

Lab Updates

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Inside this issue:

Changes in Epstein-Barr Virus (EBV)
and Lyme Serology Test Reports

Sexually Transmitted Diseases



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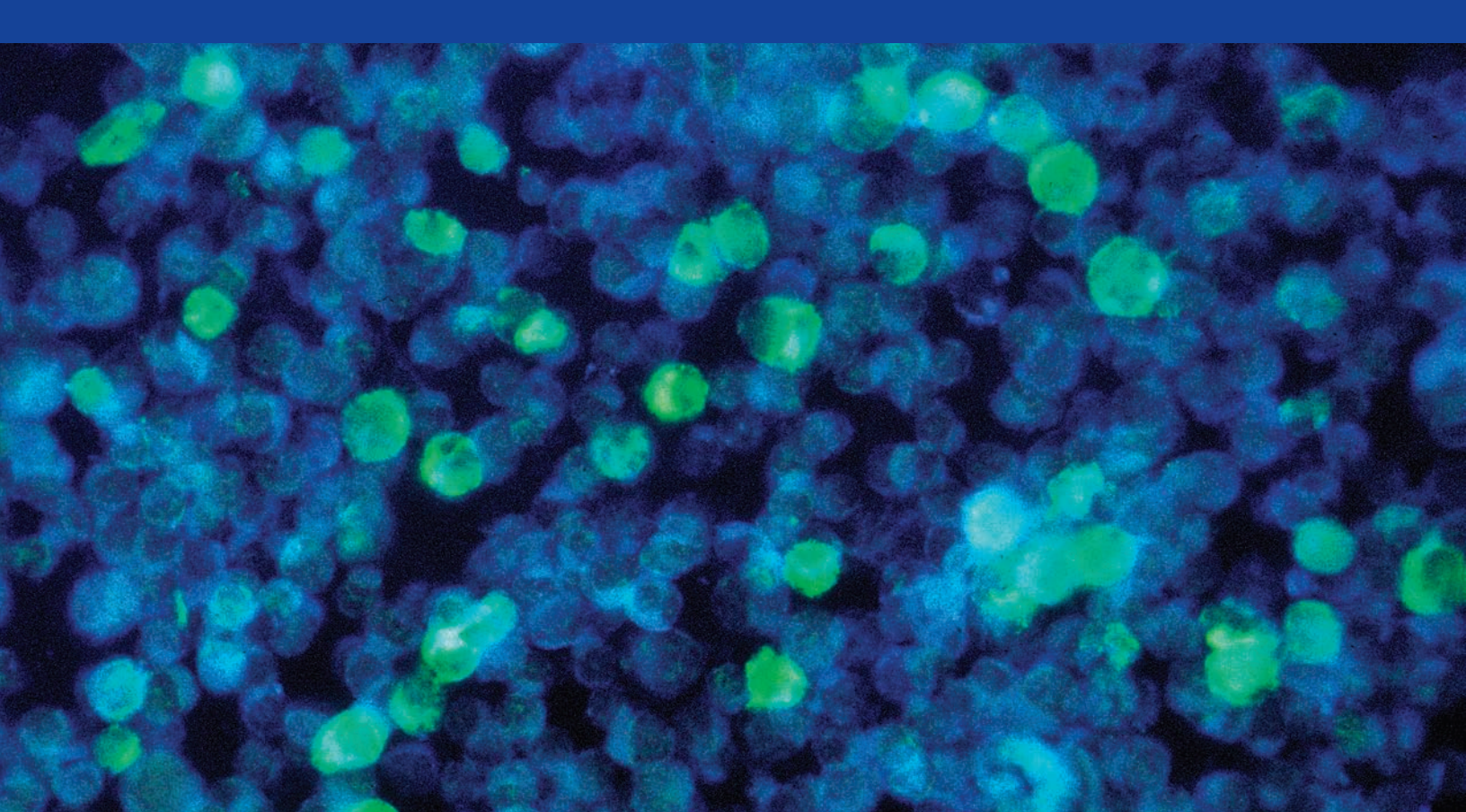


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Changes in Epstein-Barr Virus (EBV) and Lyme Serology Test Reports

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EBV IS A WIDELY DISSEMINATED HERPES VIRUS that is spread by intimate contact between susceptible persons and EBV shedders. The virus has not been recovered from environmental sources, suggesting that humans are the major reservoir. EBV primarily spreads via passage of saliva, but is not a particularly contagious disease. The virus can persist in the oropharynx of patients with IM for up to 18 months following clinical recovery. EBV has also been isolated in both cervical epithelial cells and in male seminal fluid, suggesting that transmission may also occur sexually.

Antibodies to EBV have been demonstrated in all population groups with a worldwide distribution; approximately 90-95% of adults are eventually EBV-seropositive.

EBV acquired during childhood years is often subclinical; less than 10% of children develop clinical infection despite the high rates of exposure. The incidence of symptomatic infection begins to rise in adolescent through adult years.

Although they are highly specific in the appropriate clinical setting, heterophile antibodies are somewhat insensitive. The false negative rates are highest during the beginning of clinical symptoms (25% in the first week; 5 to 10% in the second week, 5% in the third week). More specific EBV testing can be pursued in those with more prolonged illness or in those who do not fit classic diagnostic criteria. Rare false-positive heterophile tests have been reported in patients with leukemia, lymphoma, pancreatic cancer, SLE, HIV infection, and rubella. In addition, heterophile antibodies can persist at low levels for up to one year after IM. Approximately 10% of mononucleosis-like cases are not caused by EBV. Other agents that produce a similar clinical syndrome include CMV, HIV, toxoplasmosis, HHV-6, hepatitis B, and possibly HHV-7.

Measurement of EBV-specific antibodies may be warranted in patients with suspected IM and a negative heterophile test. IgM and IgG antibodies directed against viral capsid antigen have high sensitivity and specificity for the diagnosis of IM (97 and 94%, respectively).

Viral capsid antigen

IgM and IgG antibodies directed against the Epstein-Barr viral capsid antigen (VCA) are usually present at the onset of clinical illness because of the long viral incubation period. IgM levels wane approximately three months later; thus, they are a good marker of acute infection. IgG VCA antibodies persist for life and are a marker of EBV infection. Results of VCA testing need to be interpreted within the appropriate clinical context. Although the presence of IgM VCA antibodies is highly suggestive of acute EBV infection, other herpes viruses (eg, CMV) can induce IgM antibodies to cell lines that express EBV antigens. In addition, during illnesses associated with intense immune activation, serologic EBV reactivation with detectable EBV IgM VCA antibodies has been described in the absence of clinical IM.

Nuclear antigen

IgG antibodies to EBV nuclear antigen (EBNA, a protein expressed only when the virus begins to establish latency) begin to appear 6–12 weeks after the onset of symptoms and persist throughout life; their presence early in the course of an illness effectively excludes acute EBV infection. Thus, while the presence of IgM VCA antibodies suggests the likely presence of acute EBV infection, the diagnosis is most certain in the presence of IgM VCA and the absence of IgG EBNA antibodies.



Early antigen

IgG antibodies to early antigen (EA) are present at the onset of clinical illness. There are two subsets of EA IgG: anti-D and anti-R. The presence of anti-D antibodies is consistent with recent infection since titers disappear after recovery, but their absence does not exclude acute illness because the antibodies are not expressed in a significant number of patients. Anti-R antibodies are only occasionally present in IM.

EBV Panel includes all 4 of antibodies and interpretive chart will be given as a canned comment on every patient report. Also, all the below antibodies can be ordered separately.

1. EBV Serology (EBV)

- a. EBV VCA-IgM Antibodies (EBVVCAIGM)
- b. EBV-VCA-IgG Antibodies (EBVVCAIGG)
- c. EBV-Nuclear Antigen IgG Antibodies(EBVEBNA)
- d. EBV-Early Antigen IgG Antibodies (EBVEARG)

Reference Range: ≥ 1.1 Antibody Index

Canned Comment: Positive

Any Index value ≤ 0.8 will be flagged and reported with canned comment, "Negative".

Any Index value ≥ 0.9 to ≤ 1.0 will be flagged and reported with canned comment; "Equivocal".

All patient test reports will have an interpretation as below.

EBV Serological Status	EBV-NA IgG	EBV VCA IgG	EBV-EA IgG	EBV VCA IgM	Heterophile Ab
Primary Acute	NEG	POS	POS	POS	NEG
	NEG	NEG	POS	POS	POS
	NEG	POS	NEG	POS	POS
	NEG	NEG	NEG	POS	POS
	NEG	NEG	NEG	POS	NEG
	NEG	NEG	POS	POS	NEG
	NEG	POS	POS	POS	POS
	NEG	POS	POS	NEG	POS
Late Acute	NEG	POS	NEG	POS	NEG
	POS	POS	POS	POS	POS
	POS	POS	POS	POS	NEG
	POS	POS	NEG	POS	POS
	POS	POS	POS	NEG	NEG
	POS	POS	NEG	POS	NEG
Recovering	NEG	POS	POS	NEG	NEG
Previous Infection	NEG	POS	NEG	NEG	NEG
	POS	POS	NEG	NEG	NEG
Susceptible	NEG	NEG	NEG	NEG	NEG

2. Lyme Serology Screen (LYME):

Reference Range: ≥ 1.09 Index Value

Canned Comment: Positive. Presence of detectable *Borrelia burgdorferi* antibodies. This sample was sent to Western Blot confirmation for both IgM and IgG antibodies.

Any Index value < 0.9 will be flagged and reported with canned comment, "Negative". Absence of detectable *Borrelia burgdorferi* antibodies. A negative result does not exclude the possibility of *Borrelia burgdorferi* infection. If early Lyme disease is suspected; a second sample should be collected and tested two to four weeks later.

Any Index value 0.9 to 1.09 will be flagged and reported with canned comment; "Equivocal". This sample was sent to Western Blot confirmation for both IgM and IgG antibodies.

Note: The magnitude of the measured result, above the cut-off, is not indicative of the amount of antibody present.

3. Toxoplasma IgG antibodies:

Reference Range: ≥ 8.0 IU/mL

Canned Comment: Positive. The presence of IgG antibodies to *Toxoplasma gondii* is an indication of past infection.

Any Index value < 6.5 IU/mL will be flagged and reported with canned comment, "Negative". Absence of IgG antibodies to *Toxoplasma gondii*.

Any Index value 6.5 to < 8.0 will be flagged and reported with canned comment; "Equivocal". Intermediate levels of *Toxoplasma gondii* IgG antibodies. Please submit another specimen in 1–2 weeks.

4. Toxoplasma IgM antibodies:

Reference Range: ≥ 1.1 S/C Ratio

Canned Comment: Positive. The presence of IgM antibodies to *Toxoplasma gondii* is an indication of recent infection. The magnitude of the measured results above the Cutoff is not indicative of the total amount of antibodies detected.

Any S/C ratio < 0.9 will be flagged and reported with canned comment, "Negative". *Toxoplasma* IgM antibodies were not detected.

Any S/C ratio between 0.9 and < 1.1 will be flagged and reported with canned comment; "Equivocal". Intermediate levels of *Toxoplasma gondii* IgM antibodies. Please submit another specimen in 1–2 weeks



5. Cytomegalovirus (CMV) IgG antibodies:

Reference Range: ≥ 1.1 S/C Ratio

Canned Comment: Positive. The presence of IgG antibodies to Cytomegalovirus is an indication of past infection. The magnitude of the measured results above the Cutoff is not indicative of the total amount of antibodies detected.

Any S/C ratio < 0.9 will be flagged and reported with canned comment, "Negative". Cytomegalovirus IgG antibodies were not detected.

Any S/C ratio between 0.9 and < 1.1 will be flagged and reported with canned comment; "Equivocal". Intermediate levels of Cytomegalovirus IgG antibodies. Please submit another specimen in 1–2 weeks.

6. Cytomegalovirus (CMV) IgM antibodies:

Reference Range: ≥ 35.0 AU/mL

Canned Comment: Positive. The presence of IgM antibodies to Cytomegalovirus is an indication of acute infection, reactivation or persistent IgM production.

Any Index value < 30.0 AU/mL will be flagged and reported with canned comment, "Negative". Absence of IgM antibodies to Cytomegalovirus.

Any Index value 30.0 to 34.9 will be flagged and reported with canned comment; "Equivocal". Intermediate levels of Cytomegalovirus IgM antibodies. Please submit another specimen in 1–2 weeks

Effective May 19, 2009, EBV, Lyme serology, Toxoplasma IgG, IgM and CMV IgG and IgM antibody test reports now include the quantitative value along with test interpretation. Previously they were reported qualitatively as negative or positive.

If you have questions, comments or suggestions, please contact:

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Sexually Transmitted Diseases

I. Definition. A sexually transmitted disease (STD) is a disease that is transmitted through sexual contact.

II. Syphilis. This disease is caused by the spirochete *Treponema pallidum*. There has been a marked increase in syphilis cases coincident with the HIV epidemic.

A. Clinical features

1. Primary syphilis occurs about 3 weeks after infection (range: 3–90 days).

A painless papule appears at the site of contact. The papule erodes into an ulcerated, wet chancre. Moist chancres are teeming with spirochetes, rendering the patient highly infectious. Regional adenopathy may occur.

2. Secondary syphilis: Signs and symptoms of secondary syphilis occur 2–8 weeks after the chancre disappears. During this stage, spirochetes disseminate to all organs of the body. Systemic symptoms include a macular rash, generalized lymphadenopathy, fever, and malaise.

3. Latent stage of syphilis: During the latent phase of syphilis, patients are asymptomatic, but seropositive. The latent stage may last for years.

4. Tertiary syphilis: The chronic symptoms of tertiary syphilis may occur months to years after primary infection.

B. Laboratory diagnosis

1. Culture of *T. pallidum* in vitro is not available. Therefore, diagnosis is made by demonstrating *T. pallidum* directly in infected tissue or by serology.

2. Direct examination: Spirochetes may be demonstrated by specific or nonspecific staining. Darkfield examination of fluid exuded from infected lesions may be diagnostic, if the typical *T. pallidum* morphology and motility are noted.

3. Serologic testing: Antibody response can usually be demonstrated between 3–10 weeks after infection. Two types of assays are used.

a. “Nontreponemal” assays measure antibodies formed in response to *T. pallidum*-induced tissue damage; these antibodies are cross-reactive with cardiolipin.

(1) The rapid plasma reagin (RPR) assay is the most commonly used nontreponemal assay, whereas the Venereal Disease Research Laboratory (VDRL) assay is the only assay standardized for testing cerebrospinal fluid.

(2) Because biological false positives occur in nontreponemal assays, all positive results must be followed up with a treponemal assay (see II B 3 b). The titer of nontreponemal assays may fall (or disappear) in late tertiary syphilis and must be interpreted with caution in late chronic disease.





b. Treponemal assays are based on specific *T. pallidum* antigens. They are most useful in confirming positive nontreponemal assay results and may be the most accurate tests in tertiary syphilis. False-positive reactions are rare.

- (1) The microhemagglutination-*T. pallidum* (MHA-TP) assay measures agglutination of erythrocytes sensitized with *T. pallidum* antigen. The assay has been miniaturized in a microtiter plate format.
- (2) In the fluorescent treponemal antibody, absorbed (FrA-ABS) assay, antibodies in test serum react with *T. pallidum* organisms that have been fixed to a slide. Specific antibodies in the serum reactive with surface antigens on the organisms are detected using labeled antihuman antibodies.
- (3) False-negative reactions may occur in primary syphilis, but sensitivity increases with longer duration of disease.

III. Gonorrhea. Gonorrhea is caused by the Gram-negative diplococcus, *Neisseria gonorrhoeae*.

A. Clinical features

1. **Symptoms usually occur within 7 days after exposure.**
 - a. **Men** who are infected usually have urethritis, epididymitis, or prostatitis.
 - b. **Women** who are infected typically have cervicitis, urethritis, salpingitis, pelvic inflammatory disease (PID), or perihepatitis (i.e., Fitz-Hugh Curtis syndrome). PID may occur in 10% of women with cervical infection. Infertility is a major complication.
2. **Disseminated gonococcal infection** develops in 1%–2% of patients. Certain complement deficiency states increase the risk for disseminated gonococcal infection. Myalgia, arthralgia, polyarthritis, tenosynovitis, and peripheral dermatitis characterize this bacteremic illness.

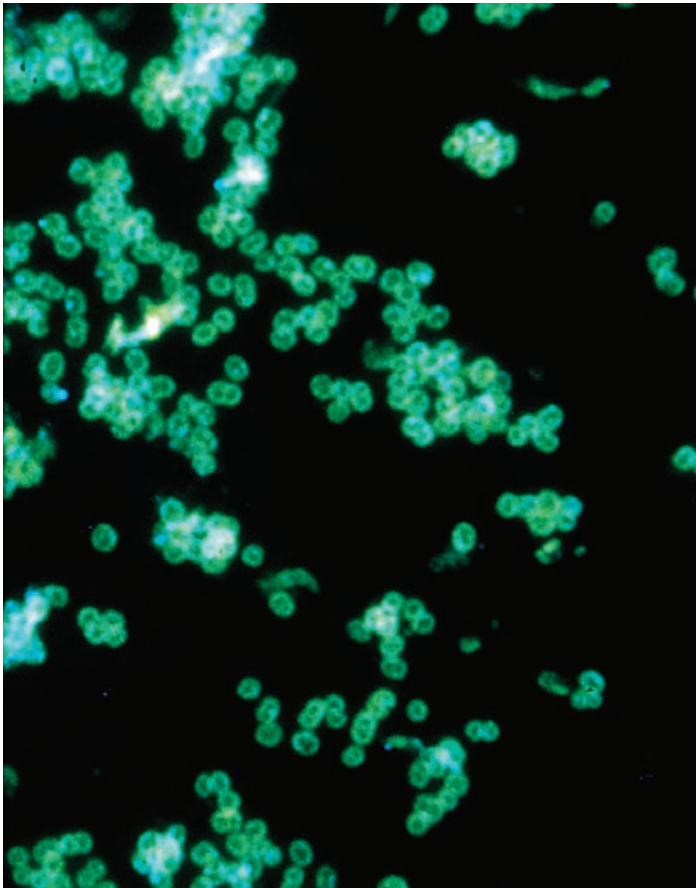


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b. Sensitivity of culture. Cultures of secretions show high sensitivity for detecting gonorrhea in men. Sensitivity of culture is somewhat lower in women (80%–90%), due in part to problems associated with appropriate specimen collection.

c. Proctitis. If proctitis is suspected, obtain sample secretions from the anal crypts. Discard swabs stained with stool.

3. Molecular genetic testing: Direct probe or molecular amplification techniques are available for the detection of *N gonorrhoeae* infection.

a. Cervical and urethral specimens may be submitted for molecular diagnosis.

b. Because molecular genetic testing offers increased sensitivity, urine may be tested by molecular amplification tests.

c. Use of molecular tests may eliminate the transport problems associated with cultures of *N gonorrhoeae*.

d. Susceptibility testing is unavailable for infections documented by molecular genetic testing.



B. Laboratory diagnosis

1. Direct examination

- a.** In men, a Gram stain of urethral discharge showing bunches of intracellular Gram-negative diplococci is diagnostic.
- b.** Genital secretions in women should not be stained because of nonpathogenic Gram-negative diplococci in normal vaginal flora.

2. Cultures of local lesions are frequently negative. Clinical diagnosis and culture of the primary site of infection is critical.

a. Handling of specimen. Isolates are fastidious and lose viability quickly. Therefore, special handling of specimens is critical. The use of charcoal-containing transport media for swabs may ensure viability for 12–24 hours.

- (1)** The requisition for throat swabs submitted to rule out gonorrhea pharyngitis must specifically indicate that *N gonorrhoeae* is suspected.
- (2)** The anticoagulant sodium polyanethol sulfonate (SPS), present in most commercial blood culture media, may inhibit isolates of *N gonorrhoeae*.

IV. Chlamydia. Chlamydia is caused by the intracellular pathogen *Chlamydia trachomatis*, which is the most common cause of bacterial STD.

A. Clinical features. The spectrum and clinical features of *C. trachomatis* infection may be indistinguishable from gonorrhea.

- 1. Intrapartum infection** may result in inclusion conjunctivitis, mucosal infection, and pneumonitis in newborns.
- 2. Lymphogranuloma venereum (LGV)** is an uncommon presentation of *C. trachomatis* infection caused by serotypes LI-3.
 - a.** In the primary stage of LGV, a painless ulcer forms at the site of inoculation.
 - b.** Subsequently, during the second phase, painful regional (typically inguinal) adenopathy develops.
 - c.** During the tertiary phase of LGV, systemic symptoms, local cutaneous changes, draining sinuses, and lymphatic obstruction may develop.

B. Laboratory diagnosis

1. Direct detection

- a.** Inclusion conjunctivitis caused by *C. trachomatis* may be accurately detected (~90%) by the direct staining of corneal specimens using a tagged monoclonal antibody.
- b.** The sensitivity for cervical or urethral specimens is only about 75% sensitive compared with culture.
- c.** Similar sensitivity has been reported for *C. trachomatis* detection by enzyme immunoassay (EIA). The specificity of EIA testing may limit its usefulness for screening low prevalence populations unless confirmatory testing is performed.
- d.** Detection of *C. trachomatis* using direct hybridization of nucleic acid probes may be comparable to detection by culture.

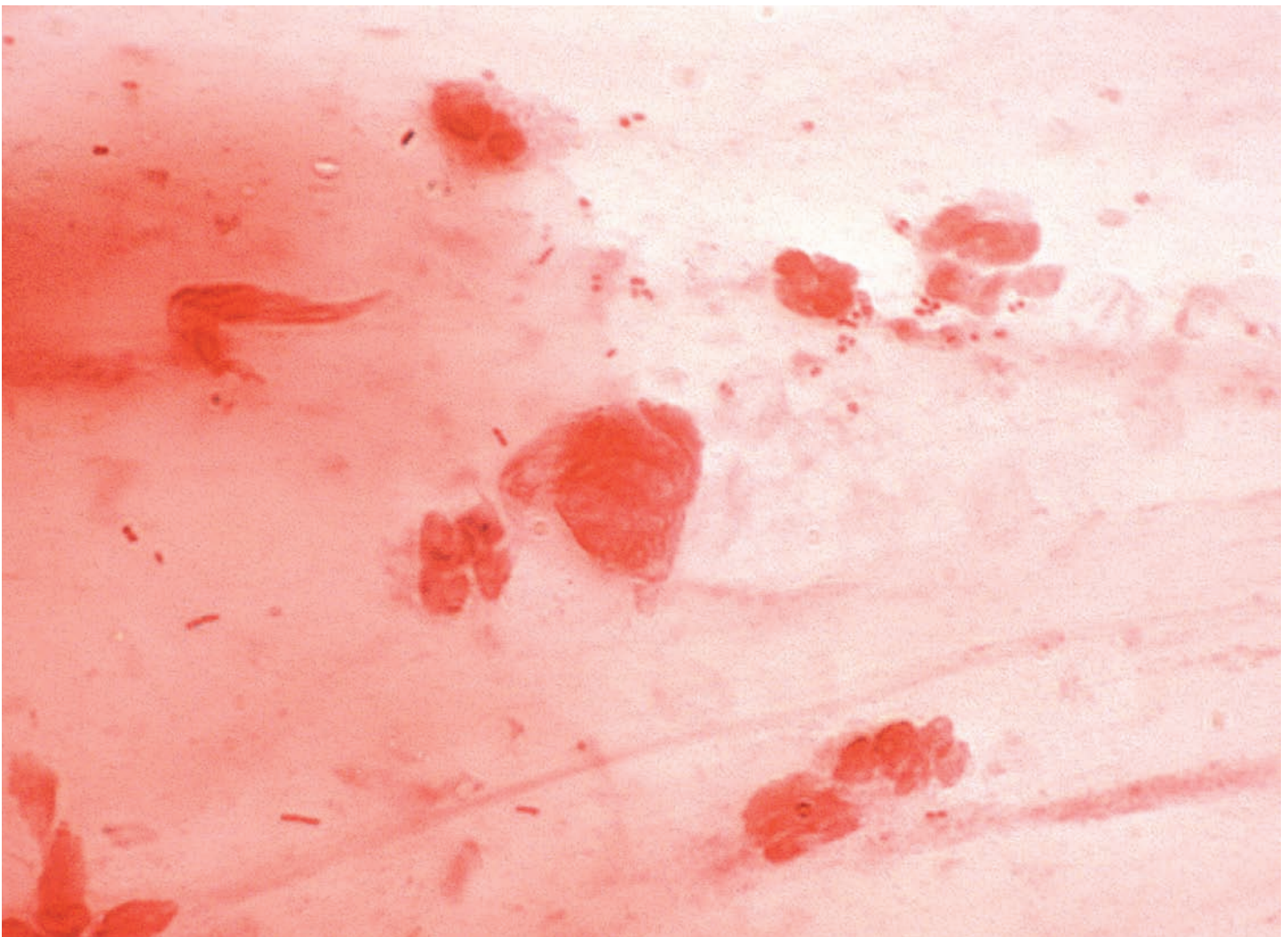


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2. Culture: It is critical to sample infected **epithelial cells** for the diagnosis of *C. trachomatis* infection. Purulent discharge should be removed before vigorously sampling the epithelial surface of infected tissue.

- a. Specimens for *C. trachomatis* culture should be placed in transport media and sent to the laboratory on wet ice.
- b. Specimens acceptable for *C. trachomatis* culture include cervical os, urethra, corneal scraping, fallopian tube biopsy, lymph node aspirate, and anal swab.
- c. Subculture of initially negative cultures increases the sensitivity of detection. Properly performed, around 80% of patients with *C. trachomatis* infection can be identified by culture.

3. Amplified nucleic acid tests

- a. Polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription-mediated amplification (TMA), and other assays have been developed to amplify specific *C. trachomatis* sequences in clinical material before detection with specific probes.
- b. Amplification techniques can identify 90%–95% of infected patients. A small number of infected patients may not be detected because of substances in the specimen that inhibit the amplification reaction.

- c. Use of urine specimens in amplified nucleic acid tests yield detection comparable with *C. trachomatis* culture and may be a suitable noninvasive specimen type.

IV. Genital Herpes. Herpes simplex virus (HSV) causes genital herpes infection. **HSV type 2** causes most cases, although a significant number of cases (10%–40%) are caused by HSV type 1.

A. Clinical features

1. **Most genital HSV infections are asymptomatic**, which may contribute to the spread of the disease.
2. **Symptomatic disease** tends to be more severe in women, especially in those patients with primary infection. Typical infection consists of a localized vesicular rash that may be preceded by pain or tenderness.
 - a. Symptoms usually occur **within 7 days after infection**. New vesicles form for a week or more. As vesicles rupture, local spread to the urethra, anus, and adjacent mucosal and cutaneous sites is common. Virus shedding may continue for weeks after symptom onset.
 - b. Herpetic lesions may be **exquisitely sensitive** with intense local inflammation including lymphadenopathy.



- c. Urethritis and dysuria are common symptoms in women.
- d. Systemic symptoms of fever, headache, and malaise also occur more commonly in women.
- e. Infection in men is frequently localized and associated with lower incidence of symptoms.

3. Infection at nongenital sites may occur by primary sexual or genital contact (pharyngitis, proctitis, neonatal infection), viremic spread (disseminated rash), or neural spread (meningitis in patients with lumbosacral symptoms).

4. Recurrent HSV infections occur in most patients due to reactivation of latent virus.

B. Laboratory diagnosis. Virus shedding is greatest in vesicular and wet ulcerative lesions compared with crusted lesions.

1. Direct detection: Cells scraped from the base of unroofed vesicles or wet ulcers may be stained for rapid identification.

a. Multinucleated giant cells demonstrated by a Tzanck stain are consistent with HSV infection, but cannot distinguish lesions from *Varicella zoster* virus.

b. The use of tagged monoclonal antibodies gives HSV results that are 80%–90% sensitive compared with culture.

2. Culture detection: Mucocutaneous lesions may be sampled by Dacron swab early in infection. Calcium alginate swabs may be inhibitory and should not be used. Swabs should be placed in viral transport media and sent to the laboratory on wet ice. Specimens are often positive within 2 days.

3. Serological testing may identify prior infection, but is of limited value in the diagnosis of acute HSV infection.

Reference: Mitchell, Michael, M.D., and Daly, Jennifer, M.D., "Sexually Transmitted Diseases". *Guide to Diagnostic Testing*. pp 214-218.

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