

REFERENCE BACTERIOLOGY

INTRODUCTION

The primary function of the Reference Bacteriology Laboratory is to assist Kansas clinical laboratories and physicians in the identification, confirmation, and characterization of bacterial isolates of medical or bioterrorism significance. Emphasis is placed on the identification of unusual isolates, potential bioterrorism agents, and other organisms which are not readily identified by automated microbiology systems.

REFERENCE BACTERIOLOGY LABORATORY TESTS

- Biochemical identification of nonfermenters, non-enteric fermenters *Listeria*, etc.
- Bioterrorism-related organism identification including *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, and *Brucella* species.
- Serotyping of *Neisseria meningitidis* and *Haemophilus influenzae* group b.
- Detection of *Bordetella pertussis* in nasopharyngeal specimens by PCR and identification of culture isolates by a direct fluorescence antibody test.
- Culture and identification for *Corynebacterium diphtheriae*.
- Lancefield grouping of beta hemolytic streptococci.
- Species determination of *Neisseria*, *Streptococcus*, and *Staphylococcus*.
- Legionella specimens are referred to CDC.
- Anaerobic identification is not performed but clinically relevant isolates can be sent to CDC for identification. Call the Bacteriology Laboratory before sending isolates.
- Staphylococcus DNA fingerprinting: available for epidemiologic investigations. When DNA fingerprinting is required call the Diagnostic Microbiology section at (785) 296-1620 for information and requirements.

Additional reference tests are available at CDC when referred through this laboratory; please contact the Bacteriology Laboratory for more information at (785) 296-1620.

GENERAL INSTRUCTIONS FOR SENDING REFERENCE CULTURES

Cultures should be clinically relevant, not contaminants or normal flora components. Only pure cultures should be submitted on agar slants with screw caps to prevent leakage in transit. **Note: culture isolates are classified as dangerous goods and must be transported in a UN 6.2 certified infectious substance mailer.** The infectious substance mailer is available from the laboratory mail room (785-296-1620). Complete a laboratory request form. Specimens must be identified by name or other unique identifier. It is helpful to know which bacterial agent is suspected when a culture is submitted. When specimens or cultures require referral to the CDC, Form 50.34- CDC Referral Form, will need to be completed by the originator of the specimen before the specimen can be sent to the CDC. A copy of the form is in Appendix A or forms can be obtained through our Sample and Data Management Office. For further information and additional forms, please call (785) 296-1620.

TYPES OF CLINICAL SPECIMENS

- A. Nasopharyngeal swabs for *Bordetella pertussis* by PCR (Note: culture is provided only by prior arrangement, see note below) The specimen of choice is a nasopharyngeal swab collected pernasally: A commercial bacterial nasopharyngeal swab collection system should be used, such as the Bacti-Swab NPG Collection and Transport System available from REMEL as catalog # 12-300. (Note: citation of a product by name is not an endorsement by the state laboratory). With the patient's head tilted back about 70° from vertical and immobilized, gently insert the swab into a nostril until resistance is encountered as the swab contacts the nasopharynx. Patient tolerance permitting, try to leave the swab in this position for 10 seconds to absorb nasopharyngeal secretions. However, the tickling sensation of the swab usually induces a cough and good clinical judgment should dictate when to remove the swab.

Sample each nares with the swab and return the swab to the transport tube. **DO NOT** prepare smears from the swab.

Label the transport tube with the patient's name and attach a barcode from the form and complete the laboratory requisition including checking the pertussis PCR box on the back of the form. Send the completed laboratory request form and the swab to the Division of Health and Environmental Laboratories. The specimen can be shipped at room temperature since viable bacteria are not required for a PCR analysis.

NOTE: Analysis by PCR is the method of choice. The laboratory does not routinely provide media for *Bordetella* culture but can do so under special circumstances, usually in conjunction with an outbreak investigation by Epidemiologic Services. Experience suggests that when attempting culture isolation for *B. pertussis* that inoculation of either Regan-Lowe and/or Bordet-Gengou media immediately upon specimen collection is essential for recovery. The bacteria usually do not survive when transported before inoculation onto culture media in the state laboratory.

- B. Blood, CSF, and Urine for *Leptospira*

During the first week of illness in humans, blood and CSF are specimens of choice for the culture of *Leptospira* sp. After the first week of illness, blood and CSF specimens rarely yield leptospire, however, at this time and for several months thereafter, the urine may contain low levels of intermittently shed leptospire.

Culture in appropriate media is important, however, the Diagnostic Microbiology Laboratory does not provide *Leptospira* culture media nor are there any

commercial media sources in Kansas. **Please call to make arrangements for specimens for culture to be sent to CDC.**

C. Throat swab for culture of Diphtheria.

Diphtheria may cause a peritonsillar pseudomembrane or throat inflammation. Collect either a swab of the membrane or inflamed area or a portion of the pseudomembrane. Transport in a sterile, dry container. Swabs should not be placed in transport media.

Submit each labeled specimen along with a completed requisition form to the Division of Health and Environmental Laboratories using overnight mail.

REPORTING

Results of biochemical or serological tests are returned with final culture reports. Most cultures are reported in five to seven days: mixed cultures and slow-growing organisms often require more time. Diphtheria toxin production is the definitive characteristic of pathogenic *C. diphtheriae*. Isolates will be sent to CDC to confirm the presence of diphtheria toxin. Significant results are telephoned prior to sending written results.

REFERENCES

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C. 20005