

## Appendix A

# LABORATORY DIAGNOSIS OF SEXUALLY TRANSMITTED DISEASES

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## INTRODUCTION

Appendix A contains current information on the laboratory methods available for the diagnosis of STDs. For procedures that can be performed in the office, step-by-step instructions and interpretation guidelines are given. In addition, the collection and handling of specimens for laboratory procedures that are not usually performed in an office setting but are essential for the care of STD patients are described. As with most procedures, the more frequently laboratory tests are performed, the better the quality of results. Thus, before adding a procedure to the office laboratory, evaluate the frequency of its use. Also, the patient population involved influences the types of tests performed. Table A-1 summarizes available tests for both the rapid presumptive diagnosis and the definitive diagnosis of the common STD agents and syndromes.

**Table A-1**

### LABORATORY TESTS AVAILABLE FOR DIAGNOSIS OF SEXUALLY TRANSMITTED DISEASES

<b>Etiologic Agent</b>	<b>Common Syndromes</b>	<b>Rapid Diagnostic Test</b>	<b>Definitive Diagnostic Test</b>
<i>Neisseria gonorrhoeae</i>	urethritis, cervicitis, proctitis, PID	Gram stain	culture; DNA probe; NAATs*
<i>Chlamydia trachomatis</i>	NGU, cervicitis, proctitis, PID	Gram stain (to determine presence of inflammation)	culture; direct FA slide; EIA; DNA probe; NAATs*
<i>Trichomonas vaginalis</i>	vaginitis, urethritis	saline wet prep	if positive, saline wet-prep is definitive; culture to increase sensitivity
<i>Candida albicans</i> , other <i>Candida</i> sp.	vaginitis, balanitis	10% KOH prep; Gram stain	if positive, rapid test is definitive; culture to increase sensitivity
<i>Gardnerella vaginalis</i> , anaerobic bacteria	bacterial vaginosis	saline wet prep, whiff test, <u>and</u> vaginal pH; Gram stain	rapid tests are definitive; culture for <i>Gardnerella</i> and anaerobes or gas liquid chromatography increase sensitivity
Herpes simplex virus	genital ulcer	None <sup>2</sup>	ulcer: direct FA slide; culture; EIA; PCR serological tests: Western blot, EIA (glycoprotein (gG1/gG2) type-specific antibody test)
<i>Treponema pallidum</i>	genital ulcer	ulcer: darkfield microscopy serological test: RPR	ulcer: if positive, darkfield is definitive; DFA-TP; serological tests: RPR, VDRL, USR, ART, (non-treponemal tests); FTA-ABS, MHA-TP (treponemal tests); TP-PA
<i>Hemophilus ducreyi</i>	genital ulcer, bubo chancroid	Gram stain of pus from inguinal bubo	culture of ulcer or bubo
<i>Chlamydia trachomatis</i> (L1-L3 immunotypes)	lymphogranuloma venereum (LGV), genital ulcer, bubo	none	culture; PCR; LCR; serological tests: complement fixation, microimmunofluorescence
<i>Calymmatobacterium granulomatis</i>	granuloma inguinale, donovanosis, genital ulcer	none	Giemsa or Wright's stain
<i>Sarcoptes scabiei</i>	dermatitis, ulcers	mineral oil wet prep	rapid test is definitive
<i>Phthirus pubis</i>	dermatitis	dry mount	rapid test is definitive
poxvirus??	molluscum contagiosum	none	none
Human papillomavirus (HPV)	genital warts (condylomata acuminata)	none	Pap stain; DNA hybridization
<i>Salmonella</i> sp., <i>Shigella</i> sp., <i>Campylobacter</i> sp.	enteritis, proctocolitis	none	cultures
<i>Entamoeba histolytica</i> <i>Giardia lamblia</i>	enterocolitis	none	wet prep or trichrome stain of fresh or concentrated stool
Hepatitis virus: A, B, C	viral hepatitis	none	serological test for specific antibody

\*Nucleic Acid Amplification Test

<sup>2</sup>Performance of Tzanck smear not generally adequate to recommend routine use.

## Section I

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# PROCEDURES AND INTERPRETATION

## GRAM STAIN

The Gram stain is a rapid test that is useful in the diagnosis of gonorrhea, candidal vulvovaginitis, and bacterial vaginosis, and in the assessment of urethritis, cervicitis, proctitis, and other infections characterized by mucosal discharge. Both the numbers of polymorphonuclear leukocytes (PMNs) and microbial flora present can be assessed.

### MATERIALS

- Microscope slides
- Alcohol or Bunsen burner
- Gram stain reagents: crystal violet, Gram's iodine, decolorizer (50:50 mixture of acetone and 95% ethanol), safranin
- Sink or staining tray with water source
- Paper towels or blotting paper
- Immersion oil
- Brightfield microscope

### PREPARATION OF SMEAR

1. Carefully roll the swab onto a slide to avoid disrupting cells. Cover only a small area of the slide.
2. Let the smear air-dry rather than drying it over a flame.
3. Heat-fix the smear briefly by passing it over a flame several times. The slide should feel warm but not hot.

### STAINING

1. Flood the slide with crystal violet for approximately 5 seconds, then rinse with a gentle stream of tap water.
2. Flood the slide with Gram's iodine for approximately 5 seconds, then rinse with a gentle stream of tap water.
3. Rinse the slide with decolorizing solution until purple no longer runs from the thinnest part of the smear. The length of decolorizing depends on the composition of the decolorizing reagent and the thickness of the smear. For a 50% acetone to 50% ethanol solution, decolorize approximately 5 seconds.
4. Flood the slide with safranin for approximately 5 seconds, then rinse with water.
5. To dry the smear, blot it gently on a clean paper towel (do not rub).

### SOURCES OF ERRORS

- "Scrubbing" rather than rolling the swab across the slide may destroy cellular morphology.
- Failure to heat-fix the slide may cause material to wash off during staining.
- Overheating the slide may cause staining artifacts and distortion of the cells.
- Use of old Gram's iodine solution (shelf life at room temperature is about 90 days).

- Over-decolorizing the slide may cause Gram-positive bacteria to appear Gram-negative.
- Under-decolorizing the slide may cause Gram-negative bacteria to appear Gram-positive.
- Reagents contaminated with bacteria or yeast may give spurious results.

## **MICROSCOPIC EXAMINATION**

1. Place a drop of immersion oil on the stained smear.
2. Examine the smear at low power (10X) to check for proper staining and to locate areas of the smear containing many cells.
3. Then use the oil immersion objective (100X) to search in these areas for bacterial morphotypes and to count PMNs. This means that each field is at 1000X magnification for identification and counting purposes.

Cells and mucus should stain pink. Yeast stains purple. Bacteria are characterized as Gram-positive (purple) or Gram-negative (pink), and as cocci (round), bacilli (rod-shaped), or coccobacilli (small in size with morphology in between rods and cocci).

## **INTERPRETATION - Male Urethral Smear**

Patient should not urinate for 2 hours before the specimen is collected. Insert a small swab 1 to 2 cm into the urethra.

### **Gonococcal infection**

- Positive:  $\geq 1$  PMN with intracellular Gram-negative diplococci of typical morphology. Extracellular Gram-negative diplococci may also be present, and numerous PMNs are usually present. Distinguish carefully between Gram-negative diplococci and Gram-negative rods.
- Negative: No intracellular Gram-negative diplococci. Extracellular Gram-negative diplococci or Gram-negative diplococci of atypical morphology may be present, but do not meet the criteria for a presumptive diagnosis of GC (wait for culture or NAAT results for final diagnosis). Mononuclear cells and PMNs may or may not be present.

### **Nongonococcal urethritis (NGU)**

- Positive:  $\geq 5$  PMNs per oil immersion field and no intracellular Gram-negative diplococci found. To make this count, examine an area of the smear with many cells and average the number of PMNs seen in three 1000X fields.

## **INTERPRETATION - Cervical Smear**

Wipe off the cervix before collecting the specimen to reduce the amount of vaginal bacteria and cells in the smear.

### **Gonococcal infection**

- Positive:  $\geq 1$  PMN clearly containing Gram-negative diplococci of typical morphology. Numerous PMNs, extracellular Gram-negative diplococci, Gram-negative rods and Gram-positive rods may be seen.
- Not diagnostic: No intracellular Gram-negative diplococci or only extracellular Gram-negative diplococci found. PMNs may be present.

## **Cervicitis**

- Positive: There is lack of consensus regarding the number of PMNs seen in women with cervicitis; the positive predictive value of a cervical Gram stain is higher with a higher cutoff (e.g. > 30 PMNs/hpf). Thus >30 PMNs per oil immersion field suggests cervicitis in a woman who is not menstruating or within 1 to 7 days of menses. To determine the count, examine an area of the smear with many cells and average the number of PMNs seen in three 1000X fields. It is important to note, however, that PMNs alone do not necessarily define cervicitis.

## **INTERPRETATION - Rectal Smear**

Use an anoscope to collect the specimen above the anal verge and sample areas containing pus.

## **Gonorrhea/Proctitis**

- Positive for gonorrhea: ≥1 PMN clearly containing Gram-negative diplococci of typical morphology. Distinguish carefully between Gram-negative diplococci and Gram-negative rods. Numerous PMNs may also be seen.
- Not diagnostic of gonorrhea but suggestive of proctitis: Absence of intracellular Gram-negative diplococci, or Gram-negative diplococci found extracellularly only, but ≥1 PMN per oil immersion field.

## **INTERPRETATION - Vaginal Discharge**

### **Bacterial vaginosis**

- Positive: 0-2+ lactobacillus morphotype; 3-4+ mixed morphotypes (small Gram-negative, positive, and variable rods; Gram-positive cocci; and/or curved Gram-negative rods). The small amount of lactobacillus morphotype is the key finding.

### **Yeast**

- Positive: Budding yeast and/or pseudohyphae; PMNs may or may not be present.

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## **SALINE AND 10% KOH WET PREPS, VAGINAL pH**

The saline wet prep is easily prepared and is used for the rapid detection of *Trichomonas vaginalis*, “clue” cells associated with bacterial vaginosis, PMNs, and yeast. The KOH prep is used to detect yeast. In addition, a characteristic amine odor may be observed in patients with bacterial vaginosis and *T. vaginalis* when vaginal secretions are combined with 10% KOH. Vaginal pH >4.5 suggests the presence of bacterial vaginosis and/or trichomoniasis.

### **MATERIALS**

- Microscope slides
- Coverslips (22 x 22 mm)
- Saline
- 10% KOH (potassium hydroxide)
- Cotton-tipped swabs
- Small test tubes
- Brightfield microscope
- pH paper with color scale (pH range of 4.0-7.0)

## DETERMINATION OF VAGINAL pH

1. Touch pH paper to vaginal wall. Avoid contact with cervical mucus which has a high pH.
2. Match pH paper to color scale to determine the pH value.

## PREPARATION OF SALINE WET PREP

Use either of the following two methods of preparation:

Method I	Method II
<ol style="list-style-type: none"><li>1. Place approximately 0.5 ml of normal saline (i.e. 0.85%) in a small test tube. The saline must be at room temperature or warmer.</li><li>2. Collect vaginal material on a swab by rubbing the swab against the vaginal walls, and emulsify in the saline to make a heavy suspension.</li><li>3. Leave the swab in the tube and go to the microscope <i>within 15 minutes</i>.</li><li>4. Place a drop of the specimen on a slide and cover with a coverslip. Be careful not to trap air bubbles under the coverslip.</li><li>5. Read the slide immediately.</li><li>6. Save the test tube suspension for repeat wet preps or Gram stain if necessary.</li></ol>	<ol style="list-style-type: none"><li>1. Place a large drop of saline on a microscope slide.</li><li>2. Collect the vaginal specimen on a swab as described in Method I, and add enough of it to the drop to make it turbid.</li><li>3. Carefully add a coverslip without trapping air bubbles.</li><li>4. Read the slide immediately.</li></ol>

## PREPARATION OF KOH SLIDE

1. Collect the vaginal specimen on a swab, then roll the swab on a small area of the slide.
2. Add a large drop of 10% KOH (potassium hydroxide) and mix with a wooden applicator or swab.
3. Sniff for a “fishy” odor.
4. Cover with a coverslip; avoid trapping air bubbles.
5. Read the slide as soon as possible.

## MICROSCOPIC EXAMINATION

1. Adjust the microscope to obtain maximum contrast. With many types of microscopes this is achieved by racking down the condenser and lowering the light.
2. Immersion oil is not used.
3. Saline prep: read before KOH. Examine under low power (10X or 20X) to focus and detect rapidly moving trichomonads or large pseudohyphae. Then examine on high dry (40X or 45X) to evaluate the presence or absence of PMNs, “clue” cells, trichomonads, yeast buds, or pseudohyphae.
4. KOH prep: Scan for pseudohyphae on low power. Confirm presence of pseudohyphae and locate yeast buds on high dry.

## INTERPRETATION - Vaginal Wet Prep

### Trichomonas vaginalis vaginitis

Trichomonads are only seen in the saline prep; they are lysed by KOH. Actively motile trichomonads are easily seen on low power (10X). High power (40X) is necessary to detect less vigorously moving organisms when only the flagella may be moving. (Note: when compared to culture, wet prep is 60%-70% sensitive for detection of *T. vaginalis*. See Section II for additional testing options for detection of *T. vaginalis*.)

Saline wet prep	KOH wet prep
≥1 actively moving trichomonad	Trichomonads are lysed
Numerous PMNs are often present	PMNs are lysed

The following errors in technique will decrease the sensitivity of the wet prep for detection of *T. vaginalis*:

- Collection of the specimen from the endocervix
- The use of cool saline
- Delay in reading the smear
- Contamination of the saline prep with KOH
- “Sloppy” preparation with too much saline, causing organisms to move rapidly across the field
- Making a preparation too thick
- Failure to read the slide with adequate microscope light contrast
- Examination of only a small area of the slide

### Yeast infection

Saline wet prep	KOH wet prep
Pseudohyphae & budding yeast sometimes visible	Pseudohyphae & budding yeast seen
Epithelial cells may obscure yeast	Epithelial cells are lysed
PMNs may/may not be seen	PMNs are lysed

### Bacterial vaginosis

Individual squamous cells rather than clusters of squamous cells should be examined.

Saline wet prep	KOH wet prep
Numerous “clue” cells*	“Clue” cells are lysed
Few or no PMNs	PMNs are lysed
*A “clue” cell is an irregularly bordered squamous epithelial cell with at least 75% of its outline obliterated by clusters of small bacteria.	

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## URINE MICROSCOPY

The microscopic examination of urine is used to assess whether or not pyuria and bacteriuria are present. Also, it can be used as an alternative to a urethral Gram stain to detect the presence of urethritis in men.

### MATERIALS

- Centrifuge and centrifuge tubes
- Microscope slides
- Coverslips (22 x 22 mm)
- Pasteur pipets
- Gram stain reagents
- Alcohol or Bunsen burner
- Brightfield microscope

### SPECIMEN COLLECTION

#### Pyuria/bacteriuria

Appropriate collection of a “clean catch” midstream urine specimen is critical. Refrigerate specimen immediately if examination will be delayed.

##### *Instructions—women*

1. Spread labia.
2. Wash urethral area from front to back with 4 soaped gauze sponges, one at a time.
3. Then rinse urethral area in the same manner with 4 damp gauze sponges.
4. Void a small amount of urine into the toilet, then continue voiding into the specimen container.

#### Urethritis

##### *Instructions*

##### Self Collection

1. Do not void for 2 hours prior to specimen collection.
2. Collect the *first* 10 to 15 ml of voided urine.

### EXAMINATION AND INTERPRETATION

#### Pyuria/urethritis

1. Centrifuge 10 to 15 ml urine for 5 minutes at 1500 rpms.
2. Discard the supernatant.
3. Place one drop of sediment on a slide using a Pasteur pipet and add a coverslip.
4. Examine with the high dry (40X) objective.

Positive for pyuria	Positive for urethritis
Midstream “clean-catch” specimen ≥ 10 PMNs/400X field	First-void specimen ≥10 PMNs/400X field

## Bacteriuria

1. Draw a small circle 5 to 10 mm in diameter on a slide with a grease pencil.
2. Do not centrifuge specimen.
3. Mix the specimen well and place 1 drop of urine into the circle on the slide.
4. Allow to air dry.
5. Heat-fix and Gram stain.
6. Examine first with the 10X objective to locate the smear, then move to the oil immersion (100X) objective (1000X magnification) to count PMNs and bacteria.

### Positive for bacteriuria

1-2 organisms/1000X field  
(correlates with  $\geq 10^5$  organisms/ml  
colony count)

All bacteria of same morphotype  
(i.e., large Gram-negative rods)

Finding a variety of bacteria in each field (Gram-negative rods, Gram-positive cocci, Gram-positive rods) or vaginal epithelial cells indicates probable contamination during collection—the smear must be interpreted with caution.

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## URINE REAGENT STRIPS

As an alternative to urine microscopy, reagent strips containing a leukocyte esterase (LE) test patch are used to detect pyuria (midstream urine) and urethritis (first-void urine). A nitrite test on the reagent strips is used to detect bacteriuria. The nitrite test is based on the ability of bacteria in the urine to reduce nitrate (from the diet) to nitrite.

### MATERIALS

Reagent strips for urinalysis with LE and nitrite test patches.

### SPECIMEN COLLECTION

The specimen collection for reagent strips is identical to that used for URINE MICROSCOPY.

- Pyuria/bacteriuria: midstream “clean catch”
- Urethritis: *first* 10-15 ml voided; patient should not void for 2 hours prior to collection.

### EXAMINATION AND INTERPRETATION

1. Use fresh, uncentrifuged urine at room temperature.
2. Dip the reagent strip into the urine and remove immediately.
3. Touch the strip to the edge of the urine container to remove excess urine.
4. Hold strip horizontally while waiting for color to develop.
5. Match the strip color to the color key on the test strip bottle: read the nitrite test at 1 minute and the LE test at 2 minutes. A positive LE is defined as  $\geq 1+$ .

## Pyuria

Positive	False positive	False negative
<p>Read LE test @ 2 min. Positive results indicative of pyuria.</p> <p>Will detect minimum 5-15 PMNs/400X magnification.</p>	<p>Test read <i>after</i> 2 min.*</p> <p>Urine contaminated w vaginal discharge containing PMNs#</p>	<p>Elevated urine glucose; high specific gravity; presence of cephalixin, cephalothin, tetracycline, or oxalic acid</p>
<p>* color may continue to develop # cannot distinguish PMNs from bladder and vagina</p>		

## Bacteriuria

Positive	False positive	False negative
<p>Read nitrite test @ 1 min. Positive results indicative of bacteriuria (<math>\geq 10^5</math> organisms/ml)</p>	<p>Interpreting pink spots or edges of the test block as positive. Test block should have uniform color.</p>	<p>Bacteria present do not reduce nitrate.* Urine not held in bladder at least 4 h before voiding.# High specific gravity, high ascorbic acid</p>
<p>* i.e., <i>Staphylococcus saprophyticus</i>, enterococcus # urine has not been held in the bladder long enough (4h) for nitrite to accumulate</p>		

*Note:* a negative nitrite result does not rule out bacteriuria.

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## MICROSCOPY FOR ECTOPARASITES

### MATERIALS

- Brightfield microscope
- Mineral oil
- Sterile scalpel
- Microscope slides
- Coverslips (22 x 22 mm)

## **SPECIMEN COLLECTION AND INTERPRETATION**

### **Crabs**

Microscopic examination of a hair shaft will reveal nits of *Phthirus pubis*. Adult lice can also be captured and examined microscopically.

### **Scabies**

A clinical diagnosis of scabies can be confirmed by demonstrating microscopically the mite, *Sarcoptes scabiei*, its eggs, and fecal pellets.

1. Locate recently developed, unexcoriated papules or burrows.
2. Place a small drop of mineral oil on the site.
3. Using a sterile scalpel, scrape the lesions 6 or 7 times to remove the tops of papules or burrows.
4. Transfer oil and scalpel material to a microscopic slide and cover with a coverslip.
5. Examine under low (10X) power. The adult female mite is 400 microns long. Eggs are large and oval in shape. Fecal pellets may be more numerous than mites or eggs.

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## **RAPID PLASMA REAGIN TEST (RPR)**

This is a nontreponemal, qualitative, serological test for the diagnosis of syphilis. It is quickly performed because it requires unheated serum or plasma and allows for the macroscopic interpretation of results. However, for accurate results, careful controls must be concurrently observed.

### **MATERIALS**

- RPR Card Test Kit containing:
  - RPR card antigen suspension
  - Needle and antigen dispensing bottle
  - RPR test cards (18 mm)
  - Dispensers
  - RPR control cards
- Known positive and negative sera for controls (optional)
- Card test rotator with humidifying cover
- Distilled water
- 1-ml serological pipets
- Plasma or serum (5-ml tube adequate)

### **STORAGE OF MATERIALS**

#### **Unopened ampules of antigen suspension**

- Store in refrigerator (2-8°C).
- Do not freeze.
- Observe manufacturer's expiration date.

### **Antigen suspension in dispensing bottles**

- Store in refrigerator (2-8°C).
- Reactivity of the antigen is usually satisfactory for 3 months. Storage at room temperature shortens satisfactory period.
- Avoid exposure to bright sunlight and temperature greater than 29°C.

### **Control cards**

- Store unopened in sealed packages in the refrigerator (2-8°C).
- Observe manufacturer's expiration date.

### **Control sera**

- Store in freezer.

### **Test cards**

- Store at room temperature in a dry, protected area.
- Avoid touching test circles on the cards.

## **PREPARATION OF MATERIALS AND QUALITY CONTROL**

### **Periodically**

- Test of needle accuracy is performed before initial use of antigen dispensing needle. Needle is siliconized.  
Never touch or wipe it as this will remove silicone coating and affect accuracy.
  1. Attach needle to 1-ml serological pipet.
  2. Fill the pipet with antigen suspension.
  3. Hold the pipet vertically and count the number of drops delivered in 0.5 ml  
Needle for the 18 mm card test is 20 gauge and should deliver 30 drops  $\pm$  1 drop.  
Do not use a needle not meeting this requirement.
- Opening new antigen ampule  
Shake vigorously 10 to 15 seconds to resuspend antigen.  
Avoid excessive shaking as this will roughen the antigen.
  1. Attach needle to the dispensing bottle.
  2. Transfer all the antigen from the ampule to the dispensing bottle.
  3. Label dispensing bottle with lot number of antigen and expiration date (3 months if stored in refrigerator).

### **Daily**

- Check temperature of test area—acceptable range 23-28°C.
- Warm all materials and specimens to room temperature (23-28°C) before using.
- Check speed of rotator by holding a pen next to the rotating shelf and counting the number of taps per minute.  
Desired speed = 100 rpm  
Acceptable speed = 95-110 rpm  
Make necessary adjustments before using.
- Check humidifying chamber for adequate moisture.

- Test antigen suspension on control cards for proper reactivity. Can be done concurrently with specimen card. To avoid cross-contamination, use 3 different Dispensstirs. Alternatively, use a different Dispensstir for each circle.
  1. Using Dispensstir, place 0.05 ml distilled H<sub>2</sub>O in each circle.
  2. Spread drop, add antigen, and rotate as in test procedure.

Acceptable results are:

<b>Reactive circle</b>	<b>Nonreactive circle</b>	<b>Reactive-minimal circle</b>
Strong clumping	No clumping, homogeneous gray appearance	Minimal to moderate clumping

- Quality control can also be performed using pooled positive and negative patient sera.
- Rinse needle with distilled water, shake to dry, and replace protective shield. This will keep the needle passage clear. *Do not touch or wipe the needle.*

### **QUALITATIVE TEST PROCEDURE**

1. Draw specimen carefully into Dispensstir. Avoid picking up cells and bubbles.
2. Hold Dispensstir vertically and squeeze firmly so that 1 drop (approximately 0.05 ml) of the specimen falls freely into test card circle. Do not touch card surface.
3. Use sealed end of Dispensstir to spread specimen to fill entire circle. Do not spread outside the circle.
4. Gently invert antigen bottle several times.
5. Hold the bottle vertically and dispense 4 to 5 drops of antigen into the dispensing bottle cap to check that the needle passage is clear.
6. Put one drop antigen onto the test circle. The drop must not cling to shaft of needle.
7. Do not mix specimen and antigen.
8. Transfer antigen from bottle cap (step 5) back into dispensing bottle.
9. Rotate test card for 8 minutes under humidifying cover.

### **INTERPRETATION AND REPORTING**

To facilitate interpretation, tilt the card by hand 3 or 4 times. Read immediately under a bright light or daylight. A fluorescent light source may cause interpretation errors.

<b>Reactive</b>	<b>Nonreactive</b>
Characteristic clumping. Includes minimal to moderate reactives. REPORT: reactive	Slight roughness or no clumping  REPORT: nonreactive

## Section II

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### Collection and Handling of Specimens

#### DETECTION OF NEISSERIA GONORRHOEAE

##### CULTURE

Gonococci are susceptible to drying and temperature extremes and most isolates require an environment rich in CO<sub>2</sub> for initial growth. Thus, close attention must be paid to these requirements to optimize recovery of the organism. Specimens may be held in transport media for a few hours or they may be directly inoculated onto culture media and incubated in the clinic in a CO<sub>2</sub>-rich environment.

##### Specimen collection

Using a swab, collect secretions from the area under examination. Cotton, dacron, and calcium-alginate swabs can be used.

##### Urethra

1. Preferably the patient should not urinate for at least 2 hours before collection.
2. Insert swab 1 to 2 cm into the urethra and gently rotate the swab.

##### Cervix

1. Using a large cotton swab, remove external vaginal secretions from the cervix before collection of endocervical secretions.
2. Using a clean collection swab, insert and rotate the swab and allow several seconds for absorption.

##### Rectum

1. Insert the swab at least 2 cm.
2. Rotate the swab to sample areas of pus but not feces.
3. Some experts suggest specimen collection with an anoscope.

##### Oropharynx

Swab the posterior pharynx and tonsillar crypts.

##### Direct inoculation of culture media

This method is recommended for recovery of gonococci because immediately after collection, the organisms are transferred to a suitable environment for growth.

##### Culture media and environment

1. Use a selective agar, such as modified Thayer-Martin medium.
2. Use a system for producing and maintaining a CO<sub>2</sub> environment, such as a biologic environmental chamber—JEMBEC, TABCO, BIOBAG.
  - Place the inoculated agar plate and a CO<sub>2</sub>-generating tablet or capsule inside a zip-lock bag.
  - Tightly seal the bag to retain the CO<sub>2</sub>-rich atmosphere.
3. If a candle jar is used to establish a CO<sub>2</sub>-rich environment, place the inoculated agar plate, agar side up, in a large jar with a lighted candle. When the lid is tightly closed and the candle has been extinguished, the atmosphere contains approximately 3% CO<sub>2</sub>. Light the candle each time the jar is opened.
4. Set incubator at 35° to 36°C.

### **Procedure for inoculation and handling**

1. Check culture medium for expiration date and for a smooth surface (free of cracks, wrinkles, and contaminating bacteria or mold).
2. Warm media to room temperature.
3. Rotate specimen swab with gentle pressure on to entire agar surface.
4. Place the culture in a CO<sub>2</sub> environment within 15 min. of inoculation.
5. Incubate at 35° to 36°C within 1 hour of inoculation.
6. Incubate the culture for 18 to 24 hours before transporting to the lab.

### **Use of transport media**

If direct inoculation of culture media is not feasible, specimen swabs are placed in non-nutritive transport medium, such as Cary Blair or Stuart's. The transport media is held at room temperature and sent to the laboratory where the culture media is inoculated and incubated as described above. Gonococci remain viable in the transport medium for up to six hours.

### **Culture method**

Colonies of *N. gonorrhoeae* appear after 24 to 48 hours of incubation. In the laboratory, identification is made by colony morphology, microscopic morphology, and positive oxidase reaction. The identification may be confirmed by carbohydrate utilization, fluorescent antibody, or agglutination tests. Confirmation of results is needed particularly for specimens taken from the pharynx, from children or if medicolegal issues are a concern (for example, evaluation of sexual assault).

### **Testing for antimicrobial resistance in *N. gonorrhoeae***

This is usually done only upon request, but is of particular importance in treatment failures or in special epidemiologic circumstances. A variety of methods are used, including agar dilution, disc diffusion, and E-test.

## **NUCLEIC ACID HYBRIDIZATION ASSAY**

### **DNA PROBE for detection of *N. gonorrhoeae***

- This test, manufactured by Gen-Probe as PACE®, is based on nucleic acid hybridization. A chemiluminescently labeled DNA probe targeted to a portion of the 16S ribosomal RNA of *N. gonorrhoeae* is mixed with the patient's specimen. Ribosomal RNA released from *N. gonorrhoeae* in the patient's specimen hybridizes with probe DNA. Non-hybridized probe is removed and the intensity of the luminescence of the DNA:RNA hybrids is measured.
- Chlamydia can be detected on the same swab using a probe specific for *C. trachomatis*.
- Urethral and endocervical swab specimens are acceptable. Always use the swab and the transport medium in the kit provided by the manufacturer. Keep specimens in transport media at 4°C or at room temperature for up to 7 days.

## **NUCLEIC ACID AMPLIFICATION TESTS (NAAT)**

Several types of NAAT are available for the detection of *N. gonorrhoeae*. These include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement assay (SDA), hybrid capture assay, and transcription mediated assay (TMA). Key general points regarding these tests are as follows:

- All exhibit equal or slightly greater sensitivities for detection of *N. gonorrhoeae* than that of culture, and all have excellent specificity as well (≥99.6%).
- All can be performed on 'traditional' swab specimens collected from the urethra and cervix.

- Most importantly, all can be performed on urine. It is important that first catch urine (FCU) be collected; this consists of the first 10-15 cc of voided urine. Ideally, patients should not have voided for at least one, and preferably two, hours prior to FCU collection. Midstream urine should *not* be used.
- Specifications for storage, transport, and stability at room temperature vary by specific assay. Users should be familiar with the instructions for the specific tests being used.
- Some tests have been approved for performance on self-collected vaginal swabs, and others are being evaluated for this purpose. Check the manufacturers' specifications for up-to-date information in this area.
- None of the NAAT are approved for use on rectal or pharyngeal detection of *N. gonorrhoeae*.

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## DETECTION OF CHLAMYDIA TRACHOMATIS

### CULTURE

#### Specimen collection

Viable chlamydial organisms are found within urethral, cervical, and rectal epithelial cells, but not in exudate or pus. Thus, a specimen containing purulent discharge is not adequate. The type of swab used for specimen collection is critical to the success of chlamydia cultures, so use only a swab provided or recommended by the laboratory. *Do not* use wooden shafted swabs. Cytobrushes, used for collection of cervical specimens, appear to result in specimens with a greater number of epithelial cells and, in some studies, more frequent recovery of *Chlamydia trachomatis*.

The following techniques provide optimum specimen collection.

#### Cervix

1. Clean the cervical os to remove debris and secretions. This is very important because these make the cell culture plates difficult or impossible to read.
2. Insert a swab into the external os and rotate it several times.
3. Remove, taking care not to contaminate the swab with material from the vaginal walls and place in transport medium.

*Note:* If swabs are obtained for other tests (Pap smear or gonorrhea), they should *precede* the swab to be used for the chlamydia test. However, if a cytobrush is used for Pap collection it can subsequently be placed in the chlamydia transport media.

#### Urethra—women

Urine-based testing should be used on women who have undergone hysterectomy.

#### Urethra—men

1. Insert a swab 2 to 3 cm into the urethra and rotate, making sure the swab is in contact with the urethral wall.
2. Remove the swab and place it in transport medium.

#### Rectum

1. Insert a swab 2 cm into the rectal canal and rotate.
2. Make sure the swab is obtained from the rectal mucosal surface.
3. Remove the swab and place it in transport medium.

## **Specimen transport**

Chlamydia are fragile organisms and do not survive at room temperature or after prolonged (>72 h) refrigeration. After placing the swab in the chlamydia transport medium, either refrigerate and deliver to the lab on wet ice within 72 hours, or immediately freeze on dry ice and maintain frozen at -70°C until delivery to the laboratory. A normal freezer temperature of -20°C will not preserve the organisms.

## **Culture method**

In the laboratory, tubes or plates containing living cells (cell culture) are inoculated with the specimen. After 2 to 3 days of incubation, the cells are stained with fluorescein-conjugated monoclonal antibodies, iodine, or Giemsa, and examined microscopically. A positive culture shows cells containing characteristic intracytoplasmic inclusions.

## **DIRECT IMMUNOFLUORESCENCE**

Several kits are available for the rapid detection of chlamydial elementary bodies in urethral, cervical, conjunctival, and rectal smears directly stained with specific fluorescein-labeled antibody (FA). Specimen collection techniques are the same as those described in the culture section, except that the specimen swab is smeared onto the glass slide provided by the manufacturer, allowed to dry, fixed in methanol, and sent to the laboratory. The fixed slides are stable and are suitable for mailing. Trained microscopists apply the specific FA reagent and read the slides using a fluorescence microscope. Low sensitivity of the test has been reported in low risk populations when the number of elementary bodies may be very small, but the test is relatively sensitive and quite specific in high-risk populations.

## **ENZYME IMMUNOASSAY (EIA)**

Advantages of this method include ease of transport and rapid results. Endocervical, urethral, or conjunctival specimens are collected on swabs provided by the manufacturer and are held in the refrigerator (4-8°C) until sent to the laboratory. Collection techniques are the same as those described in the culture section. If urine is to be tested, instruct the patient to collect only 10 to 15 ml from the initial urine stream. Send this sample to the lab. These tests have a relatively good sensitivity and specificity in high-risk populations, but less satisfactory results have been found in low-risk populations.

## **NUCLEIC ACID HYBRIDIZATION ASSAY**

A DNA probe targeted to a portion of the 16S rRNA of *C. trachomatis* forms the basis of the Gen-Probe PACE® chlamydia diagnostic test. Specimens are collected using a dacron swab and transport tube and medium provided by the manufacturer. The specimen is stable for up to one week at room temperature. For chlamydia, the test has been successfully used for cervical, urethral, and conjunctival specimens. An assay for gonorrhea can be performed on the same specimen.

## **NUCLEIC ACID AMPLIFICATION TESTS (NAAT)**

Several types of NAAT are available for the detection of *C. trachomatis*. These include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement assay (SDA), hybrid capture assay, and transcription mediated assay (TMA). Key general points regarding these tests are as follows:

- All exhibit considerably greater sensitivities for detection of *C. trachomatis* than that of non-amplified DNA probe (PACE2), EIA, or DFA-based tests, and to a lesser extent, greater sensitivity than that of culture. All have excellent specificity as well (≥99.6%).

- All can be performed on ‘traditional’ swab specimens collected from the urethra and cervix.
- Most importantly, all can be performed on urine. It is important that first catch urine (FCU) be collected; this consists of the first 10-15 cc of voided urine. Ideally, patients should not have voided for at least one, and preferably two, hours prior to FCU collection. Midstream urine should *not* be used.
- Specifications for storage, transport, and stability at room temperature vary by specific assay. Users should be familiar with the instructions for the specific tests being used.
- Some tests have been approved for performance on self-collected vaginal swabs, and others are being evaluated for this purpose. Check the manufacturers’ specifications for up-to-date information in this area.
- None of the NAAT are approved for use on rectal or pharyngeal detection of *C. trachomatis*.

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## DETECTION OF HERPES SIMPLEX VIRUS

### CULTURE

#### Specimen collection

The stage of the lesion and the quality of the specimen collected significantly affect culture sensitivity. Sensitivity decreases with increasing lesion age. Thus, herpes simplex virus (HSV) is recovered most frequently from vesicular lesions and infrequently from crusted lesions. Primary lesions are also more likely to yield virus than are recurrent lesions.

*Note:* When collecting the specimen, emphasis is on collection of cells from the *base* of the lesion.

Culture sensitivity according to stage of lesion	
Vesicle	90% +
Pustule	80-90%
Ulcer (≤5 days)	60-75%
Ulcer (>5 days)	50%
Crust	20-30%

#### Vesicular or pustular lesion

1. Unroof the vesicle with an 18-gauge needle.
2. Using a moistened swab, abrade the base of the lesion in order to obtain a good sample of cells.
3. Immediately place the swab in viral transport media.

#### Crusted lesion

1. Remove the crust.
2. Scrape the *base* of the lesion with a moistened swab. Avoid making the lesion bleed.
3. Immediately place the swab in viral transport media.

#### Specimen transport

Depending on the type of transport medium used, the specimen should either be refrigerated or held at room temperature until transported to the laboratory.

**If refrigerated**

- Deliver to the lab on wet ice or a coldpak within 72 hours.
- When delivery to the lab is delayed >72 hours, maintain the specimen on dry ice or at -70°C. (Normal freezer temperature of -20°C will not preserve the virus.)

**Culture method**

In the laboratory, tubes containing living cells (cell culture) are inoculated with the specimen and incubated. Positive HSV cultures are usually detected between 1 and 7 days. By day 5, about 90% of positive cultures will be detected. The cell cultures are examined microscopically for cytopathic effects characteristic of HSV. Type-specific monoclonal antibodies are then used to confirm the identification and to type the isolate as HSV-1 or HSV-2.

**DIRECT IMMUNOFLUORESCENT**

This method is used to demonstrate viral antigen in a direct smear made from an external genital lesion. Collect cells from the base of the lesion as described in the CULTURE section above, roll the swab on the slide provided by the manufacturer, add fixative, and send to the laboratory. Fluorescein-labeled antibodies (FA) to HSV-1 and HSV-2 are added to the slide, and trained microscopists read the slides using a fluorescence microscope. Mixed infections of both HSV-1 and HSV-2 can be detected by this method. Sensitivity of the test varies with the age of the lesion and the number of cells collected. This test should not be used for detecting viral shedding from the cervix.

**SEROLOGICAL TESTS****Enzyme immunoassay (EIA)**

- Glycoprotein G1 and glycoprotein G2-specific EIAs differentiate seronegative and HSV-1 / HSV-2 seropositive samples
- Commonly available commercial test due to ease of test performance and relatively low cost
- Examples include: Focus Technologies HerpeSelect™–2ELISA which detects type-specific antibodies for HSV-1 and HSV-2 infection and is easy to use, fast and automated; and, POCKit which detects antibodies to HSV-2 only and can detect early seroconversion

**Western blot**

- Distinguishes antibody response to HSV-1 and HSV-2 infection
- Detects early seroconversion
- Not widely available and remains largely a research tool

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## **DETECTION OF SYPHILIS**

### **CULTURE**

No cultural methods are available.

### **DIRECT SMEARS**

#### **Darkfield microscopy**

Darkfield microscopy is used to demonstrate *Treponema pallidum* in material from lesions or lymph nodes. The presence of *T. pallidum* constitutes a definitive diagnosis of syphilis. Since *T. pallidum* is identified by characteristic morphology and motility, the preparation must be fresh and the organisms actively motile. Considerable expertise is required not only for the correct identification of the spirochetes but also for proper use of a darkfield microscope. For these reasons, the test should only be performed in a setting where it is routinely done.

#### **Direct fluorescent antibody (DFA-TP)**

As an alternative to darkfield microscopy, fixed smears from lesions, serous fluid, or lymph node aspirates may be sent to reference laboratories for staining with fluorescein-conjugated antibody to *T. pallidum*. The results, however, are usually not available for days to weeks and thus, may not be helpful in guiding patient management.

### **SEROLOGICAL TESTS**

#### **Nontreponemal or reagin tests**

This group of common nontreponemal tests measure antibody to a nonspecific cardiolipin lecithin antigen. The tests are moderately specific for syphilis (false-positives occur), but highly sensitive. Because they are easily performed, the nontreponemal tests are useful screening tools. The tests can be quantitated to obtain a titer and, thus, are useful in monitoring patient response to therapy.

- VDRL: “Venereal Disease Research Laboratory,” the standard test against which other nontreponemal tests are compared.
- RPR card test: “Rapid Plasma Reagin,” a rapid field test performed in STD clinics.
- USR: “Unheated Serum Reagin,” a modification of the VDRL test.

#### **Treponemal Tests**

These two common treponemal tests measure antibody specific for *T. pallidum*. They are both highly specific and highly sensitive. Treponemal tests are not currently used for general screening because they are expensive and time consuming to perform. Their use is limited to confirmation of positive reagin tests (to identify false-positive diagnoses) and in the diagnosis of late syphilis when reagin tests may be nonreactive.

- FTA-ABS: “Fluorescent Treponemal Antibody-Absorption”
- MHA-TP: “Micro-hemagglutination–*Treponema pallidum*”
- TP-PA: “*T. pallidum* particle agglutination”

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## **DIAGNOSIS OF CHANCROID**

### **CULTURE**

Isolation of *Hemophilus ducreyi* from a genital lesion or lymph node provides a definite diagnosis of chancroid. However, it is difficult to isolate the organism and culture of *H. ducreyi* may not be offered by all laboratories.

Request media from the laboratory in advance so the specimen can be plated immediately after collection. Gonococcal agar base supplemented with bovine hemoglobin, fetal calf serum and vancomycin is recommended.

### **Specimen collection**

1. Clean the lesion thoroughly with sterile nonbacteriostatic saline.
2. Then moisten a cotton-tipped swab with saline and swab the lesion.
3. Press and roll the swab on the agar plate and immediately deliver to the laboratory.

### **Culture method**

In the laboratory, the plates are incubated in a candle jar lined with damp gauze. Colonies of *H. ducreyi* appear from 2 to 9 days after inoculation.

### **DIRECT GRAM STAIN**

Gram stain of a lymph node aspirate may be helpful in making the diagnosis of chancroid when tiny, chaining Gram-negative rods are seen. Gram stain of a lesion is generally not recommended because of the frequent polymicrobial nature of these lesions.

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## **DIAGNOSIS OF LYMPHOGRANULOMA VENEREUM (LGV)**

### **SEROLOGICAL TESTS**

Serological testing, by microimmunofluorescence (MIF) or the more widely available LGV complement fixation test, is used to establish the diagnosis of LGV. A fourfold rise in titer by complement fixation indicates active infection, while a single titer of 1:64 or greater supported by clinical finding suggests infection. Specific antibody to the LGV immunotypes of *Chlamydia trachomatis* can be demonstrated by MIF.

### **CULTURE**

Lymph node aspirate may be sent for chlamydia culture. Isolation of the LGV immunotypes (L1, L2, or L3) is diagnostic.

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## **DIAGNOSIS OF GRANULOMA INGUINALE (GI) (Donovanosis)**

### **SMEAR**

A touch prep of a lesion biopsy or tissue smear stained with Giemsa or Wright's stain is used to demonstrate infection with *Calymmatobacterium granulomatis*. Large mononuclear cells with characteristic intracytoplasmic Donovan bodies are diagnostic.

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## **DETECTION OF TRICHOMONAS VAGINALIS**

As described in the section on saline microscopy of vaginal fluid, trichomoniasis is usually diagnosed by visualization of motile trichomonads on saline microscopy of vaginal fluid. This method has an estimated sensitivity of 60% relative to an expanded diagnostic standard that includes culture and polymerase chain reaction (PCR). With regard to these specific tests:

- Saline microscopy should be performed immediately on fresh specimens of vaginal fluid to enhance the likelihood of detection. Even with appropriate performance, sensitivity of this test generally does not exceed 60% to 65%.
- Culture for *T. vaginalis* can be performed using various media, the most widely available being the InPouch system which is inoculated with the swab used to collect the specimen. This system can be used to culture the urethra in men and women as well as vaginal fluid.
- PCR is available for *T. vaginalis*, and can be applied to vaginal, urethral, or urine specimens.
- Antigen detection assays for *T. vaginalis* are under evaluation.