS, for those smears with very few organisms present.

#### To record coordinates:

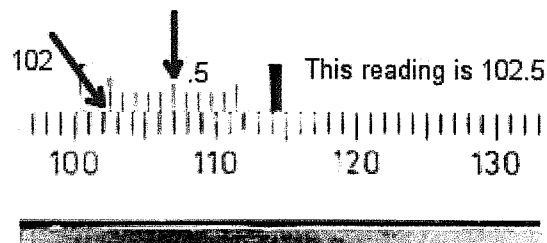
1. Place the organism/object in question as close to the center of the field of view as possible.
2. Record whether the patient label is on the right or the left side of the slide.
3. Record which microscope you are using to read the slide. (example: Olympus, blood room)
4. Looking at the X axis (top scale of the microscope stage), determine which value the first line of the top sliding scale coincides with. In the example below it is 37.
5. Read across the same scale until the line on the top scale is directly opposite of the line on the bottom scale. In the example below it is the 4<sup>th</sup> line over, = .4
6. Therefore the first coordinate in the example below is 37.4.
7. Look at the Y axis along the side of the stage and repeat this process.

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### Vernier Scale (X axis)



### Vernier Scale (Y axis)



#### D. Guidelines to be used by Second Reader of Gram-stains:

1. Verify that the specimen result has been recorded by the correct patient label on the Gram Stain Worksheet.
2. Ensure slide was prepared and stained properly - not over or under decolorized, not too thick to be read accurately. If there is any concern about proper staining, the smear should be repeated.
3. Independently review the slide and determine presence and quantity, or absence of cells and organism.
4. To distinguish between organism and artifact it may be helpful to perform a cytospin (if appropriate sample) or consult with a senior technologist or supervisor.
5. If questionable or very small numbers of organisms were seen, ensure that correct coordinates have been documented.
6. Record coordinates for but do not report: Questionable organisms that cannot be confirmed or organisms seen only in one area of the slide (especially near the edge) which are possible contaminants.
7. Ensure that positive sterile specimens are phoned to all necessary locations. Refer to "Microbiology Master Reporting Chart".

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**Ideally the checker should review the smear independently, and without prior knowledge from the first reader. For problem organisms, or when the numbers of organisms are very few, the checker may first be shown the field in question before further reading of the slide. The reader and the reviewer should initial the entry on the worksheet.**

Note: These guidelines apply to all shifts. If there is no other member on your shift who can act as a second reader for a gram stain smear (e.g. night shift), report and leave slides and corresponding worksheet for review by the dayshift ASAP the following morning.

For Gram stains requiring review the next morning:

- Enter results
- Skip a line
- Enter "Results to be considered preliminary until review in A.M."
- Give "Preliminary" status.
- Save.
- View your report for accuracy.

E. **DO NOT CHANGE ANY RESULTS** that have been reported unless you have discussed the matter with a supervisor.

## VI. Examination of Gram-stained smear

### A. Gross examination

Note where the stained material is on the slide and whether or not the smear is too thick or improperly decolorized.

### B. Scan using 10x objective (low power).

1. Gain general impression of smear and look for best areas (where inoculum is adequate).

#### 2. Look for cells using low power (10x objective)

##### a. Epithelial Cells:

Report the presence (quantitate) or absence of squamous epithelial cells only on Respiratory specimens. (See specific source-driven guidelines IV.) Do not mention epithelial cells on smears other than respiratory specimens.

Quantitate (#) squamous epithelial cells as follows:

≤10/low power field  
>10/low power field

##### b. PMN's:

Report presence (quantity) or absence of PMN's from all specimen types, including bone, bone marrow, and lymph node specimens.

Estimate PMNs/low power field (lpf).

If only PMN cell fragments are seen (PMNs lacking a cell membrane) on smears, report:

"(#) cell fragments"



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- c. **Nucleated white cells other than PMNs (NPMNs):**  
Reported as "nucleated cells, not PMNs". These are reported on Sterile Fluid specimens only.

C. **Quantitation (#) of PMNs, and other cells:**  
**use low power (10x objective) to quantitate, but confirm cell morphology using 40x or 100x**

1. Expectorated sputum and tracheal aspirates with >10 epithelial cells/low power field as follows:

<25/low power field.....(**not** read for bacteria)  
>25/low power field.....(read for bacteria)  
too numerous to count (TNTC)/low power field.....(read for bacteria)

2. For all other sources:

0/low power field  
<1/low power field  
1-10/low power field  
10-25/low power field  
>25/low power field

D. **Look for presence of hyphae**

Because of their large size, uneven distribution, relatively small numbers present, **and characteristic uneven or negative staining**, hyphae can easily be overlooked or missed on high power. However, low power scanning can sometimes spot these elements. This is especially true of respiratory specimens, including lung tissue and sinuses.

E. **Be alert to problems with smear/stain preparation**

- a. Underdecolorization – should be suspected when cells (esp. PMNs) have gram-positive nuclei  
**Repeat the smear.**
- b. Overdecolorization – should be suspected when entire smear stains gram-negative  
**Repeat the smear.**
- c. Abnormal color of stain or gram-variable organisms of same morphology. **A repeat smear may be helpful, consult with second reader.**
- d. Contamination – suspect if distribution of organisms on slide is grossly uneven, **or if organisms are only present on one area of the slide or near the edge of the slide. Whenever possible, repeat the smear or do a cytospin preparation. Do not report organisms unless confirmed by one of these methods.**

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### F. Quantitation of bacteria (using 100x oil objective):

1. Under oil immersion, examine carefully for various morphologies and types of bacteria and yeast. Report relative numbers of gram-negative bacilli, gram-negative diplococci, gram-positive cocci, gram-positive bacilli, and yeast.

many = several per oil immersion field  
moderate = one per field  
few = one per several/many fields  
very few = one or two/slide

**Note: For sterile specimen types when only a few organisms are seen on the entire slide, document organism coordinates in LIS as a hidden comment.**

2. For all specimen types (including respiratory and non-sterile exudates) examine for, and report, all morphotypes seen.

Example:

(#) PMNs  
(amount) gram-negative bacilli  
(amount) gram-positive cocci in pairs and chains  
(amount) gram-positive bacilli  
(amount) yeast

### G. Cytospin preparation

**Refer to Cytospin protocol for additional details.**

1. Prepare on "**sterile**" fluid or "**sterile**" abscess only if:
  - $\geq 10$  PMNs/low power field, but no organisms seen on direct smear.....(add cytospin - test code: GSCY)
  - unsure whether or not organisms are seen on direct smear  
.....(add cytospin – test code: GSCY)
  - when requested on a sterile fluid by physician.....(add cytospin – test code: GSCY)
2. Prepare routinely on all:
  - CAPD fluid, Peritoneal Dialysate fluid (PD), Ascites fluid if  $\geq 10$  ml received
  - CSF obtained from shunt
  - BALs and Protected Bronchial Brush specimens

**Note: For grossly bloody specimens, dilute by placing 1 drop of specimen into a sterile tube containing up to 1 ml of sterile saline. Use the diluted suspension to perform the cytospin.**

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### 3. Cytospin Procedure Report

- a. If no organisms are seen on routine Gram stain, but organisms are seen on cytospin, report:  
**(No need to report cell picture)**  
  
(few, moderate or many) (organism description)
- b. If no organisms are seen on routine Gram stain and cytospin preparation, report:  
**(No need to report cell picture)**  
  
No organisms seen
- c. If cytospin prep is done to confirm positive findings of routine Gram stain, report the results for both smears.
- d. Report results of a cytospin if performed on a CAPD fluid, Peritoneal Dialysate fluid (PD), Ascites fluid or CSF shunt as follows:  
  
(few, moderate or many) PMNs  
(report quantity of NPMNs if present. Do not report absence of NPMNs)  
(few, moderate or many) (organism description)

## VII. Smears of respiratory specimens

### A. Sputa and tracheal aspirates

1. If  $\leq 10$  epithelial cells/lpf **the specimen is acceptable for culture.** Record and report:
  - c.  $\leq 10$  epithelial cells/low power field
  - d. # PMNs/low power field
  - e. Amount and description of each type of organism.

Examples:

$\leq 10$  epithelial cells/low power field  
>25 PMNs/low power field  
gram-negative bacilli  
many  
gram-positive cocci in pairs  
few

$\leq 10$  epithelial cells/low power field  
1-10 PMN's/low power field  
gram-positive cocci in chains  
few

**Retrieve sputum and CYEP from Biosafety cabinet. Label BAP/CHOC/MAC with patient label and place at Set-up bench to be plated.**

## GRAM STAINED SMEARS

2. If >10 epithelial cells/low power field **the specimen is unacceptable for culture**. Read for PMN's and select appropriate choice:

≤25 PMNs/low power field ...not read for bacteria\*

>25 PMNs/low power field (but not TNTC) ...read for and report bacteria\*

TNTC PMNs/low power field ...read for and report bacteria\*

\*The following comment will be automatically added to the report when the appropriate choice is selected from the keypad:

" The presence of large numbers of squamous epithelial cells indicates that the specimen is contaminated with oropharyngeal microorganisms. Specimen not cultured for organisms colonizing the upper respiratory tract airways or mouth."

The specimen will be rejected for routine bacterial culture.

Cancel the AER test in the LIS. BCYE will still be set up to culture for *Legionella*.

**Note: The patient location must be notified that the specimen is not being cultured. Inpatient reports must be printed to the floor. Outpatient reports may be faxed to the location. If a fax number is unavailable, the report must be phoned.**

- B. Exceptions to IV.A.2 include the following:

1. **Cystic Fibrosis (CF) sputum or deep throat specimens:**
  - Quantitate epithelial cells as in IV.B.2.a. and PMNs as in IV.B.2.b.
  - Specimens from CF patients with <10 EPIs are always read for bacteria.
  - Specimens from CF patients with >10 EPIs and/or <10 PMNs ... report cell picture with "Not examined for bacteria".
  - **These are never rejected for culture.**
2. If ***Nocardia*** or **aerobic *Actinomycetes*** is requested prepare two smears (1 Gram stain, 1 modified Kinyoun stain). Perform Gram stain, read cell picture and enumerate all bacteria. Examine the entire smear on high power. If characteristic organisms are observed (filamentous, branching, beaded staining gram-positive bacilli), report:
  - (#) cell picture
  - branching gram-positive bacilli
  - (quantity)A "MODK" test will reflex when the branching gram-positive bacilli is reported in the LIS.  
Give slide for modified Kinyoun stain to Mycology/Mycobacteriology personnel ASAP. If appropriate staff is not available, leave slide clearly marked for modified Kinyoun in the fungal stain box. Leave a note for the Mycology technologist.
3. Smear preparations that are too thick to be read ... repeat smear.

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- C. **Bronchial washes, Unprotected Bronchial Brushes, Deep Tracheal Lavages and Transtracheal aspirates, Endotracheal aspirates:**
1. Always read for cells and bacteria, report cells and bacteria regardless of epithelial count.
  2. Quantitate epithelial cells as in IV.B.2.a. and PMNs as in IV.B.2.b.
  3. **These are never rejected for culture.**
- D. **BALs and Protected Bronchial Brush specimens:**
1. Prepare cytospin smear.
  2. Quantitate epithelial cells as in IV.B.2.a.
  3. Report relative amounts (few, moderate, many) of PMNs and relative amounts and types of bacteria seen.
  4. Report the percent of PMNs which you observe to contain intracellular organisms.
  5. **These are never rejected for culture.**

## VIII. GC smears (GC specified)

- A. Report the cell picture. (PMNs, cell fragments)
- B. Examine for and report presence (few, moderate or many) or absence of gram-negative diplococci, intracellular or extracellular. The presence of a cell membrane is necessary for the use of the word "intracellular". If PMN cell fragments are seen, but no intact PMNs, report: "(#) cell fragments.
- C. Note: Gram stains of cervical/vaginal swabs are not routinely read for gram-negative diplococci in our lab. If a request is made, prepare a smear but do not read. Cancel as: "Gram stain not performed due to poor correlation with culture" (XBCX). The smear may be examined for the presence of intracellular gram-negative diplococci if further discussion with the physician indicates it is appropriate to do so. **For requests made to gram stain vaginal swabs for the diagnosis of bacterial vaginitis, refer to VII.**
- D. Examples of reports:
- >25 PMNs/low power field  
intracellular gram-negative diplococci  
few
- many cell fragments  
extracellular gram-negative diplococci  
many
- 0 PMNs/low power field  
no intracellular or extracellular gram-negative diplococci seen
- 0 PMNs/low power field

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Scant inoculum on slide. Specimen is not adequate to determine presence of Intracellular gram negative diplococci.

### IX. Urine smears<sup>2</sup>

- A. Report cell picture, i.e., PMNs/low power field
- B. Report organisms
  - 1. ">100,000 (organisms)/ml" if  $\geq 1$  per oil immersion field."
  - 2. "<100,000 (organisms)/ml" if <1 per oil immersion field."
  - 3. "No organisms seen"

### X. Interpretation of Vaginal Smears Using Nugent Criteria<sup>3</sup>

- A. Slides should be delivered to the Mycology Laboratory (or placed in the fungal smear box).
- B. Heat fix and stain as usual.
- C. Examine for presence (quant.) or absence of:

PMNs/low power field  
gram-positive bacilli consistent with lactobacilli/oil immersion field  
gram-negative to gram-variable bacilli consistent with *Gardnerella*/oil immersion field  
gram-variable curved bacilli consistent with *Mobiluncus*/oil immersion field  
Clue cells/high dry field  
Yeast/oil immersion field

- D Interpretation of the Gram stain

Large gram-positive bacilli (large with flat ends) are the lactobacilli morphotype. The *Lactobacillus* and *Gardnerella* morphotypes often decolorize and appear gram-negative. This should not be confused with predominantly gram-negative flora. Enteric gram-negative bacilli rarely predominate in the vagina. Clue cells are squamous epithelial cells that have a dense coating of adherent bacteria. The epithelial cells are irregular, almost polygonal, in shape and are about 100 x 50  $\mu\text{M}$  in size. The dense adherent bacteria almost entirely obscure any cellular detail. In normal epithelial cells, the ellipsoidal cell nucleus (10 x 5  $\mu\text{M}$ ) is commonly visible.

- E. Quantitation

Each morphotype and clue cell is quantitated from 0 to 4+ with regard to the number of morphotypes per oil immersion field, except clue cells which should be counted on high dry and PMNs per low power field:

0 = none seen  
1+ = less than one per field  
2+ = 1 – 4 per field  
3+ = 5 – 30 per field  
4+ =  $\geq 30$  per field

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### F. Calculating the Nugent score

Score	<i>Lactobacillus</i>	<i>Gardnerella/Bacteroides</i>	<i>Mobiluncus</i>
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3 or 4+
3	1+	3+	
4	0	4+	

Example:

	Score
<1 PMN/low power field	
1+ gram-positive bacilli consistent with <i>Lactobacilli</i>	3
4+ gram-negative bacilli consistent with <i>Gardnerella</i>	4
2+ gram-variable bacilli consistent with <i>Mobiluncus</i>	1
0 yeast	

Nugent Score = 8

The scoring criteria sums the weighted quantitation of the 3 morphotypes to yield a score of 0 to 10. The criterion for bacterial vaginosis is a score of  $\geq 7$ , a score of 4-6 is considered intermediate, and a score of  $\leq 3$  is considered normal.

## XI. Reporting Gram Stain Results

### A. Entering Reports in LIS

1. Access the "Gram Stain Worklist" in the LIS.  
 Gram stain reports for all specimens should be entered using this list. In addition, this list should be checked at the beginning, end, and throughout each shift. All specimens on the list must be accounted for.
2. When entering gram stain results for sterile fluids, obtain the following information from the Gram Stain Worksheet and enter into the LIS:
  - Enter volume of fluid received
  - If specimen was bloody, indicate that in the LIS
  - If specimen was spun, indicate that in the LIS
3. When entering gram stain results for tissue samples remember to enter the grind charge, as appropriate.
4. When entering gram stain results for smears that have been reviewed by a second reader indicate the checkers initials in the LIS.
5. When entering gram stain results which were called to a patient location document in the LIS the following:
  - The first and last name of the person taking the report
  - That the report was read back and verified

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### B. Telephone Calls

Consult "Microbiology Master Reporting Chart" for additional details

- **Positive sterile fluid, sterile tissue, organ abscesses – call to the patient location or ordering Physician**
- **Call MCHD when –dc are reported from gram stain of CSF**  
On Dayshift: call MCHD @ 753-5164  
After hours: contact On-Call Medical Examiner @753-5999

### C. Printing/Faxing Reports

- **Cancelled Gram Stain:**           Inpatients – print report to floor  
  Outpatients – fax report to patient location
- **Positive CSF:**                   **Print 2 instant reports. Tape 1 to the benchtop at the Sterile station. Give the other report to the Bacteriology supervisor. On evenings/nights place in the Bacteriology supervisor's Mailbox.**

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### References:

- <sup>1</sup>Forbes, Betty A., D. F. Sahm, A.S.Weissfeld. 1998. Diagnostic Microbiology, 10<sup>th</sup> ed. St. Louis, Missouri.
- <sup>2</sup>Cumitech 2A, Laboratory Diagnosis of Urinary Tract Infections; American Society for Microbiology; March 1987.
- <sup>3</sup>Nugent R P, et. al. Reliability of Diagnosing Bacterial Vaginosis is Improved by a Standardized Method of Gram Stain Interpretation. J Clin Micro. 29:297-301, 1991.