## Microbiology

Organisms not readily identified by routine methods may be submitted for identification or confirmation by various nucleic acid, biochemical, or serologic tests. Examples of such organisms include unusual or atypical Gram-negative bacilli, yeasts, fungi, atypical mycobacteria, mycoplasmas, and viruses.

Organisms must be in pure culture, actively growing on agar slants or in transport medium before they are sent. The selection and extent of tests used for identification vary according to the origin of the specimen from which the microorganism was isolated and the type of infection suspected or produced. This information must be provided on a Microbiology Request Form before processing can begin. In virtually all instances, organisms other than viruses may be transported at room temperature. Viral cultures, as a rule, require refrigeration only. If necessary, call NorDx for additional information. Cultures of pathogenic microorganisms must be considered infectious and be shipped as infectious substances.

ANTIMICROBIAL SUSCEPTIBILITY TESTS
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests require a pure and viable culture of the infecting bacteria.

The MIC and MBC provide a measure of the minimum concentration, in µg/mL, of an antimicrobial required to inhibit or to kill, respectively, a particular organism.

When only MIC is requested, antimicrobials appropriate to the organism and source will be chosen from the following: amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, ceftazidime, chloramphenicol, clindamycin, ciprofloxacin, erythromycin, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, oxacillin, penicillin, piperacillin/tazobactam, ticarcillin/clavulanic acid, trimethoprim, trimethoprim/sulfamethoxazole, and vancomycin. MIC for other agents will be provided only by special request. Requests for MBC should designate which antimicrobials are to be tested. MBC test results are expressed in terms of concentrations killing 99.9% or more of the inoculum.

SERUM BACTERICIDAL TITER (SBT)
This test requires a pure and viable culture of the infecting bacteria. Also, a minimum of 3.0 mL of serum drawn 30 minutes after completion of infusion for intravenous dose and 60 minutes after intramuscular or oral dose is to be sent frozen in a screw-capped, sterile vial on dry ice. In most instances, bactericidal titers ("peak titers") of at least 1:8 are desirable. It is important to remember that an SBT measures the combined activity of all antimicrobials present at the time of serum collection. Please include a Microbiology Request Form complete with all information necessary to process the specimen.

SEROLOGY (Chlamydia trachomatis, Chlamydia psittaci, and Chlamydia pneumoniae)
Information:

The organisms within the genus Chlamydia are divided into three species:

1. Chlamydia trachomatis, consisting of 15 biotypes, is responsible for a wide variety of infections including inclusion blennorrhea and pneumonia in infants and inclusion conjunctivitis and trachoma in adults and children. Chlamydiae have also been implicated in some cases of nongonococcal urethritis, proctitis, cervicitis, and pelvic inflammatory disease (PID). Among the 15 biotypes are three that produce lymphogranuloma venereum (LGV).
2. Chlamydia psittaci causes pneumonia when transmitted to humans from infected psittacine birds.
3. Chlamydia pneumoniae (formerly called TWAR) has properties distinct from Chlamydia trachomatis and Chlamydia psittaci. This species is responsible for approximately 10% of cases of community-acquired pneumonia.

The micro-immunofluorescence assay utilizes purified elementary bodies as the substrate. This new test is able to distinguish antibodies specifically reactive to each Chlamydial species. Thus, antibodies directed to Chlamydia pneumoniae can be specifically detected. Cross-reacting antibodies to the specific chlamydial antigens are uncommon.

Serology Interpretation:
- Species: Chlamydia trachomatis and Chlamydia psittaci
- IgG Titer >= 1:64
- Comment: IgG end point titers of >= 1:64 are considered presumptive evidence of infection.
- IgG Titer: < 1:64
- Comment: IgG end point titers <1:64 suggest that the patient does not have a current infection. This may be found in patients with either no history of chlamydial infections or those with past infection whose antibody levels have dropped below detectable levels.

- Species: Chlamydia pneumoniae
- IgG Titer: >= 1:512
- Comment: IgG end point titers of >= 1:512 are considered presumptive evidence of current infection.
- IgG Titer: >= 1:64 and < 1:512
- Comment: A single specimen end point titer >= 1:64 and <1:512 should be considered evidence of infection at an undetermined time. If a second specimen drawn 10-21 days after the original draw exhibits a titer of >= 1:512 or a fourfold increase over that of the initial specimen, current infection is indicated. Unchanging titers >= 1:64 and <1:512 suggest past infection.
- IgG Titer: < 1:64
- Comment: IgG end point titers <1:64 suggest that the patient does not have a current infection. This may be found in patients with either no history of chlamydial infection or those with past infection whose antibody levels have dropped below detectable levels.

- Species: Chlamydia trachomatis, Chlamydia psittaci, and Chlamydia pneumoniae
- IgM Titer: >=1:10
- Comment: IgM end point titers of >= 1:10 are considered presumptive evidence of infection.
- IgM Titer: <1:10
- Comment: IgM end point titers <1:10 suggest that the patient does not have a current infection.

This may be found in patients with either no history of chlamydial infections or those with past infection whose antibody levels have dropped below detectable levels.

VIROLOGY
(Specimen Handling, Mailing, and Test Interpretation)

Virus Culture:
1.Culturette ® swabs: Swabs should be mailed on foam refrigerant but not frozen. Place coolant in the bottom of large NorDx's Styrofoam® container. Next, enclose swab in tightly sealed plastic bag and place this bag on top of refrigerant. DO NOT BEND OR BREAK SWAB. Pack vacant space with any type of packing material. Mark "REFRIGERATE ONLY" on the side of the outside mailing carton to prevent it from being placed in a freezer if received during off hours or weekends.
2. Urine and cerebrospinal fluid: Send 2.0 mL in a screw-capped, sterile vial. Specimens should be handled under sterile conditions to avoid contamination. Specimens should be considered infectious.
3. Miscellaneous (sputum, brain biopsy, blood): Specimen should be in a screw-capped, sterile container and handled under sterile conditions to avoid contamination. For blood cultures, collect at least 5.0 mL of blood in a sterile, EDTA evacuated tube. Specimens should be considered infectious.

Viral Serology: Indirect immunofluorescence assay (IFA) and enzyme immunoassay (EIA) comprise almost all serologic assays in diagnostic virology. For serologic testing, indicate tentative diagnosis and specific tests desired.
1. Send 1.0 mL in a screw-capped, sterile vial. Specimen should be handled under sterile conditions. Forward promptly in a NorDx's Styrofoam® container. Specimen does not need to be sent refrigerated or frozen. Specimens should be considered infectious.
2. Test interpretation:
- Congenital infections: Serial sera from both the mother and infant are required for laboratory diagnosis. Antibody levels in the infant which are passively acquired from the mother will decrease markedly within 2 - 3 months. Active infection, however, is indicated by antibody levels that are unchanged or increased in serial sera over 2 - 3 months. The absence of antibody to a particular virus in the mother rules out intrauterine infection. In addition, for cytomegalovirus and herpes simplex virus, IgM antibodies may be determined by the immunofluorescence test on a serum specimen from the infant (see below).
- Other infections: The laboratory diagnosis of virus infections requires a minimum of 1.0 mL of serum. A fourfold or greater rise in antibody titers between the acute and convalescent phase sera or the presence of antibody of the IgM class is indicative of infection. The timing for obtaining the sera is of utmost importance. Instructions and a reminder on obtaining convalescent phase serum will be included with the report of the previous specimen, whether it be an acute or a convalescent phase specimen. In most instances, a serologic test performed on a single serum specimen will not differentiate recent infection from one that occurred sometime in the past.
- IgM antibodies:
(a) Congenital infections: Immunoglobulin M is the first class of antibody produced after exposure to microbial agents. Qualitative detection of IgM is especially useful in the diagnosis of congenital and neonatal infections since, unlike IgG, this immunoglobulin does not cross the

placental barrier, and so any IgM detected in a neonate represents actively produced antibody.
(b) Other infections: Except in congenital diseases, immunoglobulin M antibodies can be detected for approximately 3-4 weeks after primary infections, although, in some cases, detectable IgM antibodies can persist for several months. Immunoglobulin M antibodies have also occasionally developed following reinfections (rather than primary infections) and have persisted for a period of several months.
(c) For our assays, IgM is physically separated from whole serum to avoid interference of rheumatoid factor-containing specimens in the test procedures.
- Immune status:
(a) Varicella-zoster virus (VZV) (chickenpox) antibody (screening test): Immune status to VZV is determined by an enzyme immunoassay (EIA). This test is equal in sensitivity to the fluorescent antibody to membrane antigen procedure.
(b) Measles and mumps virus antibody: Any detectable titer is evidence of previous infection. The detection of IgM class antibodies to these agents indicates acute phase infection.
(c) Rubella virus antibody: A microparticle enzyme immunoassay is used to detect both IgG (evidence of immunity) or IgM as an indication of recent acute infection with this virus. In addition, the test has the capability of detecting acute phase infection by determining IgG antibody increases in paired sera or the assay of IgM antibodies in a separate procedure.