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I. GENERAL INFORMATION

## A. Microbiology Services Introduction

The Medical Section of the SDPHL offers a listing of services we provide to our clients in the areas of bacteriology, serology, parasitology, mycobacteriology, mycology, and virology. The mission of this laboratory is to provide assistance to public and private health care providers in their investigation and control of diseases. It is believed that this mission can be best accomplished by the accurate analysis of clinical and reference specimens submitted by the community health departments, hospitals, other clinical laboratories, and physicians in private practice.

In addition to providing this manual, our laboratory supports several statewide investigations. When deemed necessary, both environmental and non-human specimens are tested to help investigate outbreaks of diseases. We also assist the Centers for Disease Control and Prevention (CDC) by participating in surveillance studies and by providing them with the results of our laboratory findings for a number of communicable diseases.

The State Health Laboratory provides specimen collection test kits, request forms and approved postal mailers. These are available upon request.

The "Quick Reference" section gives basic information about the tests offered and the corresponding computer codes to complete the test requisition forms. Collection instructions are also included with the collection kits. Collection requirements for particular tests are discussed in the "Detailed Reference" Section as well.

For unusual test requests, please contact the Laboratory to ensure proper sample submission.

#### B. Laboratory Contacts

1. Director, State Health Laboratory

Michael Smith

Laboratory telephone: 605-773-3368

FAX: 605-773-6129

Internet e-mail address: Mike.Smith@state.sd.us

2. Deputy Director

Jerry Hofer Laboratory Phone 605-773-3368 Fax 605-773-8201

Internet e-mail address: Jerry.Hofer@state.sd.us

3. Laboratory Section Phone Numbers

a.	Main Laboratory.	 605-773-3368

b. Microbiology/Parasitology/Mycology......605-773-4968

d.	Serology	605-773-5573 605-773-4969
e.	Virology/Rabies	605-773-6769
	DE0E/D0D	005 770 4000

f. PFGE/PCR......605-773-4238

g. Mailroom......605-773-3183

4. Rabies: Questions regarding rabies exposure (after hours)

Linda Schaefer

Office phone: 605-773-3914 Cell phone: 605-280-4810

Nick Hill

Office phone: 605-773-6528 Cell phone: 605-280-4810

5. Website Address for the Public Health Laboratory

www.state.sd.us/doh/Lab

## C. Hours of Operation

Monday through Friday – 8:00 A.M. to 5:00 P.M.

Saturday, Sunday and Holidays – Only by prior arrangement as in the event of a rabies specimen.

## D. Mailing Address

South Dakota Public Health Laboratory 615 East 4<sup>th</sup> Street Pierre, SD 57501

## E. Who can request laboratory services

- 1. All licensed physicians, dentists and optometrists.
- 2. All public health nurses and physicians assistants.
- 3. Veterinarians.
- 4. Local Health Departments.
- 5. Communicable Disease Specialists.
- 6. Indian Health Service clinics and hospitals.

Reports will be issued to the requesting agency (above).

F. General Requirements for Collecting and Submitting Specimens

See the <u>Quick Reference</u> section for brief information concerning the collection of specimens. Under the column labeled "kit" in this chart there is a Y or N;

Y= Yes, kit available.

N = No, kit not available.

G. Specimen Kits

Blood Lead: Capillary Blood Collection Kit

Chlamydia/Gonorrhea

Gen-Probe: Urine Collection Kit or Unisex Swab Collection Kit for Endocervical and

Male Urethral Specimens.

Enteric Cultures (1 vial): Para-Pak Enteric Plus

Mycobacteria: Sputum, fluid, etc: sterile screw-cap vial

Gastric Washings: 2-bottle set containing sterile water for wash and

empty sterile bottle to receive washings.

Urine: sterile bottle with buffer

Ova & Parasites (2 vials): Para-Pak 10% buffered neutral formalin

Para-Pak zinc-PVA fixative

Pertussis: Regan-Lowe tubes

Viral specimens: Viral transport media

Every effort has been made to provide suitable kits so that specimens will reach the Laboratory in the best possible condition. Physicians and laboratories should be familiar with the various kits available. Infectious disease material must be sent in approved containers. The importance of selecting the proper kit cannot be too strongly emphasized.

The containers and kits provided are for the purpose of submitting specimens to the South Dakota Public Health Laboratory only. The use of these kits for any other purpose or for use at any other facility constitutes misuse of state property.

H. Identification of Specimens

The request form supplied by the South Dakota State Health Laboratory must accompany <u>each</u> specimen to be tested and must include:

Patient/Specimen Information

- 1. The patient identifier, either the patient's name or a unique identifier.
- 2. The submitter's name and address, zip code + 4 and phone number.
- 3. The patient's date of birth.
- 4. County or city of patient residence.
- 5. Date of collection.
- 6. Patient history and specific diseases suspected should be included particularly for viral testing.
- 7. Specimen's collected by a clinic/provider to be billed to a Health Department Program must have prior approval from that program. Mark the CD Billing code box at the top of the form and write in the billing code provided by Health Department program manager.

Important: The specimen tube or container must be clearly labeled with the patient identifier shown on the request form. Unlabeled or mislabeled specimens will not be tested.

## I. Reportable Diseases in South Dakota

Category I: <u>Report immediately</u> on suspicion of disease	Category II: _Rep	port within 3 days
Anthrax (Bacillus anthracis)	Acquired immunodeficiency syndrome (AIDS)	Legionellosis (Legionella spp.)
Botulism (Clostridium botulinum)	Arboviral encephalitis, meningitis and infection (West	Leprosy/Hansen's disease (Mycobacterium leprae)
Cholera (Vibrio cholerae)	Nile, St. Louis, Eastern and Western equine, California	Listeriosis (Listeria monocytogenes) **
Diphtheria (Corynebacterium diphtheriae)	serotype, Japanese, Powassan)	Lyme disease (Borrelia burgdorferi)
Enterohemorrhagic E. coli (EHEC) shiga-toxin	Brucellosis (Brucella spp.) **	Malaria (Plasmodium spp.)
producing (Escherichia coli), includes E. coli O157:H7	Campylobacteriosis (Campylobacter spp.)	Melioidosis (Burkholderia pseudomallei)
**	Chancroid (Haemophilus ducreyi)	Mumps (paramyxovirus)
Influenza, novel strains	Chicken pox/Varicella (herpesvirus)	Nipah virus (paramyxovirus)
Measles (paramyxovirus)	Chlamydia infections (Chlamydia trachomatis)	Pertussis (Whooping cough) (Bordetella pertussis)
Meningococcal disease, invasive (Neisseria meningitidis)	Cryptosporidiosis (Cryptosporidium parvum)	Psittacosis (Chlamydophila psittaci)
**	Cyclosporiasis (Cyclospora cayetanensis)	<b>Q fever</b> (Coxiella burnetii)
Plague (Yersinia pestis) **	Dengue fever (flavivirus)	Rocky Mountain spotted fever (Rickettsia rickettsii)
Poliomyelitis (picornavirus)	Drug resistant organisms:	Salmonellosis (Salmonella spp.) **
Rabies, human and animal (rhabdovirus)	- Methicillin-resistant Staphylococcus aureus	Shigellosis (Shigella spp.) **
Ricin toxin	(MRSA), invasive	Staphylococcus enterotoxin B
Rubella and congenital rubella syndrome (togavirus)	- Vancomycin-resistant and -intermediate	Streptococcal disease, Group A, invasive
SARS (Severe Acute Respiratory Syndrome,	Staphylococcus aureus (VRSA and VISA) **	Streptococcal disease, Group B, invasive
coronavirus)	- Drug resistant Streptococcus pneumoniae (DRSP),	Streptococcus pneumoniae, invasive (<5-years of age)
Smallpox (Variola)	invasive	Syphilis (Treponema pallidum)
Tularemia (Francisella tularensis) **	Ehrlichiosis (Ehrlichia spp.)	Tetanus (Clostridium tetani)
Typhoid (Salmonella typhi) **	Epsilon toxin of Clostridium perfringens	Toxic shock syndrome
Viral Hemorrhagic Fevers (filoviruses, arenaviruses)	Giardiasis (Giardia lamblia / intestinalis)	Transmissible spongiform encephalopathies
Outbreaks:	Glanders (Burkholderia mallei) **	Trichinosis (Trichinella spiralis)
- Acute upper respiratory illness	Gonorrhea (Neisseria gonorrhoeae)	<b>Tuberculosis</b> (Mycobacterium tuberculosis and M. bovis)
- Diarrheal disease	Haemophilus influenzae type b disease, invasive **	active disease and latent infection (positive skin test)
- Foodborne	Hantavirus pulmonary syndrome (hantavirus)	Typhus fever (Rickettsia prowazekii)
- Illnesses in child care settings	Hemolytic uremic syndrome	Vaccine Adverse Events
- Nosocomial	Hepatitis, acute viral A, B, C, D, and E	Yellow fever (flavivirus)
- Rash illness	Hepatitis, chronic viral B and C	
- Waterborne	Hepatitis B infection, perinatal	** Send isolate to SD Public Health Laboratory
Syndromes suggestive of bioterrorism and other public		(in addition to submitting an isolate please submit full
health threats	Human immunodeficiency virus infection (HIV)	disease report by one of the methods listed in "how to
Unexplained illnesses or deaths in humans or animals	Influenza: 1. hospitalizations and deaths, 2. lab	report" section)
	confirmed cases (culture, DFA, PCR), 3. weekly	
	aggregate report of total rapid antigen positive (A and	
	B) and total tested	

The South Dakota Department of Health is authorized by SDCL 34-22-12 and ARSD 44:20 to collect and process mandatory reports of communicable diseases by physicians, hospitals, laboratories, and other institutions.

#### How to report:

Secure website: www.state.sd.us/doh/diseasereport

Telephone: 800-592-1804 confidential answering device, or 800-592-1861 or 605-773-3737 for communicable disease staff person during

normal business hours; after hours Category I diseases or other emergencies, call 605-280-4810. Fax: 605-773-5509

**Mail** or **courier**, address to: Infectious Disease Surveillance, Department of Health, 615 East 4th Street, Pierre, SD 57501; marked "Confidential Disease Report"

What to report: Reports must include as much of the following as known:

- Disease or condition Date
- Case name, age, date of birth, sex, race, address and occupation
- Pertinent laboratory results and specimen collection date
- Date of disease onset
- Attending physician's name, address and phone number
- Name and phone number of the person making the report.

**CANCER** (SDCL 1-43-14) Report to SD Cancer Registry; call 800-738-2301; Reportable cancers list found at http://doh.sd.gov/SDCR/PDF/ReportableList.pdf

The reportable list has been divided into Category I and Category II diseases. Category I diseases are reportable immediately by telephone on the day of recognition or strong suspicion of disease. Category II diseases are reportable by telephone, mail, courier, or facsimile within three days after recognition or strong suspicion of disease.

Reportable disease reports must include the following, or, as much of the following as is known to the person making the report:

- 1. The disease or condition diagnosed or suspected;
- 2. The case's or carrier's name, age, sex, race, address, and occupation;
- 3. The date of onset of illness and whether the person is hospitalized and, if so, where;
- 4. Any pertinent laboratory results;
- 5. What, if any, public health measures have been taken;
- 6. The name and address of the attending physician; and
- 7. The name and telephone number of the person making the report.

If the reportable disease is an epidemic or outbreak, the report must also include the diagnosis or principal symptoms, the approximate number of cases, the place or community within which the cases occurred or are occurring, and the name and telephone number of the reporting physician or person.

Several methods of reporting are provided:

- 1. By telephone, call 1-800-592-1804 and respond to the departments' automatic answering-recording device, or, call 1-800-592-1861 or 773-3737 during normal business hours and provide the required information to an authorized department representative, or, by facsimile 773-5509;
- By mail, place the report in a sealed envelope addressed to the Department of Health, Office of Disease Prevention, 615 East 4th Street, Pierre, SD 57501, and marked "Confidential Medical Report";
- 3. By courier, deliver the report to the department in a sealed envelope and addressed as in 2);
- 4. By telephone for after hours, holidays, or weekends to report Category I diseases, call cellular phone (280-4810).

#### J. Definitions/Abbreviations

- **CDC Centers for Disease Control**
- **CF Complement Fixation**
- CNS Central Nervous System
- CSF Cerebrospinal Fluid
- EIA Enzyme Immunoassay
- FA Fluorescent Antibody

Etiological agent - Cause of disease

GC - Gonorrhea

ID - Identification

IFA - Indirect Fluorescent Antibody

M.TB - Mycobacterium Tuberculosis

TPA/TP-PA – Treponema pallidum agglutination Assay for Antibodies to

Treponema Pallidum

NP – Nasopharyngeal

PCR- Polymerase Chain Reaction

RSV - Respiratory syncytial virus

RPR - Rapid plasma reagin

RMSF - Rocky Mountain Spotted Fever

SDPHL - South Dakota Public Health Laboratory

SLE - St. Louis Encephalitis

VDRL - Venereal Disease Research Laboratory

WEE - Western Equine Encephalitis

Zn-PVA - Zinc Polyvinyl alcohol

**II. QUICK REFERENCE CHART** 

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page:
Adenovirus Culture	Virology	VOI	Nasopharyngeal or throat swab or washing conjunctival swab, fecus, urine.	Y	Container and transport varies with specimen. Ship refrigerated or frozen	76-81
AFB (Acid Fast Bacilli)See – Mycobacterium						
AIDS – See HIV 1/2						
Anaerobic Bacteria	Microbiology	BMD	Pure Culture	N	An02 transport system. Do not refrigerate or freeze.	28-30
Anthrax: See Bacillus Anthracis						
Arbovirus Serology	Virology	VAS	Acute and convalescent sera drawn 2-4 weeks apart.	N		62-66
Ascaris-see Parasites						
Bacillus Anthracis	Microbiology	BMD	Pure culture, lesion, blood, and respiratory specimens.	N		22-27
Bacillus Cereus	Microbiology	BMD	Clinical Specimens related to food borne outbreak.	N		42-42
Bacteroides	Microbiology	BMD		N		
Blastomyces Culture	Mycology	BMI	Pure culture.	N	Suspected blastomyces should be labeled as such.	57-58
Blood lead	Blood lead	BLT	EDTA whole blood, capillary or venous.	Y	Capillary blood collection kit.	83-86
Bordetella pertussis –see Pertussis	Microbiology	BPC	Nasopharyngeal swab or aspirate	Υ	Reagen Lowe Transport Media	31-32
Borrelia Burgdorferi – See Lyme Disease						
Botulism – see Clostridium botulinum.						
Brucella Culture	Microbiology	BMD	Pure culture.	N	Submit on chocolate agar slant.	22-27

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Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Brucella Serology	Serology	SBR	Acute and convalescent sera.	N		62-66
Campylobacter	Microbiology	CAM	Pure culture or stool.	Υ	Para-Pak Enteric Plus kit.	37-38
Chickenpox – see Varicella Zoster						
Chlamydia	Serology	GP1	Female cervical, male urethral, and urine.	Y	Gen Probe Aptima Combo 2 collection kit. May ship at room temperature.	44-47
Chlamydia/Gonorrhoeae Panel	Serology	GPB	Female cervical, male urethral and urine.	Y	Gen Probe Aptima Combo 2collection kit. May ship at room temperature.	44-47
Cholera-see Vibrio						
Clostridium botulinum	Microbiology	BMD	Serum, feces, food wound culture.	N	Call for specific instructions.605-773-4968	33-36
Clostridium perfringens	Microbiology	BMD	Reference culture	N	Anaerobic transport. Do not refrigerate or freeze.	42-42
CMV – See Cytomegalovirus						
Coccidioides	Mycology	ВМІ	Reference Culture	N	Suspected coccidiodies should be labeled as such.	57-58
Corynebacterium diphtheria-see diptheria						
Coxsackie culture (Enterovirus)	Virology	VEI	Throat swab, feces, CSF pericardial fluid	Y	Viral transport media.	76-81
Coxiella burnettie – see Q Fever						
Cryptosporidia	Parasitology	ВСР	Stool	Y	Para-Pak formaline vial.	59-61
Cyclospora	Parasitology	BCS	Stool	Y	Para-Pak formaline vial.	59-61

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Cytomegalovirus Culture	Virology	VCI	Urine, lung biopsy, tissues, throat wash or swab.	Y	Viral transport media	76-81
Cytomegalovirus Serology	Virology	CMG	Cytomegalovirus IgG  Acute and convalescent sera	N	Transport at 4 C. Do NOT freeze.	62-66
Cytomegalovirus Serology	Virology	СММ	Cytomegalovirus IgM  Acute and convalescent sera	N	Transport at 4 C. Do NOT freeze.	62-66
Diphtheria Culture	Microbiology	BSD	Throat, lesion, or membrane swab. Pure culture.	Y	Dry swab in gel pack or sterile red top tube	22-27
E.coli 0157:H7 – See Escherichia coli						
Echovirus Culture	Virology	VEI	Throat swab, feces CSF	Y	Viral transport media.	76-81
Enteric Pathogens- Salmonella shigella, campylobacter, and E.Coli 0157:H7	Microbiology	BEP	Stool	Y	Para-Pak Enteric Plus kit. Vibrio and Yersinia tested on request.	39-41
Enterobius Vermicularis	Parasitology	BPT	Scotch tape prep	N		59-61
Enterovirus culture	Virology	VEI	Throat, stool, CSF, vesicular fluid, tissue, or blood.	Y	Viral transport media.	76-81
Escherichia coli 0157:H7 confirmation	Microbiology	BEE	Sorbitol negative colonies of E.coli	N		39-41
Escherichia coli 0157:H7 culture	Microbiology	BEP	Stool	Y	Para-Pak Enteric Plus kit. Send refrigerated.	39-41
Escherichia coli-shigatoxin producing culture.	Microbiology	STX	Stool or shigatoxin producing colonies.	Y	Para-Pak Enteric Plus kit. Send refrigerated.	39-41
Fluorescent Treponemal Ab-see Syphilis						
Foodborne illness outbreak					Contact Office of Disease Prevention 605-773-3737	42-43
Francisella tularensis-see Tularemia						
Fungus/Yeast Culture	Mycology	ВМІ	Pure culture.	N	Suspected Coccidioidies, Blastomyces and Histoplasmosis isolates should be so labeled as such.	57-58

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Giardia lamblia	Parasitology	ВОР	Stool	Υ	Para-Pak formaline/PVA vials.	59-61
Gonorrhoeae	Serology	GP2	Female cervical, male urethral and urine.	Y	Gen-Probe Aptima Combo 2 collection kit	44-47
Gonorrhoeae culture	Microbiology	BGR	Urogenital, throat, rectal, eye Isolate for confirmation	N N	Thayer Martin or chocolate agar shipped with increased CO2.	48-50
Haemophilus species culture	Microbiology	HFLU	Reference culture	N	Chocolate agar. Identification typing of <i>H.influenzae</i> only.	22-27
Hantavirus Serology (Sin Nombre)	Virology	HPS	Serum (whole blood clots and tissue to CDC). Acute and convalescent sera.	N	Contact laboratory before sending. 605-773-6769	62-66
Hepatitis A Antibody – IgM	Serology	HAM	Serum	N	Used to indicate acute phase of infection.	62-66
Hepatitis A Antibody – Total	Serology	HAV	Serum	N	Used to indicate acute and past infection. Confirms previous exposure and immunity.	62-66
Hepatitis B Surface Antibody	Serology	VHG	Serum	N	Used to indicate clinical recovery and/or immunity.	62-66
Hepatitis B Surface Antibody (post-vaccination)	Serology	VSG	Serum	N	Used to establish immunity after immunization.	62-66
Hepatitis B Surface Antigen	Serology	VSB	Serum	N	Indicator of acute infection. May be used for needlestick injuries and prenatal screens. Also may indicate chronic infection.	62-66
Hepatitis B. Core Antibody – Total	Serology	VHC	Serum	N	Indicates infection at some undefined time.	62-66
Hepatitis B Core Antibody – IgM	Serology	VCM	Serum	N	Early indicator of acute infection.	62-66
Hepatitis B Be Antibody	Serology	VHH	Serum	N	Prior approval needed before submitting.	62-66
Hepatitis B Be Antigen	Serology	VHE	Serum	N	Prior approval needed before submitting.	62-66

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Hepatitis B Acute Profile	Serology	HAP	Serum	N	HBsAg, HBcAb-IgM, HAV-IgM, HCV	62-66
Hepatitis B Chronic Profile	Serology	НВС	Serum	N	HBsAg, HBsAb, HBcAb	62-66
Hepatitis B Diagnostic Profile	Serology	HBD	Serum	N	HBsAg, HBc-lgM, HBsAb	62-66
Hepatitis B Pre-Vaccination Screen	Serology	HBV	Serum	N	HBsAb, HBcAb	62-66
Hepatitis B Pre-Natal Screen	Serology	HPN	Serum	N	HBsAg, reflex to HBeAg if positive	62-66
Hepatitis C Antibody	Serology	HCV	Serum	N	Indicates presence of acute or past infection.	62-66
Herpes Simplex Culture	Virology	VHI	Throat, nasopharyngeal swab. Scraping from mouth, genitalia epidermal sites vesicles, CSF.	Y	Viral transport media available. Includes typing for HSV I and HSV II.	76-81
Herpes Simplex Serology	Virology	HSQ	Acute and convalescent sera	N		62-66
Histoplasma Culture	Mycology	ВМІ	Reference culture.	N	Suspected Histoplasma should be labeled as such.	57-58
HIV-1/2 (serum)	Serology	HIV	Serum	N	Positives confirmed with a HIV-1 Western blot.	67-68
HIV-1/2 (oral fluid)	Serology	ORA	Oral fluid	Y	Orasure collection kit. Positives confirmed with a HIV-1 Western blot.	67-68
Influenza Culture	Virology	VRI	Throat swab, NP aspirate or swab, Nasal wash	Υ	Viral transport media available. Send on cool pack.	76-81
Influenza RT-PCR	Virology	IAB	Throat swab, NP aspirate or swab, Nasal wash	Υ	Viral transport media available. Send on cool pack.	82
Intestinal Parasites	Parasitology	ВОР	Stool	Υ	Para-Pak (2 vials)	59-61
Lead, blood – See Blood Lead						
Legionella Culture	Microbiology	BMD	Sputum, bronchial washings, or lung biopsy.	N	Contact laboratory before sending. 605-773-4968	22-27

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Legionella Serology	Virology	VLS	Acute and convalescent sera collected 3-6 weeks apart.	N	Detects IgG and IgM antibodies.	62-66
Listeria	Microbiology	BMD	Reference isolate	N		22-27
Lyme Disease	Virology	VLM	Acute and convalescent sera	N	Positives confirmed with a western blot.	
Measles See Rubeola						
Measles, German See Rubella						
Meningitis-bacterial (Haemophilus influenza, Neisseria meningitidis)	Microbiology	BMD	Pure isolate	N	Submit on chocolate agar. Includes identification and serotyping.	22-27
Meningitis-aseptic – See Enterovirus culture.						
Miscellaneous Bacterial Culture	Microbiology	BMD	Pure isolate	N	Include isolation information.	22-27
Miscellaneous Viral Culture	Virology	VOI	Appropriate specimen for virus suspected.	Y	Viral transport media available. Requires history and clinical information. Contact lab for assistance. 605-773-6769.	76-81
Miscellaneous Viral Serology	Virology	MISC	Appropriate specimen for virus suspected.	N	Contact SDPHL for assistance. 605-773-6769.	62-66
MRSA – Methicillin Resistant Staph aureus	Microbiology	MRSA	Reference culture.	N	Call for special arrangements for PFGE if indicated. 605-773-4238.	
Mumps Culture	Virology	VOI	Throat swab, saliva, urine, CSF	N		76-81
Mumps Serology-Diagnostic	Virology	VUM	Acute sera	N		62-66
Mumps Serology-Immunity	Virology	VMS	Serum	N	Used for immunity check.	62-66
Mycobacterium sp. Culture Tuberculosis	ТВ	TTB	Sputum, urine, gastric washings, pleural fluid, tissue, body fluids, blood, and bone marrow.	Y	Containers provided.	53-56
Mycobacterium Reference Culture	ТВ	ТОТ	Reference isolate.	N		53-56

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Mycology Identification	Mycology	ВМІ	Reference Isolate.	N		57-58
Neisseria Gonorrhoeae. See Gonorrhoeae						
Neisseria Meningitidis	Microbiology	NMEN	Reference isolate for serotyping.	N		22-27
Neisseria Species	Microbiology	BMD	Reference isolate for identification and serotyping as necessary.	N		22-27
Norovirus (Norwalk-Like)	Virology	NORO	Stool	N	Send on cool packs, not frozen.	87-88
Notifiable Disease Reporting					Contact the Office of Disease Prevention. See Reportable Disease list for contact information.	
Ordering Supplies	Mailroom				Contact mailroom at 605-773-3183.	
OVA and Parasites-see Parasites						
Parainfluenza Virus Culture	Virology	VRI	Throat swab NP swab and aspirate.	Y	Viral transport media available. Includes typing of types 1,2 and 3.	76-81
Parasites - Intestinal	Parasitology	ВОР	Stool	Y	Para-Pak (2 vials) Collect 3 stools 48-72 hours apart.	59-61
Pertussis Culture	Microbiology	BPC	Nasopharyngeal swab	Y	Regan-Lowe transport media available. Incubate at 36 C overnight if possible. Hold/transport at 4 C or ice pack.	31-32
Pertussis PCR	Microbiology	PPR	Nasopharyngeal swab or aspirate	Y	Regan-Lowe transport media available. Incubate at 36 C overnight if possible. Hold/transport at 4 C or ice pack.	31-32
PFGE-Pulsed Field Gel Electrophoresis	Microbiology	PFGE			Used for characterization of isolates in outbreaks. Call for further information. 605-773-4238.	
Pinworm	Parasitology	BPT	Scotch tape prep.	N		59-61
Plague - See Yersinia Pestis						
Pneumococcus-See Strep. Pneumonia						

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Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Poliovirus Culture	Virology	VEI	Throat washing or swab, NP wash or swab, feces, or rectal swab.	Y	Viral transport media available.	76-81
Q Fever – Quantitative Serology	Virology	VQS	Acute and convalescent sera	N		62-66
Rabbit Fever See Tularemia)						
Rabies Direct FA Serology	Virology	VRB	Brain tissue of suspect animal or large animal head of smaller animals	N	Contact the SDPHL before sending. 605-773-6769.	73-75
Rabies Serum Titer	Virology	VRT	Serum	N	Used for checking antibody levels in persons who have been immunized.	62-66
Rapid Plasma Reagen – RPR	Serology	RPR	Serum	N	Positive will be confirmed with the TPA.	69-72
Reportable Disease Reporting					Contact the Office of Disease Prevention. See Reportable Disease List for contact information.	6-7
Respiratory Virus Culture	Virology	VRI	Throat washing or swab NP swab or aspirate.	Y	Viral transport media available.	76-81
Respiratory Syncytial Virus (RSV) Culture	Virology	VRA	NP or throat swab or washings	Y	Viral transport media available. Send with a cold pack.	76-81
Respiratory Syncytial Virus (RSV) Direct FA	Virology	VR2	Nasopharyngeal aspirate preferred.	N		76-81
Rickettsia Serology Panel (Q-fever, RMSF, and Typhus)	Virology	VRK	Acute and convalescent sera.	N	Detects IgG antibodies. Diagnostic only with paired sera.	62-66
Rocky Mountain. Spotted Fever Serology-Quantitative	Virology	VSF	Acute and convalescent sera.	N		62-66

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Rubella Serology – German Measles	Serology	VRE	Single Serum.	N	Used to establish immunity.	62-66
Rubella IgM Serology	Virology	VRM	Acute Serum.	N	Detects IgM antibodies. Draw blood 1-3 weeks after onset on symptoms.	62-66
Rubeola Culture-Measles	Virology	VOI	Throat or NP swab or washing.	Υ	Viral transport media available.	76-81
Rubeola Serology	Virology	VRO	Single serum	N	Used to establish immunity.	62-66
Rubeola IgM Serology	Virology	VMM	Acute serum.	N	Collect 3-4 days after rash appears. Used for recent infection.	62-66
Rubeola Serology Diagnostic	Virology	VMD	Acute and convalescent sera	N	Titer IgG antibodies.	62-66
Salmonella Culture	Microbiology	SAL	Feces or rectal swab.	Υ	Para-Pak Enteric Plus kit.	39-41
Salmonella Serotyping	Microbiology	SAL	Reference isolate.	N		
Shigatoxin	Microbiology	STX	Feces, Rectal Swab	Y	Para-Pak Enteric Plus Kit or primary SMAC plate or enrichment broth. Send refrigerated.	89-90
Shigella Culture	Microbiology	SHIG	Feces or rectal swab	Υ	Para-Pak Enteric Plus kit.	39-41
Shigella Serotyping	Microbiology	SHIG	Reference isolate.	N		
Shingles - See Varicella Zoster						
St. Louis Encephalitis Serology	Virology	SLE	Acute and convalescent sera drawn 2-4 weeks apart.	N		62-66
Staphylococcus Aureus	Microbiology	BMD	Appropriate source.	N	Isolates from documented outbreaks only.	22-27
Staphylococeus Aureus-MRSA	Microbiology	MRSA	Reference isolate.	N	Call for special arrangements for PFGE. 605-773-4238.	
Streptococcus Species	Microbiology	BMD	Reference culture	N		
Streptococcus, Group A	Microbiology	BMD	Invasive isolates	N		51-52
Streptococcus pneumoniae	Microbiology	BMD	Antibiotic resistant isolates from sterile sites	N		22-27

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
Syphilis Serology-RPR	Serology	RPR	Serum	N	Positives will be confirmed with TPA.	69-72
Syphilis Serology – Confirmation	Serology	TP-PA	Serum	N	RPR and TPA performed.	69-72
TB - See Mycobacteria Culture						
Tick Borne Diseases – See Rickettsia, Lyme, and Arboviruses.						
TPA – See Syphilis						
Tuberculosis See Mycobacteria Culture						
Tularemia Culture	Microbiology	BMD	Reference isolates, Blood, sputum, Lymph nodes, NP washings, and gastric aspirates.	N	Submit on chocolate agar.	22-27
Tularemia Serology	Serology	STU	Acute and convalescent sera.	N		62-66
Typhoid Fever - See Salmonella						
Typhus Serology-Quantitative	Virology	VTY	Acute and convalescent sera.	N		62-66
Undulant Fever – See Brucella						
Varicella Zoster Serology – Immunity	Virology	VNZ	Single serum.	N	Used to establish immunity.	62-66
Varicella Zoster Serology – Diagnostic	Virology	VND	Acute and convalescent sera.	N	Titer of IgG antibodies.	62-66
Vibrio Culture	Microbiology	BVC	Feces or rectal swab	Y	Para-Pak Entric Plus kit.	39-41
Vibrio Confirmation	Microbiology	BMD	Reference isolate.	N		

Disease/Test	Lab Section	Test Code	Specimen	Kit	Special Handling	Detailed Info. Page
West Nile Virus Serology IgM	Virology	WNM	Acute Serum or CSF	N	605-773-6769.	62-66
West Nile Virus Serology IgG	Virology	WNG	Acute & Convalescent Sera	N	605-773-6769.	62-66
Western Equine Encephalitis Serology	Virology	VAS	Acute and convalescent sera	N		62-66
Whooping cough - See Pertussis						
Yersinia Culture	Microbiology	BYC	Feces or rectal swab.	Υ	Para-Pak Plus Enteric kit.	39-41
Yersinia Confirmation	Microbiology	BYC	Reference culture.	N		39-41
Yersinia Pestis	Microbiology	BMD	Blood, aspirated fluids from lymph nodes or bubo. Reference Isolate	N	Blood culture bottles or sterile container.	

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III. C	DETAIL	ED REFI	ERENCE	<b>SECTION</b>
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#### **Aerobic Bacteriology**

#### Introduction

The Aerobic Bacteriology Section serves primarily as a referral laboratory for bacteria that are unusual or difficult to identify. In this context, aerobic bacteriology refers to the examination of a wide variety of microorganisms. Reference cultures will be accepted from public and private health care providers. Pure culture isolates are required for serotyping and identification of reference specimens. Clinical specimens are accepted for the isolation and identification of specific pathogen if this testing is unavailable at the sending facility. Refer to Chart II – 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

Refer to sections on *Bordetella* (whooping cough), Gonorrhea, *and streptococcus* Group A for specific information on these organisms.

Services available in the Aerobic Section include, but are not limited to, the following:

- Serotyping of Neisseria meningitidis from sterile sites.
- Culture, PCR, direct fluorescent antibody staining of nasopharyngeal specimens for *Bordetella pertussis*.
- Biochemical identification of non-fermentative gram-negative bacilli and fermentative gram-negative bacilli not included in the family Enterobacteriacease, Vibrionaceae, or Aeromonadaceae.
- Grouping of beta hemolytic streptococci and biochemical identification of clinically significant strains of other gram-positive cocci on referred isolated only.
- Biochemical identification or confirmation of any bacterial isolate (other than anaerobic, enteric, or mycobacterial) that is unidentifiable at the local level, due to unusual or special requirements of the organisms. These may include growth requirements, aberrant biochemical results, additional test requirements, as well as the rarity or hazardous nature of the suspected organism.

The Aerobic Bacteriology Section does not perform antimicrobial susceptibility testing for patient treatment.

The SDPHL requests that laboratory personnel send isolates of *Bacillus anthracis*, *Brucella* species, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Corynebacterium diphtheriae*, *Francisella* species, *Francisella tularensis*, \**Haemophilus influenzae*, *Listeria monocytogenes*, \**Neisseria meningitidis*, and *Yersinia pestis* to the South Dakota Public Health Laboratory for surveillance purposes.

#### \*Sterile sites only

Special services are available through the Microbiology Section, and include:

- Serotyping of certain bacteria under specific circumstances.
- Toxigenicity testing of Corynebacterium diphtheriae.
- Pulsed field gel electrophoresis (PFGE) for coagulase-positive staphylococci from documented outbreaks.
- Typing of *Streptococcus pneumoniae* isolates from patients who have received pneumococcal vaccine or from documented outbreaks.
- Antimicrobial susceptibility testing under special circumstances.

All specimens for special services must be accompanied by a clinical history documenting the need for special testing.

# Chart II – 1 Aerobic Specimens Requiring Special Handling

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
Bacillus anthracis anthrax	Aseptically collect specimens from lesion, contaminated hair products, or sputum. Subculture isolates to blood or nutrient agar slants. Use extreme caution.	Blood culture bottles for blood and spinal fluid, TB plastic tube for sputum. Sterile containers for other specimens. Ship in double-walled shipping container or equivalent.	USE BIOLOGICAL SAFETY HOOD. Do not create aerosols. Send by registered* mail. Notify Section before shipping.
Bordetella pertussis – w	hooping cough. Refer to E	BORDETELLA SECTION.	
Brucella species  Brucellosis or undulant fever	Aseptically collect multiple blood samples, infected tissues, abscess material, bone marrow, or liver biopsies. Subculture isolates to sheep blood, nutrient or Brucella agar slants. Use extreme caution.	Blood culture bottles, vented and incubated under 5 to 10% CO <sub>2</sub> . Use sterile container. Ship in double walled shipping container or equivalent.	USE BIOLOGICAL SAFETY HOOD. Refrigerated clinical specimen if delay is anticipated. Send by registered* mail. Notify Section before shipping.
Burkholderia mallei B. pseudomallei melioidosis	Aseptically collect blood, sputum or pus. Subculture isolates to nutrient or infusion agar slant.	Blood culture bottles for blood, TB plastic tube or sputum, sterile container for pus. Ship in double- walled mailing container or equivalent.	Send by registered* mail. Notify Section before shipping.
Corynebacterium diphtheriae Diphtheria	Collect throat or skin lesion swabs. Insert swab into silica gel pack or red top tube.	Ship in double walled shipping container or equivalent.	
Francisella tularensis Tularemia or rabbit fever	Collect specimens aseptically. Specimens include material from lesions, lymph nodes, sputum, gastric aspirates, nasopharyngeal washings and blood cultures. Use extreme caution.	Sterile container, no transport medium. Ship in double walled shipping container or equivalent.	DO NOT ATTEMPT ISOLATION. Send by registered* mail. Notify Section before shipping.

<sup>\*</sup> Federal regulations require that these organisms must be shipped by a system that requires of provides for notification of receipt, such as registered mail. This is required so that packages can be tracked and undelivered packages may be located quickly. Special safety labeling on the outside of the container is also required.

# Chart II – 1 Aerobic Specimens Requiring Special Handling

Shipping Poquiromonte

double-walled shipping

Special Dequirements

Organism or Disease	Collection instructions	Snipping Requirements	Special Requirements
Haemophilus ducreyi	Collect specimens from lesions or inguinal bubo and inoculate onto	Heavy growth of 24 to 48 hour culture scraped with sterile swab.	Primary culture must be done at the local level.
chancroid	enriched chocolate agar and incubate at 35 to 37°C in 5 to 10% CO <sub>2</sub> .	transport as subsurface stabs in chocolate agar. Ship in double-walled shipping container or equivalent.	
Haemophilus influenza	<b>ae</b> – Isolates from sterile sites	required for surveillance pur	poses.
Leptospira	Collect blood during first	Obtain media for culture	Obtain permission to
Species			
Оросия	week of illness, spinal fluid during acute phase, urine (three consecutive specimens) from 2 <sup>nd</sup>	and/or transport from the CDC. Telephone (404) 639-3905 to request.	ship specimen in advance. Complete clinical history required.

**Listeria monocytogenes** – Isolates from sterile sites required for surveillance purposes. Isolates from all sites are requested.

and alcohol, acid or

blood at room temperature.

Collection Instructions

Organism or Disease

**Neisseria gonorrhoeae – gonorrhea -** Refer to GONORRHEA AND CHLAMYDIA, DNA PROBE TECHNOLOGY, and GONORRHEA, CULTURE METHOD, SECTIONS.

from urine for direct FA to the CDC. Ship in

formalin fix smears. Hold container or equivalent.

Neisseria meningitidis – Isolates from sterile sites are required for surveillance purposes.

Staphylococcus	Isolates from	Isolated organisms on	Documentation must
aureus	documented outbreak.	nutrient or infusion agar	accompany specimens.
	Only coagulase positive	slants. Ship in double-	Notify Section before
	staphylococci accepted.	walled shipping	shipping. Pulsed Field
		container or equivalent.	Gel Electrophoresis
			(PFGE) is performed.

**Streptococcus pneumoniae** – Isolates from sterile sites are required for surveillance purposes. **Streptococcus Group A** – Isolates from sterile sites are required for surveillance purposes.

Miscellaneous	Use blood, chocolate or	Ship in double-walled
Bacteria	TSA slant or Cary-Blair	shipping containers or
	transport.	eguivalent.

<sup>\*</sup> Federal regulations require that these organisms must be shipped by a system that requires or provides for notification of receipt, such as registered mail. This is required so that packages can be tracked and undelivered packages may be located quickly. Special safety labeling on the outside of the container is also required.

## **Specimen Collection**

Aseptically collect specimens from sites such as autopsy material, surgically obtained tissue, urine, and the respiratory and urogenital tract using appropriate techniques for the individual type of specimen. Aseptically collect blood samples and inoculate directly into appropriate commercial blood culture bottles. Preferably, all specimens should be cultured at the local laboratory using recommended isolation procedures. (For exceptions refer to Chart II – 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.) To ensure purity, isolates should be subcultured onto appropriate media before transportation to the SDPHL..

Isolated organisms should be submitted on non-carbohydrate-containing agar slants such as infusion, nutrient, Trypticase soy, blood, or chocolate.

Telephone the Microbiology Section at (605) 773-3368 to make special arrangements in urgent situations or unusual circumstances. **Always** telephone in advance when submitting large numbers of isolates, as in an outbreak situation, or when the organism being submitted is classified as a biologically hazardous organism. For specimens requiring special handling refer to Chart II – 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

#### **Specimen Identification**

- 1. Complete all the provider and patient information areas. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form **will not be tested.**

#### **Shipment of Specimens**

- 1. Pack the specimen in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container. Place the cap on securely.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
- 3. Ship to the South Dakota Public Health Laboratory in Pierre.
- 4. Use first-class postage on US mail.
- 5. If Burkholderia mallei, Burkholderia pseudomallei, Bacillus anthracis, Brucella species, or Francisella tularensis is suspected, send by registered mail. This is so that packages can be tracked and undelivered packages may be located quickly. Before shipping, telephone the **Microbiology Section**.

#### **Reporting Procedures and Interpretation of Results**

Most cultures are reported within 5 to 10 working days. Mixed cultures or fastidious bacteria may require more time for identification. Final results on isolates submitted to the CDC for confirmation or further testing require a longer interval for completion.

#### Reporting of Results

Organisms are reported by genus and species.

Organisms are identified to a genus and species level only when culture, morphology, and biochemical test results support the species identification. Genus and species designations are those consistent with designations in the American Society for Microbiology" *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*. Some organisms encountered in aerobic bacteriology can be identified accurately only to the genus level and are reported as such. Organisms normally encountered as contaminants or those believed to lack clinical significance may be reported only to the genus level especially if the culture was not accompanied by clinical information to the contrary.

Organisms reported as "unidentified" are those which do not fit the description of recognized genera and/or species. These organisms are not routinely forwarded to the CDC for further study unless the nature of the isolate, source of isolation, and/or the clinical history of the patient warrant further identification efforts, or a special request is made to forward the isolate (such a request requires justifying information from the submitter).

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the Office of Disease Prevention in the State Health Department are sent reports on the following organisms:

Bacillus anthracis

Bordetella pertussis

Brucella species

Clostridium botulinum

Clostridium tetani

Corynebacterium diphtheriae

Escherichia coli O157:H7

Shiga-toxin positive Escherichia coli

Francisella tularensis

Haemophilus influenzae, from sterile body sites

Listeriosis, (Listeria monocytogenes)

Legionella species

Neisseria gonorrhoeae

Neisseria meningitidis, from sterile body sites

Salmonella species

Shigella species

Streptococcus pneumonia, from sterile body sites

Vibrio cholerae

## **Criteria for Unacceptable Specimens**

# All specimens

- 1. The specimen was not properly identified with the patient's name/or and the tear strip control number.
- 2. The patient identifier on the specimen did not match the identifier on the form.
- 3. The specimen was broken in transit.
- 4. The type of specimen was improper for the test requested.
- 5. The specimen was non-viable.
- 6. A mixed specimen was submitted.

#### Anaerobic Bacteria

#### Introduction

Anaerobic bacteria are a frequent cause of serious infections. The Microbiology Section generally accepts only clinical isolates for identification. In certain cases, clinical material for primary isolation is accepted for cultivation of pathogenic microorganisms. Contact the Microbiology Section regarding submission of these specimens.

Isolation and identification techniques used include cultural procedures, morphological and biochemical characterization. Anaerobic isolates are not tested for antimicrobial susceptibilities.

#### **Specimen Collection**

Since anaerobic organisms make up a major part of the body's indigenous flora, clinical specimens for anaerobic culture must be collected by methods that avoid contamination with normal flora. Aspirates collected with a syringe or tissue specimens are recommended for anaerobic culture.

The Microbiology Laboratory accepts anaerobic organisms isolated from the following sources:

- Aspirated pus.
- Tissue (biopsy, surgery, autopsy).
- Transtracheal aspirates.
- Direct lung aspirates.
- Body fluids.
- Sulfur granules from suspected cases of actinomycosis.

Anaerobic organisms isolated from the sources listed below are unacceptable for testing. If you submit an isolate from one of these sources, include information that establishes its clinical significance.

- Throat, gingival or nasopharyngeal swabs.
- Skin.
- Voided urine.
- Sputum or gastric contents.
- Superficial wounds.
- Rectal swabs, feces or small bowel contents (except for special testing).
- Vaginal or cervical swabs.

The culture must be maintained in an anaerobic environment. Submit a PURE, actively growing culture in a screw-cap tube of liquid or semi-solid media such as motility media, thioglycollate, or chopped meat broth. Pick a single colony and inoculate a tube of media. At the same time, check the oxygen requirement of the organism by streaking a single colony to an aerobic blood plate. Incubate the confirmed anaerobe for 24 to 48 hours or until visible growth is present. Overlay with ¾ inch of sterile Vaspar\* Tighten the cap and seal securely with parafilm or waterproof tape to prevent leakage.

Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3-ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

## Specimen Identification

- 1. Complete all the provider and patient information areas. Include pertinent clinical, biochemical, and epidemiological information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

#### **Shipment of Specimens**

- 1. Pack the specimen in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
- Use first-class postage on US mail.
- 4. Ship to the South Dakota Public Health Laboratory in Pierre.

Note: Do not mail specimens on plates. Specimens submitted on plates are acceptable only if they are properly closed in an anaerobic transport bag and delivered by courier to the laboratory.

## **Reporting Procedures and Interpretation of Results**

Most anaerobic cultures are reported within 7 working days, but fastidious, slow-growing, or nutritionally deficient organisms or mixed cultures may require several days longer. Reports on cultures forwarded to CDC for further identification and/or confirmation may be delayed several months due to high volume workload.

## **Reporting of results**

Organisms are reported by genus and species and subspecies when appropriate.

Organisms are identified to genus, species, and subspecies level when appropriate and only if culture, morphology, and biochemical test results support the identification. Genus, species and subspecies designations are consistent with designations in the American Society for *Microbiology's Manual of Clinical Microbiology*, and the *International* Code of *Nomenclature of Bacteria*. Some anaerobes, particularly members of the genus Clostridium and many of the non-sporeforming gram-positive rods, can be identified accurately only to the genus level. Generally, *Lactobacillus* organisms are identified only to the genus level.

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the Office of Disease Prevention in the State Health Department are sent reports on the following organisms:

Clostridium botulinum.

Clostridium perfringens, if isolated from a foodborne outbreak.

Clostridium tetani.

## **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified with the patient's name.
- 2. The patient identifier on the specimen did not match the identifier on the form.
- 3. The specimen was broken or leaked in transit.
- 4. The type of specimen was an improper specimen type for anaerobic culture.
- 5. The specimen was not submitted under proper anaerobic conditions.
- 6. The transport media was unsatisfactory for anaerobic transport.
- 7. The specimen was non-viable.
- 8. A mixed specimen was submitted.

#### Bordetella Pertussis

(Whooping Cough)

#### Introduction

The Bacteriology Section accepts cultures for the isolation of both *Bordetella pertussis* and *B. parapertussis*. Nasopharyngeal swabs should be submitted in Regan Lowe transport medium. Nasopharyngeal aspirates along with swabs are acceptable specimens for PCR testing for *B. pertussis*. Specimens may be sent from public and private health care providers. A kit containing the materials and instructions necessary for collecting a nasopharyngeal swab specimen are available from the SDPHL. Do not order more kits than are needed as they contain a medium with a short shelf life.

Nasopharyngeal swabs are the specimens of choice for culture, and should be collected as soon as possible after onset of illness, preferably before antibiotic treatment. Dacron swabs are superior to other types of swabs and should be used to collect specimens as follows: pass swab very gently through the nostril until it reaches the posterior nares and leave in place for 15 to 30 seconds (this may induce a cough and in practice only a few seconds may be possible).

## **Specimen Processing**

- 1. Label the Pertussis Transport Medium with the patient's name.
- 2. Inoculate Pertussis Transport Medium
  - a. Warm the medium to room temperature.
  - b. A nasopharyngeal swab should be taken and inserted into the tube.
  - c. Tightly replace the cap on the medium.
  - d. Incubate the media at 36C overnight and then send on ice packs. Send immediately if an incubator is unavailable.
- 3. Nasopharyngeal aspirates should be placed into a sterile leak proof container with a minimum volume of 500 ul for required for testing.

## **Specimen Preparation – Reference Cultures**

Subculture isolated organisms for the identification or confirmation to appropriate media slants, and incubate until growth is apparent.

Do not mail reference cultures on plates. Plates are acceptable only if a courier delivers them directly to the laboratory.

## **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information sections on the SDPHL requisition slips.
- Using indelible ink, label each specimen with the date of collection and the
  patient's first and last name. Unlabeled specimens or specimens where the
  patient identifier on the specimen does not match the identifier on the form will
  not be tested.

#### **Shipment of Specimens**

1. Wrap the transport medium in absorbent material. Pack cultures in a double-walled shipping container or equivalent.

- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer mailing container.
- 3. Ship the specimens to the South Dakota Public Health Laboratory in Pierre.
- 4. Use first-class postage on US mail.

## Reporting and Interpretation of Results

Cultures are held 7 days from the date of inoculation and are read daily. Nasopharyngeal swabs received in transport medium tubes are inoculated immediately onto Regan-Lowe and Bordet-Gengou plates when received. A positive culture report is based upon typical cellular and colonial morphology and is confirmed by fluorescent antibody testing.

PCR, culture and direct FA procedures are recommended for diagnosis of B. pertussis whenever possible. False negative culture results may occur from any procedures that render the organisms nonviable, such as improper handling of plates and transport medium after collection or prolonged antibiotic treatment prior to collection of specimen.

#### **Criteria for Unacceptable Specimens**

#### All specimens

- 1. The specimen was not properly identified with the patient's name.
- 2. The patient identifier on the specimen did not match the identifier on the form.
- 3. The specimen or slide was broken in transit.
- 4. Out-dated media or dehydrated media was used.
- 5. The specimen was not viable.
- 6. A mixed specimen was submitted.
- 7. Quantity of nasopharyngeal aspirate not sufficient (PCR test)

#### **Botulism**

#### Introduction

Botulism is a neuroparalytic disease caused by the toxin of *Clostridium botulinum*. The classical disease is foodborne and results from the ingestion of food in which *C. botulinum* has grown and produced toxin. In rare cases, botulism may also result from the production of toxin by *C. botulinum* growing in a wound. A third type of botulism, referred to as infant botulism, seems to result from ingestion of the organism or its spores. There is evidence that indicates the ingested organisms grow and produce toxin in the infant gastrointestinal tract.

Toxin assays are performed at the SDPHL per LRN protocols on food and environmental samples only. Procedures for toxin assays and isolation and identification of *Clostridium botulinum* on human samples are available through a cooperative agreement with adjacent State Public Health Laboratories, Office of Disease Prevention and the CDC. Please call before submitting samples so that all necessary arrangements may be made.

Serum, feces, vomitus, gastric contents, and pus or wound biopsies are tested when botulism is a possible diagnosis. Suspected foods are tested only when patient testing has resulted in a confirmed case of botulism. Foods are rarely tested in cases of infant botulism. Possible sources of spores for infants are multiple, including dust and foods.

## **Specimen Collection**

<u>For clinical specimens and foods</u> – Refer to Chart II – 2 COLLECTION OF SPECIMENS FOR BOTULISM TESTING.

<u>Culture isolates</u> – Submit a pure, actively growing culture in a screw-capped tube of liquid or semi-solid medium such as motility medium, thioglycollate, or chopped meat broth.

## **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information sections on the SDPHL requisition slips. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

Chart II – 2

Collection of Specimens for Botulism Testing

Clinical Diagnosis	Specimen	Amount of Specimen	Test(s) Performed
Foodborne	Serum	10 to 15 ml optimal. 2 ml minimum.	Toxin assay.
	Food	100 gm if available. Do not remove from container.	Toxin assay. Isolation and identification of <i>C. botulinum.</i>
	Feces	100 gm if available.	
	Gastric contents	100 gm if available.	
	Vomitus	100 gm if available.	
Wound	Serum	10 to 15 ml optimal. 2 ml minimum.	Toxin assay.
	Feces	100 gm if available	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Pus from wound or biopsied material	Collect in anaerobic collector. <b>Do not refrigerate.</b>	Isolation and identification of <i>C. botulinum.</i>
Infant	Feces	25 gm if available.	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Bowel	Use water enema, 20 ml.	

- Collect specimens before antitoxin is given.
- Collect feces, vomitus, and gastric contents in sterile containers. Do <u>not</u> use Cary-Blair transport medium. Ship refrigerated at 2 to 8°C.
- Leave suspect foods in original containers. Leave unopened containers sealed. Ship refrigerated at 2 to 8°C.
- Separate serum from blood cells. Ship refrigerated at 2 to 8°C.
- Collect specimens for wound botulism in an anaerobic collector. Do not refrigerate.

## **Shipment of Specimens**

#### 1. Serum, food, feces, gastric contents, vomitus, and bowel specimens

Wrap the specimen to cushion it. Place the specimen in a leak-proof, insulated container, and pack with cool packs. Do not pack with dry ice. The specimens should stay cold, but not frozen, until they reach the laboratory. Place the requisition form in a plastic bag to prevent wetting and contamination.

#### **Wound botulism specimens**

Place the pus or biopsied tissue from suspected wound botulism in an anaerobic collector and ship in a double-walled mailing container. Do not refrigerate.

#### Reference isolates

Submit a PURE, actively growing culture in screw-cap tube of liquid or semi-solid medium such as motility medium, thioglycollate, or chopped meat broth that has been overlaid with Vaspar \*. Ship it in a double-walled mailing container. Do not refrigerate.

- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
- 3. Ship the specimen by the quickest means available to the South Dakota Public Health Laboratory in Pierre. Suggestions for rapid delivery include courier service, taxi, bus, or plane. Follow the courier's shipping regulations.
- 4. Notify the Microbiology Section at (605) 773-3368 to the method of transportation and when the specimens are scheduled to arrive.

#### **Reporting Procedures and Interpretation of Results**

The South Dakota Public Health Laboratory issues preliminary results to the State epidemiologist, the Office of Disease Prevention and the patient's physician by telephone within 24 hours. Communication continues until the testing is complete. Additional specimens may be requested and additional tests performed depending on the patient's condition and laboratory results. A written report is made when all tests are complete.

Results of the toxin tests are reported
Negative for Clostridium botulinum toxin.
Positive for <i>Clostridium botulinum</i> toxin Type

<sup>\*</sup> Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3 ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

# Results of cultures (original specimen or isolate) are reported Culture negative for Clostridium botulinum. Culture positive for Clostridium botulinum, Type

Toxin is identified according to the Centers for Disease Control and Prevention's (CDC) *Laboratory Methods in Anaerobic Bacteriology.* Seven toxigenic types of C. *botulinum* are recognized based on the antigenically distinct toxins produced by the different strains classified in this species (A, B, C, D, E, F, and G). Cases of human botulism are usually associated with toxin Types A, B, and E. Type E is usually associated with foodborne outbreaks involving seafood. Infant botulism is predominately Type A or B.

The results of all specimen requests are reported to the health care provider submitting the specimen, and the Office of Disease Prevention in the SD State Health Department.

# **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified.
- 2. The identifier on the specimen does not exactly match the identifier on the form.
- 3. The specimen was broken or leaked in transit.
- 4. The specimen submitted was an improper type.
- 5. The patient's symptoms do not warrant performance of the test requested.
- 6. The specimen was submitted improperly.
- 7. A stool was not submitted refrigerated.
- 8. A wound culture or isolate was not submitted under anaerobic conditions.
- 9. A wound culture specimen or isolate was submitted refrigerated.

#### Campylobacter

#### Introduction

Campylobacter are microaerophilic organisms that are identified by morphological and biochemical characteristics. They are small, curved or spiral gram-negative rods with a corkscrew-like motility. *Campylobacter* organisms may be isolated from fecal specimens or blood cultures. Antimicrobial susceptibilities are not performed on these isolates.

Both stool specimens for suspected Campylobacter infections and reference isolates for confirmation are tested at the South Dakota State Public Health Laboratory

# Campylobacter illness is a notifiable disease.

# **Specimen Collection**

For a suspected infection, use the enteric bacteria collection kit. One method for collecting a reference isolate for confirmation is to harvest the pure growth from a plate with a swab. Inoculate a Cary-Blair and leave the swab in the medium. Send the Cary-Blair refrigerated. Another method is to inoculate appropriate agar and send in campy pack with ↑ CO2.

#### **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas on the SDPHL requisition slip. Include pertinent clinical and biochemical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

# **Shipment of Specimens**

- 1. Wrap the specimen in absorbent material. Place it in a leak proof insulated container and pack with wet ice or freezer packs. Place the requisition form in a plastic bag to prevent wetting or contamination.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
- 3. Ship cultures to the South Dakota Public Health Laboratory in Pierre.
- Use first-class postage on US mail.

Specimens submitted on plates are acceptable only if they are properly closed in a *Campylobacter* transport bag and delivered by courier to the laboratory.

#### **Reporting Procedures and Interpretation of Results**

Most *Campylobacter* cultures are reported within 5 to 7 working days after receipt in the laboratory.

# **Reporting of Results**

Organisms are reported by genus and species.

Organisms are identified to the genus and species level only when culture, morphology, and biochemical test results support the species identification. *Campylobacter* species designations are consistent with the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International* Code of *Nomenclature* of Bacteria.

The results of all specimens are reported to the health care provider submitting the specimen and the Office of Disease Prevention in the SD State Health Department.

# **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified with the patient's name.
- 2. The patient identifier on the specimen does not match that on the form.
- 3. The specimen was broken or leaked in transit.
- 4. The specimen was non-viable.
- 5. The specimen was submitted under improper atmospheric conditions.
- 6. A mixed specimen was submitted.

#### **Enteric Bacteriology**

#### Introduction

The Bacteriology Section examines feces and other specimens for the presence of enteric pathogens, namely *Salmonella* serotypes, *Shigella*, *Campylobacter*, and *E.coli* O157:H7, on a routine basis. Testing for Shiga-like toxin (SLT), *Yersinia* and *Vibrio* are performed upon request.

All isolates of *Salmonella*, *Shigella*, *Campylobacter*, *E.coli* O157:H7, *Yersinia enterocolitica*, and *Vibrio* recovered from specimens by other clinical laboratories in South Dakota should be referred to the Bacteriology Section. Referred isolates will be further characterized by various methods, such as biochemical reactions, serogrouping/serotyping or Pulsed-field gel electrophoresis (PFGE), depending upon the organism. Tests for occult blood and fecal WBC counts are not performed at the SDPHL.

# Specimen Collection/Labeling/Requisition Form

- A. Feces Specimens for Salmonella, Shigella, Campylobacter, Yersinia, E.coli O157, Listeria, Staphylococcus aureus, and Vibrio:
  - 1. Feces specimens for the above agents should be collected in the appropriate culture collection kit, green-colored top (do not use the Parasitology O&P kit, because it contains formalin that kills bacteria). Collect freshly passed feces as soon as possible after onset of illness and before antimicrobial therapy has been initiated (stools for S. aureus must be collected within 24 hours after onset). Select portions of feces which contain blood or mucous, if present. Use the \*spoon\* in the lid of the collection vial to facilitate placing a portion of the feces into the vial, adding specimen only to the fill line on the vial; tighten the cap securely, and invert several times. Two to three specimens collected on different days may be necessary for diagnosis.
  - 2. Rectal swabs containing detectable feces may be collected and placed in a culturette with Stuarts, Cairy-Blair, or other commercially available transport medium (not provided by the SDPHL), if a feces specimen cannot be obtained.
  - 3. Store and ship refrigerated.
- B. Feces specimens for *Clostridium perfringens*, *Clostridium botulinium*, and *Bacillus cereus*:
  - 1. For these agents, collect fresh stool specimens and place in a leak-proof, non-crushable, clean container (not provided by SDPHL).
  - 2. For *C.perfringens* and *B.cereus*, stool specimens must be collected within 48 hours from the time symptoms begin.
  - 3. Store and ship refrigerated.

- C. Each stool specimen must be clearly labeled with the patient's name and accompanied by a properly completed accession form. The form must include the following information:
  - 1. Patient identifier (name or number)
  - 2. Patient birthdate
  - 3. Date of specimen collection
  - 4. Agent suspected, if applicable
  - Submitters name and address
  - 6. Symptoms
- D. Referred Cultures (for identification)

Submit an overnight, pure culture of the isolated bacteria on carbohydrate-free media (TSA and TSI are acceptable). Label tube with the patient's name and complete the accession form. The form must include the following information:

- 1. Patient identifier (name or number)
- 2. Patient birthdate
- Date of collection
- 4. Source of specimen
- 5. Agents suspected
- 6. Submitter's name and address

The patient identifier (name, number, or both) indicated on the requisition form should match that written on the specimen or culture. Unlabeled or mismatched specimens or cultures will not be tested.

#### **Shipment of Specimens**

Ship clinical specimens for routine screening of enteric pathogens and all reference cultures under refrigeration to the South Dakota Public Health Laboratory in Pierre.

- 1. Routine screening specimen Wrap the specimen in absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in a leak-proof insulated container and pack with cold packs. Place the form in a plastic bag to prevent wetting or contamination.
  - The specimen must be received in the laboratory within 48 hours. (Storage at a temperature of  $-20^{\circ}$ C is acceptable for 3 days.)
- 2. Reference isolates Pack the specimen with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in

- a double-walled shipping container or the equivalent. Place the form in the outer container. Place the cap on securely. Refrigeration is not required.
- 3. Affix the mailing label, return address, and specimen label to the container.
- 4. Ship by fastest method to SDPHL.
- 5. When an unusually large number of specimens are anticipated (as in outbreaks), telephone the laboratory before mailing the specimen so that necessary preparations may be made. Notify the laboratory by telephone when one or more specimens for *Yersinia pestis* or *Vibrio* cholerae are being submitted.

Note: **Do not mail specimens on plates.** Specimens submitted on plates are acceptable only if a courier delivers them to the laboratory. (Plates should be sealed).

#### **Reporting and Interpretation of Results**

Negative stool specimen results are reported within 3 to 6 working days. Preliminary positive results are telephoned to the submitter; final results are reported within 4 to 6 working days.

Serotyping and confirmation or identification results are usually reported within 3 to 5 working days for Salmonella, Shigella, Aeromonas, Vibrio, and Yersinia spp., and within 4 to 6 working days for E.coli O157:H7. Other organisms will be reported, as appropriate, per request.

The following enteric pathogens, whether isolated from stool specimens or submitted as referred cultures are identified/confirmed to the species or serotype level:

Salmonella sp. or serotype Aeromonas sp. Shiqella sp. and serotype Vibrio sp.

Campylobacter sp. Yersinia enterocolitica and serotype E.coli O157 positive/negative, H7 positive/negative, and/or SLT positive/negative

# Unacceptable Specimens

- 1. Specimens submitted in wrong preservative, e.g., PVA or 10% formalin.
- 2. No patient identifier on specimen or culture.
- 3. Specimens received more than 7 days after collection (they will be tested, but a disclaimer will be added to the report indicating that negative results are questionable).
- 4. Specimen submitted in unsuitable collection kit.

#### Foodborne Illness

#### Introduction

Testing for foodborne illness is available at the South Dakota Public Health Laboratory. The laboratory examines food samples for the presence of disease-producing bacteria only in cases of documented illness under investigation by public health officials.

A foodborne disease outbreak is defined as three or more persons with vomiting or diarrhea who attended the same event or consumed the same meal. Single isolate cases or complaints are not considered outbreaks. EXCEPTION: ONE CASE OF BOTULISM IS SUBJECT TO NOTIFICATION AND INVESTIGATION. Refer to BOTULISM in Section II.

When you suspect a possible foodborne disease, notify your health department immediately so investigation procedures and sample collection can be started if necessary. Contact your health department whenever any enteric disease outbreak is suspected in a daycare center, a restaurant, or other facility. Additional assistance in the investigation is available by contacting the Office of Disease Prevention at 773-3917.

Outbreak investigation involves the cooperation of several disciplines within the health department, including the epidemiologist, the health department, and the laboratory. The investigation requires interviewing patients, collecting food samples and clinical specimens, and laboratory testing. Communication between the various members is essential for the prompt and precise handling of an outbreak.

A wide variety of organisms can cause gastrointestinal illness. Listed below are some of the organisms that are implicated in foodborne outbreaks:

- Bacillus cereus
- Campylobacter jejuni
- Clostridium botulinum
- Clostridium perfringens
- Escherichia coli 0157
- Listeria monocytogenes
- Salmonella species
- Staphylococcus aureus
- Vibrio species
- Yersinia enterocolitica

Organisms that can cause an enteric illness outbreak not associated with food include:

- Cryptosporidium
- Giardia
- Shigella species

# **Collection and Shipment of Specimens**

The health department should be notified when an outbreak is suspected.

The laboratory accepts food samples, environmental samples, and clinical specimens as deemed necessary by the Office of Disease Prevention. Clinical specimens should be collected from a representative number of ill persons and an equal number of exposed but well persons.

If there is a danger to the community, the Office of Disease Prevention will take action to prevent further spread of the disease.

Alert the South Dakota Public Health Laboratory when a foodborne illness is suspected so that preparations for handling the food samples and associated specimens can begin.

Complete a requisition form for the food samples and a form for each clinical specimen. Deliver the foods and clinical specimens to the laboratory as quickly as possible.

#### Reporting Procedures and Interpretation of Results

Communications among the SD public health officials, the SDPH Laboratory, and the health care providers are continuous from the time an outbreak is reported until the results are reported. Work-up of specimens requires a constant exchange of information between the laboratory and the epidemiology team. Additional testing is performed as needed.

Examination of food samples requires from 2 to 7 working days depending upon the suspected pathogen. Examination of foods heavily contaminated with *Staphylococcus* may be completed in 48 hours. The presence of low numbers of pathogenic organisms or organisms damaged by processing may take up to 2 weeks for isolation, identification and/or serotyping. Isolation and identification of *Salmonella* species, *Shigella* species, C. *perfringens*, and other more commonly encountered organisms usually require 1 week to identify. Environmental samples and swabs from food handlers are usually reported within 1 to 3 days.

Pulsed field gel electrophoresis for S. *Aureus* (from documented outbreaks) is performed and requires additional time for completion.

# Reporting of results of food samples

**Positive Results:** The name of the pathogen is reported by genus and species. The number of the pathogen per gram is reported if available.

**Negative Results:** No pathogen isolated.

#### See individual sections for reporting of results of clinical samples.

Confirmation that a food is involved in an outbreak is made by isolating the same pathogen or toxin in ill patients' specimens and in the implicated food(s). Without clinical specimens, a food can be confirmed as the vehicle of infection only if toxins are detected in it. In addition, a food can be epidemiologically suspect if food-specific attack rates are significantly higher in persons who have consumed a food as opposed to those who have not. In addition, confirmation of a foodborne disease can be made if significant numbers of pathogens known to cause food poisoning syndrome are isolated from the food, or if an enteric pathogen, such as *Salmonella* or *Shigella*, is present in any number.

The results of all specimens and food samples are reported to the health care provider who submitted the specimen. The Office of Disease Prevention is also sent reports if a foodborne illness is detected.

#### **Criteria for Unacceptable Specimens**

Unsatisfactory specimens will be assessed on an individual basis.

The specimen should be properly identified, and the specimen identifier should match the information on the form.

# Gonorrhea (Neisseria gonorrhoeae) and Chlamydia trachomatis Gen-Probe Aptima Combo 2 Assay

#### Introduction

Chlamydia (C.) trachomatis is the most common treatable sexually transmitted infection affecting females of reproductive age in the United States today, with an estimated four million new cases each year. Up to 80% of infected females have few or no symptoms, and asymptomatic infection in females can persist for up to 15 months. Complications of untreated chlamydial infection in females include: acute pelvic inflammatory disease; ectopic pregnancy; chronic pelvic pain; and infertility.

Gonorrhea affects both males and females with symptoms ranging from purulent discharge to very mild symptoms. Symptoms may pass unnoticed with the health consequence that asymptomatic carriers contribute significantly to the public health problem of gonorrhea.

The Serology Section uses the Gen-Probe Aptima Combo 2 Assay to detect both Chlamydia and gonorrhea infections utilizing urine from either males or females, or female endocervical swabs and male urethral swabs.

This method is not recommended for medicolegal cases. Alternate sources such as throat or rectal swabs are sent to the Utah Public Health Laboratory for testing. The SDPHL will also continue to perform culture tests for *N. gonorrhoeae* under these circumstances if requested. Antimicrobial tests are not performed. The South Dakota Public Health Laboratory does not perform culture tests for *Chlamydia*.

Only swabs supplied with the specimen collection systems should be used for specimen collection.

#### **Specimen Collection and Storage**

The APTIMA Combo 2 Assay is designed to detect the presence of *C. trachomatis* and *N. gonorrhoeae* in endocervical and male urethral specimens, and in female and male urine specimens. The SDPHL can also test eye specimens for Chlamydia only. Only the swabs and the specimen transport tubes contained in the APTIMA Combo 2 Assay Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens should be used to collect patient swab specimens. A unisex swab is used for both male and female specimens. These collection kits are intended to be used only with the GEN-PROBE APTIMA Combo 2 Assay. Performance has not been established with other products.

Swab specimens must be transported to the laboratory in the swab specimen transport medium and tube. Swab specimens must be transported to the laboratory at 2° to 30°C and tested within 60 days of collection.

Urine specimens can be transported to the laboratory at 2° to 30°C in the urine specimen transport tube. Urine specimens must be transferred into the GEN-PROBE specimen transport tube within 24 hours of collection. After transfer, urine specimens can be stored at 2° to 30°C for up to 30 days after collection.

#### A. Instructions for collection:

- 1. Endocervical swab specimens:
  - Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). Discard this swab.
  - b. Insert the specimen collection swab (blue shaft swab in the package with green printing) into the endocervical canal.
  - c. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
  - d. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
  - e. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
  - f. Carefully break the swab shaft at the scoreline; use care to avoid splashing of the contents.
  - g. Re-cap the swab specimen transport tube tightly.
- 2. Male urethral swab specimens:
  - a. The patient should not have urinated for at least one hour prior to specimen collection.
  - b. Insert the specimen collection swab (blue shaft swab in the package with the green printing) 2 to 4 cm into the urethra.
  - c. Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling.
  - d. Withdraw the swab carefully.
  - e. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the specimen transport tube.
  - f. Carefully break the swab shaft at the scoreline; use care to avoid splashing of the contents.
  - g. Re-cap the swab specimen transport tube tightly.
- 3. Urine Specimens:
  - a. The patient should not have urinated for at least one hour prior to specimen collection.
  - b. Direct patient to provide a first-catch urine (approximately 20 to 30 mL of the initial urine stream) into a urine collection cup free of any preservatives. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.

- c. Remove the cap and transfer 2 mL of urine into the urine specimen transport tube using the disposable pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine transport tube label.
- d. Re-cap the urine specimen transport tube tightly. This is now known as the processed urine specimen.

#### B. Specimen transport and storage before testing:

1. Swab specimens:

After collection, transport and store the swab in the swab specimen transport tube at 2° to 30°C until tested. Specimens must be assayed with the APTIMA Combo 2 Assay within 60 days of collection. If longer storage is needed, freeze at -20° to -70°C for up to 90 days after collection.

2. Urine Specimens:

After collection, transport the processed urine specimens in the GEN-PROBE APTIMA Combo 2 urine specimen transport tube at 2° to 30°C until tested. Processed urine specimens should be assayed with the APTIMA Combo 2 Assay within 30 days of collection. If longer storage is needed, freeze at -20° to -70°C for up to 90 days after collection.

# **Specimen Identification**

- 1. Complete **all** the provider and patient information areas.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

#### **Shipment of Specimens**

- 1. Pack the specimen in a double-walled shipping container or the equivalent. Pack with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container. (Specimens can be shipped at 2 to 30°C.)
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
- 3. Ship the specimen to the South Dakota Public Health Laboratory in Pierre.
- Use first-class postage on US mail.
- 5. Transport the specimen so that it arrives within 48 hours.

# **Reporting and Interpretation of Results**

The goal of the Serology Section is to test and report all Chlamydia/Gonorrhea Probe specimens within a 24 hour turnaround period, with the exception of borderline or equivocal

specimens which will take 48 hours. Copies of all positive Chlamydia and Gonorrhea reports are sent to the Sexually Transmitted Disease (STD) Program.

Results are reported as follows:

**POSITIVE** – Positive for chlamydia trachomatis and/or Neisseria gonorrheae.

**NEGATIVE** – Negative for chlamydia trachomatis and/or Neisseria gonorrheae.

**EQUIVOCAL** – Borderline for chlamydia trachomatis and/or Neisseria gonorrheae. Submit another specimen.

**UNSATISFACTORY** – Specimen comprised in some manner making it unsatisfactory for testing. The reason for each Unsatisfactory specimen will be detailed on the report.

#### **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified with the patient's name or identifier.
- 2. The patient identifier on the specimen did not match the identifier on the form.
- 3. The specimen was collected by use of swabs and/or tubes (collection kit) other than by the Gen-Probe kit.
- 4. The specimen was collected from a site other than endocervical, urethral or urine.
- 5. The specimen was too old for testing.
- 6. The specimen had no collection swab in the transport tube upon receipt in the laboratory.
- 7. The specimen had two collection swabs in the transport tube.
- 8. The specimen was received in an out-of-date collection kit.
- 9. The media had leaked in transport or something has been added to the tube for example the tube was too full or was a strange color.

#### **Gonorrhea, Culture Method**

Neisseria gonorrhoeae, Gonococcus, GC

#### Introduction

Screening for the presence of *Neisseria gonorrhoeae* is routinely performed using a nucleic acid hybridization technology. Refer to *GONORRHEA* AND *CHLAMYDIA* BY DNA PROBE METHOD, Section II. The culture method is recommended for medicolegal cases and is acceptable for throat and rectal swabs as well as genitourinary. Specimens are accepted for primary isolation of *N. gonorrhoeae*, as well as reference cultures for identification confirmation.

Gonorrhea is isolated from culture and then identified by biochemical methods.

Antimicrobial tests are not performed on gonorrhea isolates.

#### **Specimen Collection**

Sites that may be cultured are the cervix, anus, urethra, vagina, and oropharynx. If more than one site is cultured, a separate laboratory request form and culture plate are required for each specimen. Chocolate agar plates or Thayer-Martin plates are acceptable media for submitting gonorrhea cultures.

# **Endocervical**

Insert speculum into vagina using only water as a lubricant and visualize the cervix. Remove excessive cervical mucus if present. Insert cotton-tipped swab into the endocervical canal. Move from side to side. Allow 15 to 30 seconds for secretions to be absorbed.

#### Rectal

Insert a cotton-tipped swab 2 to 3 cm into anal canal. If the cotton tip is inadvertently pushed into feces, use another swab to obtain a specimen. Move the swab from side to side in the anal canal. Allow several seconds for secretions to be absorbed.

# Urethral

Obtain fresh exudate from meatus on sterile cotton-tipped swab, or if no exudate is present, use a calcium alginate swab inserted 2 to 3 cm into the anterior urethra.

#### **Oropharynx**

Swab the posterior pharynx and the region of the tonsillar crypts.

#### **Procedure**

- 1. Mark the bottom of the culture plate (not the lid) with the specimen identification.
- 2. Wear disposable gloves. Collect specimen from site to be cultured.
- 3. Roll swab in a large "Z" pattern on the culture place. (With a sterile wire loop, cross-streak immediately with a second "z" at a different angle.)
- 4. Place the specimen immediately into a C0<sub>2</sub> enriched environment using the C0<sub>2</sub> Tablet/Plastic Bag method.

- a. Place the culture into the bag with the C0<sub>2</sub> generating table within 15 minutes of inoculation.
- b. When using the individually wrapped tablet, tear the foil just enough to expose the tablet and place it in that fashion in the bag. Do not open the tablet until ready to put it into bag.
- c. Expel excess air from the bag itself; seal it tightly.
- d. Assure that no portion of the bag is left open.
- e. Incubate plates within 1 to 2 hours at 35 37C overnight.
- 5. Transport the specimen to lab as soon as possible to arrive within the 72-hour limit. Suggested transport is as follows:
  - a. Hand deliver same day; or
  - b. Incubate under appropriate temperature and C0<sub>2</sub> conditions for 18 to 24 hours. Transport to laboratory.
  - c. For Friday clinics proceed as for 5.a. or 5.b. transporting specimens on Saturday to arrive on Monday or incubate specimens all weekend (72 hours), and transport on Monday to arrive on Tuesday. The latter suggestion is aimed at areas having problems with specimens being mailed on Saturday and delayed in transit as long as Tuesday or Wednesday. When using this alternative method (incubating 72 hours), all specimens should be <u>clearly marked</u> as having been incubated for 72 hours. If not marked, they will be reported as "unsatisfactory too long in transit." Contact the laboratory before instituting this procedure.

# **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

# **Specimen Shipment**

- 1. Incubate the specimen at 35-37 C overnight.
- 2. Transport the specimen to arrive at the laboratory within 72 hours after collection.
- 3. Pack the specimen in a double-walled shipping container or the equivalent. Place the specimen in the inner container, cushioned to protect against breakage. Place the form in outer container. Place the cap on securely.
- 4. Affix the mailing label, return address and specimen label.
- 5. Ship the specimen to the South Dakota Department of Health Laboratory in Pierre.

#### Reference Cultures

To submit reference cultures of *Neisseria gonorrhoeae*, transfer a well-isolated colony from the primary isolation plate to a fresh plate. Incubate under  $CO_2$  overnight or until growth is visible. Place the culture in a  $CO_2$  environmental transport system and pack as above. Ship to the South Dakota Public Health Laboratory.

#### Reporting and Interpretation of Results

Positive specimens are reported within 1 to 4 working days after arrival in the laboratory. Negative and unsatisfactory specimens are incubated for a total of 72 hours before reporting.

Positive cultures of *Neisseria gonorrhoeae* are reported immediately by telephone.

Gonorrhea results are reported as		
No Neisseria gonorrhoeae isolated		
Neisseria gonorrhoeae isolated		

**Reporting of unsatisfactory specimens:** Unsatisfactory specimens are examined for a total of 72 hours. Any unsatisfactory specimen that can be reported as positive, regardless of the unsatisfactory condition, will be reported as positive. (Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.) Negative unsatisfactory specimens will be reported as unsatisfactory with the reason given.

The results of all specimens are reported to the provider who submitted the specimen. In addition, the Office of Disease Prevention is sent reports on specimens testing positive for gonorrhea.

#### **Criteria for Unacceptable Specimens**

Specimens may be reported as unsatisfactory for the isolation of *Neisseria gonorrhoeae* for the following reasons:

- a. Loss of Carbon Dioxide
  - 1) No C0<sub>2</sub> generating tablet.
  - 2) Bag not sealed.
  - 3) Improper C0<sub>2</sub> bag used.
- b. Media Conditions
  - 1) Out of date.
  - 2) Frozen.
  - 3) Dehydrated.
- c. Incubation
  - 1) Too long in transit.
  - 2) Collected and mailed on same day.
- d. Other
  - 1) No specimen received.
  - 2) Improper inoculation.
  - 3) No apparent inoculation.
  - 4) No date of collection.
  - 5) Overgrowth by contaminants.
  - 6) Failure to properly identify specimen.
  - 7) Broken in transit.
  - 8) Laboratory accident.

#### **Streptococcus Group A**

#### Introduction

The principal streptococcal species producing communicable disease in humans, Lancefield Group A *streptococci* or *Streptococcus pyogenes*, is primarily a resident of the pharynx. This microorganism causes a variety of respiratory tract diseases, including pharyngitis, rhinitis, tonsillitis, pneumonia, and scarlet fever. In addition, group A streptococci are found in pyoderma and impetigo lesions, in wound infections, and in the blood of patients with erysipelas, cellulitis, and septicemia. Nonsuppurative disease such as acute rheumatic fever and acute glomerulonephritis may follow streptococcal pharyngitis.

Reference cultures are accepted from public and private health care providers for the confirmation of *Streptococcus* Group A. In cases of systemic infections or necrotizing fasciitis, or if acute rheumatic fever or glomerulonephritis are suspected, referred cultures will be forwarded to the Centers for Disease Control and Prevention for "M" and "T" typing.

#### **Specimen Collection**

#### **Throat Cultures**

Rub the tonsils and pharynx with a swab while avoiding the tongue or uvula tissues. Touch any visible exudate with the swab. (The two most common pitfalls that result in inadequate specimens are swabbing the tongue or uvula tissues rather than the pharynx, and inadequate exposure of the pharynx.)

#### **Nasal Culture**

Take nasal cultures with sterile cotton-tipped flexible wire nasopharyngeal swabs specifically designed for this purpose. Raise the tip of the nose with one hand and introduce the swab along the floor of the nasal cavity under the middle turbinate until you reach the pharyngeal wall; do not use force. If any obstruction is encountered, do not take the nasopharyngeal culture on that side.

#### **Skin Culture**

Collect skin lesion specimens by removing the crust of the pustule or vesicle. Rub a sterile swab, firmly but gently, into the lesion. This may cause some discomfort to the patient, but it is important to ensure maximal recovery of *Streptococci*.

#### **Wound Cultures**

Collect wound cultures in the same manner as skin cultures.

**Inoculate the specimen directly on the surface of trypticase soy agar (TSA) slants.**Discard the swab. Ship it to the laboratory immediately. If a delay in shipment is anticipated, incubate the culture at 35 to 37° for no longer than 24 hours or hold at room temperature.

#### **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas on the SDPH Laboratory requisition form. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the

patient identifier on the specimen does not match the identifier on the form will not be tested.

#### **Specimen Shipment**

- 1. Pack the specimen in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container. Ship at ambient temperatures.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label on the container.
- 3. Ship to the South Dakota Public Health Laboratory in Pierre.
- 4. Use first-class postage on US mail.

# Reporting and Interpretation of Results

Specimens are reported		
Group A Streptococci identified		
No Group A Streptococci identified		

Results are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the Office of Disease Prevention in the case of invasive Group A Strep.

#### **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified with the patient's name or identifier.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen was broken in transit.
- 4. The media had expired or was dehydrated.
- 5. There was no growth on the specimen. (The specimen is incubated in the laboratory for 24 hours before reporting. No growth indicates that the slant was not inoculated.)

#### Mycobacteriology

Mycobacterium Tuberculosis, TB

#### Introduction

The South Dakota Public Health Laboratory provides isolation and identification testing of all *Mycobacterium* species (including *M. Tuberculosis* and non-tuberculosis mycobacteria). Molecular testing for *M. Tuberculosis* is available on request, on pulmonary samples only. Public and private health care providers may submit sputum and other clinical specimens and reference specimens. Positive isolations or identifications of *M. Tuberculosis* are reported to the South Dakota Tuberculosis Control Section of the SD DOH.

Sputum and specimens from other sources are concentrated and stained with fluorochrome and/or Cold Kinyoun acid fast stain, and are cultured for isolation and identification. Middlebrook 7H-10 plates, Lowenstein-Jensen slants, and Bactec TB media (12 B) are used for isolation. Species identification is accomplished by routine biochemical characterization, pigment studies, growth studies and nucleic acid probe tests.

Indirect drug sensitivity tests are routinely performed, by radiometric methods (Bactec), on first time isolates of *M. Tuberculosis*. The drugs tested are streptomycin, isoniazid, rifampin, ethambutol and PZA. Specimens for sensitivity testing on pathogenic mycobacterium other than *M. Tuberculosis* (MOTT) are referred to other laboratories as warranted.

# **Specimen Collection**

#### **Clinical Specimens**

**Sputum**: Collect a series of 3 to 5 single, early morning samples. A volume of 5 to 10 ml is adequate for each sample.

<u>Induced (or nebulized) sputum</u>: These specimens are usually very watery and should be labeled as "induced" so that they will not be mistaken for saliva. Saliva is an unsatisfactory specimen.

**Bronchial washings**: Collect up to 40 ml.

<u>Gastric lavage specimens</u>: Collect early in the morning or 8 hours after eating or drug therapy. Buffer immediately with 100 mg of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) or other alkaline buffer. Deliver to the laboratory quickly. Kits supplied by the SDPHL contain buffer.

**Tissue**: Aseptically collect and transport to the laboratory at once.

<u>Urine</u>: A series of single, mid-stream specimens, voided in the early morning, should be submitted, rather than a 24-hour pooled specimen.

**Feces**: Only fecal specimens from confirmed or suspected AIDS or other immunocompromised patients will be accepted. Collect a minimum of 1 gram of feces.

**Blood**: Collect 10 ml of blood in a sterile tube containing heparin.

<u>Other specimens</u>: Collect aseptically following the proper procedure for the type of specimen. Other specimens include pleural fluid, pus, joint fluid, laryngeal or wound swab, and spinal fluid. DO NOT use any transport medium.

#### **Reference Specimens**

You can submit either the isolate on the original (primary) medium or a subculture on an appropriate medium after growth is visible. Laboratories electing to submit original cultures should make sure visible growth is evident before mailing and should hold a subculture in their laboratory. Contaminated cultures will be accepted only upon PRIOR approval.

# **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas on the SDPH Laboratory requisition slip. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

# **Specimen Shipment**

- 1. Pack the specimen in a double-walled shipping container or equivalent. Pack it with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container, not around the specimen or culture. Place the cap on securely.
- 2. Affix the mailing label, return address, and specimen label to container.
- 3. Ship the specimen to the South Dakota Public Health Laboratory in Pierre.

#### Reporting and Interpretation of Results

Clinical specimens are tested for the presence or absence of *Mycobacterium* species by smear, liquid culture and standard solid culture methods. Negative cultures are incubated for 6 weeks before the specimen is reported as negative. Isolates from clinical specimens and reference cultures are identified to the genus and species level.

**Smears**: The provider is notified by telephone of smear results within 24 hours of receipt of the specimen. A hard copy is mailed to the provider.

Smears are reported		
Fluorochrome Negative		
Positive for acid fast bacilli:		

<u>Cultures</u>: If growth occurs at any time during the 6-week incubation period, identification procedures begin. Turn around time is 1 to 2 weeks after growth for identification by probe and 3 to 4 weeks after growth for identification by conventional methods. Another 2 weeks is required for drug susceptibility tests to be completed.

Cultures are reported		
Negative No Mycobacterium tuberculosis found.		
Positive for M. Tuberculosis  Mycobacterium tuberculosis complex.		
Positive for Mycobacterium species	Mycobacterium (species detected).	

Drug susceptibility testing is performed only on the *M. Tuberculosis*-complex organisms. Results are available within 2 to 3 weeks after the organism has been isolated and identified. Each new isolate is automatically tested at the time of identification. The test is repeated if the organism is still being cultured from the patient after 3 months and the quantitative growth is 1+ or greater. Susceptibility testing is performed with the Bactec bottles. If resistance is shown to any of the drugs listed below, the organism is sent to the Centers for Disease Control and Prevention (CDC) for testing with additional drugs.

Drugs are reported					
Indirect sensitivity	Indirect sensitivity performed by radiometric method				
Drug (mcg/ml)	Result	S = Susceptible			
Streptomycin (2.0)		R = Resistant			
INH (0.1)		% Resistance cannot			
Rifampin (2.0)		be determined by the Bactec radio			
Ethambutol (2.5)		metric method			
PZA (100)					

Molecular testing is reported		
Negative*	MTB not detected	
Positive for M. Tuberculosis*	MTB detected- RIF resistance detected or,	
	MTB detected- RIF resistance indeterminate or,	
	MTB detected- RIF resistance not detected	

<sup>\*</sup>The following disclaimer will be included on all molecular results. "Testing results are for Research Purposes only. Non-pulmonary sites are currently not approved for testing by MTD method. Culture results pending."

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the TB program coordinator in the Office of Disease Prevention.

# **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly identified with the patient's name or identifier.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen was broken or leaked in transit.
- 4. The specimen was submitted in a non-regulation container.
- 5. The specimen was submitted in 5% formalin, Cary-Blair or other preservative.
- 6. There was no specimen in the bottle.
- 7. The patient information was not complete.

#### Mycology

#### Introduction

Until relatively recent times mycology cultures were performed only in a few cases. It was sufficient to determine that a person had a pathogenic fungus and to identify that pathogen. Most other mycology cultures were reported as "No pathogenic fungi isolated." Other fungi, even if identified, were designated as saprophytes.

Today many persons are immunocompromised or immunosuppressed. Persons with diabetes who have become ketonic, cancer patients who are receiving chemotherapy, transplant recipients who must take immunosuppressive drugs, and persons who have developed AIDS are likely at some time to develop fungal diseases.

With any of these persons, mycoses can develop rapidly. Along with diseases caused by the common pathogens, it has become increasingly evident that many organisms formerly considered to be saprophytes are causing serious and in some cases life-threatening disease processes in these immunocompromised individuals.

Reference cultures of isolates are accepted for the identification of yeast and cutaneous, subcutaneous, and systemic fungi.

Yeasts are identified on characteristic microscopic morphology on selected media and by their assimilation of carbohydrates in the API 20 C assimilation kit. Fungi are identified by their growth rate, the size and color of the hyphae, and by the arrangement and origin of the conidia they produce. Biochemical tests are used if appropriate.

The Mycology Section identifies aerobic actinomycetes and fungus-like bacteria. They are identified primarily by biochemical tests.

Antimicrobial testing is not performed in this laboratory.

# **Specimen Collection**

<u>To submit reference cultures</u>, subculture isolated colonies from primary culture media to fresh media and incubate until visible growth appears. Fungal and yeast cultures may be shipped in screw-capped tubes of Sabouraud's agar or other appropriate media. Seal tightly with parafilm or waterproof tape. Plates are acceptable only if they are sealed, placed in a plastic bag and hand-delivered to the laboratory (never mail plates).

# **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

#### **Specimen Shipment**

1. Pack the specimen in a double-walled shipping container or equivalent. Pack it with absorbent material to prevent breakage and absorb the fluid if breakage or leakage should occur. Place the form in the outer container.

- 2. Affix the mailing label, return address, and specimen label to the outer container.
- 3. Ship the specimen to the South Dakota Public Health Laboratory in Pierre.
- 4. Telephone the Mycology Section before mailing clinical material or cultures of Histoplasma capsulatum or Coccidioides immitis. Known cultures of these two organisms must be shipped by receipted mail so that packages can be tracked and undelivered packages may be located quickly.

# **Reporting and Interpretation of Results**

Clinical specimens are reported within 6 weeks.

# **Reporting of Results**

Organisms are reported by genus and species.

Organisms are reported using the genus and species designations consistent with descriptions in the American Society for Microbiology's *Manual of Clinical Microbiology* and Davise Larone's *Medically Important Fungi: A Guide to Identification.* 

For molds, if no conidia are formed after 4 weeks and sporulation cannot be induced, the culture is reported as Mycelia Sterilia (fungi that do not form conidia or spores) or Hyaline fungus no sporulation.

The results of all specimens are reported to the health care provider who submitted the specimen.

# **Criteria for Unacceptable Specimens**

#### All Specimens

- 1. The specimen was not properly identified with the patient's name or identifier.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen was broken in transit.
- 4. The specimen was non-viable.
- 5. A mixed specimen was submitted.

#### **Parasitology**

#### Introduction

Accurate clinical diagnosis of intestinal parasitic diseases is difficult and requires laboratory confirmation. Demonstration of the diagnostic stages of invading parasites by direct microscopic examination of specimens is the most reliable method of establishing a diagnosis of most parasitic infections.

Diagnostic specimens for examination of the presence of human parasites and reference specimens are accepted from public and private health care providers. Routine screening of asymptomatic individuals is not recommended.

<u>Fecal material</u> is examined for amoebic cysts, intestinal flagellates, and the ova of round worms, tapeworms, hookworms, and flukes. Stained and unstained slides are prepared and examined after a concentration procedure has been performed.

<u>Fecal specimens for the identification of Cryptosporidium or Cyclospora</u> are tested by light microscopic examination using a modified acid fast stain.

<u>Perianal slides</u> are examined for *Enterobius vermicularis* (pinworm) infection.

Specimen Collection- Para Pak Kit (available from SD Public Health Lab) or equivalent Kit should contain 2 vials: 1) 10% buffered neutral formalin
2) Zn-PVA fixative

#### Fecal material

- 1. Because the host passes parasites intermittently, multiple specimens should be examined. Collect three specimens 48-72 hours apart.
- 2. Collect stool specimen in a clean, dry container. Do not mix with urine, water, dirt or paper.
- 3. Add stool to vials according to instructions with the kit. Mix well and do not overfill.
- 4. Both vials must be filled for intestinal parasites.

# **Specimen Identification**

- 1. Complete <u>all</u> the provider and patient information areas. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be examined.

#### **Shipment of Specimens**

 Pack the specimen in a double-walled shipping container or the equivalent. Pack with absorbent material to prevent breakage and absorb the fluid if breakage or leakage should occur. Place the form in the outer container.

- 2. Place slides in a slide mailing-container before packing them in the shipping container.
- 3. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the container.
- 4. Ship to the South Dakota Public Health Laboratory in Pierre.
- 5. Use first-class postage on US mail.
- 6. Telephone the Parasitology Section when an outbreak is suspected.

# **Reporting and Interpretation of Results**

Specimens are reported within 1 to 3 working days of receipt. If the specimen is sent to the Centers for Disease Control and Prevention, reporting will be delayed by several weeks.

Most parasites found, both pathogenic and non-pathogenic species, will be reported by their scientific names. Genus and species designations are consistent with the American Society for Microbiology's *Manual of Clinical Microbiology* and the *Atlas of Human Parasitology*.

# Results of fecal specimens are reported

**Parasites found:** <u>All</u> parasites found, pathogenic and non-pathogenic, are reported.

No parasites found: No parasites found.

# Results of pinworm specimens are reported

**Positive:** Positive for *Enterobius vermicularis* eggs.

**Negative:** No pinworms found.

# Results of specimens for Cryptosporidium are reported

**Positive:** Positive for *Cryptosporidium oocysts* 

**Negative:** Negative for *Cryptosporidium oocysts* 

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the Office of Disease Prevention.

#### **Criteria for Unacceptable Specimens**

#### All specimens

- 1. The specimen was not properly identified with the patient's name or identifier.
- 2. The patient identifier on the specimen does not match identifier on the form.
- 3. The specimen or slide was broken in transit.
- 4. The specimen leaked in transit.

#### Fecal specimens, including *Cryptosporidium*

1. The specimen contains interfering substances such as barium, bismuth, gall-bladder dye, urine, or water.

# Pinworm cellulose tape

1. The cellulose tape was contaminated with fecal material or talcum powder.

#### **Infectious Disease Serology**

#### Introduction

Diagnostic and immune status serologic assays are performed for various viral, rickettsial, fungal, chlamydial, and mycoplasmal agents. The assay methods vary depending upon the specific agent for which testing is requested. For specific agents and assay methods refer to Chart V —1 SEROLOGICAL TESTS AVAILABLE.

Serological testing for infectious agents that are not performed by the South Dakota Public Health Laboratory may be available at the Centers for Disease Control and Prevention (CDC) in Atlanta. Consult with the appropriate section at the laboratory before submitting specimens for testing. According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory.

## **Specimen Acceptance Policy**

Serologic testing is available to all public and private health care providers.

# **Type of Specimen Required**

<u>Immunity Screening</u> – A single, whole clotted blood or serum is required for immunity screening.

<u>Diagnostic Testing</u> – As a rule, acute and convalescent sera must be submitted for diagnostic serological testing. The acute serum should be collected as soon after the onset of illness as possible. For the majority of the serological testing offered by the SDPH Laboratory, the convalescent serum should be collected 14 days from the time the acute specimen was collected.

# Chart V – 1 Serological Tests Available from the Laboratory

Testing for infectious agents not listed in this chart may be available at the CDC. Consult with the Laboratory concerning testing not listed.

Agent or Disease Suspected	Specimen Needed	Test Method	Normal Reference Range <sup>1</sup>
Brucella	Acute and convalescent sera	Agglutination	Negative
Francisella tularensis (tularemia)	Acute and convalescent sera	Agglutination	Negative
Hantavirus	Acute and convalescent	EIA IgG	Negative
		EIA IgM	Negataive
Herpes simplex virus	serum	EIA	Negative
HSV I IgG, HSV II IgG			
Human immunodeficiency virus Type 1 (HIV-1/2) <sup>3</sup>	Whole, clotted blood, serum, or oral fluid.	Screening – EIA Confirmation – WB	NON- REACTIVE
			NON- REACTIVE
Legionella pneumoniae	Acute and convalescent (28 days) sera (IgG)	IFA	<1:128
(referred)			
Measles virus (Rubeola)	Immunity Screening – Whole clotted blood or serum	EIA (IgG)	POSITIVE (Immune)
Measles virus (Rubeola)	Diagnostic – Acute and	IFA (IgG)	No Change in Titer
	Convalescent (14 days) sera	EIA (IgM capture)	NEGATIVE
Mumps virus	Immunity Screening – Whole clotted blood or serum	EIA (IgG)	POSITIVE (Immune)

# 

Agent or Disease Suspected	Specimen Needed	Test Method	Normal Reference Range <sup>1</sup>
Mumps virus	Diagnostic – Acute and convalescent (14 days) sera	IFA	No Change in Titer
Q Fever (Coxiella burnetii) Phases 1 and 2	Acute and convalescent (28 days) sera	IFA	<1:64
Rocky Mountain Spotted Fever (Rickettsia rickettsii)	Acute and convalescent (28 days) sera	IFA	<1:64
Rubella virus	Immunity Screening – Whole clotted blood or serum	EIA (IgG)	POSITIVE (Immune)
Rubella virus	Diagnostic – Acute and convalescent (14 days) sera	EIA (IgG) EIA (IgM) (referred)	No Change in Titer Negative
St. Louis encephalitis virus	Acute and convalescent (14 days) sera	EIA (IgG) EIA (IgM)	No Change in Titer Negative
Typhus (Rickettsia typhi)	Acute and convalescent (28 days) sera	IFA	<1:64
Western Equine encephalitis virus	Acute and convalescent (14 days) sera	EIA (IgG) EIA (IgM)	No Change in Titer Negative
West Nile Virus	Acute and convalescent (14 days) sera	EIA (IgG) EIA (IgM)	No Change in Titer Negative

# **Abbreviations**

EIA	Enzyme Immunoassay	lgG	Class Immunoglobulin
WB	Western Blot	lgM	Class Immunoglobulin
IFA	Indirect Fluorescent Antibody	Quant	Quantitation, Quantitated

#### **Specimen Collection**

#### **Blood**

- Collect an acute serum as soon after the onset of the illness as possible. A
  convalescent serum should be collected 14 days after the collection of the acute
  serum. Exceptions to this general rule of collection of specimens are noted in
  Chart V 1.
- 2. Draw at least 5 to 7 ml of blood into a red-stoppered vacuum tube allowing the tube to fill completely. Allow the tube to stand at room temperature to ensure complete clotting of blood. Blood should not be taken for 1 hour after a meal to avoid chylous serum.
- 3. Store the specimen in a refrigerator until it is sent to the laboratory. If the serum is to be sent to the laboratory, separate the serum from the blood clot by centrifuging the whole clotted blood at 1,500 to 2,000 rpm at room temperature for 10 minutes. Pipette the serum into a new red-stoppered vacuum tube or a sterile plastic screw-capped vial. A minimum of 1 ml of serum should be sent to the laboratory for testing.

Serum-separating tubes may be used to collect the specimens for serological testing. These specimens should be sent to arrive in the laboratory within 48 to 72 hours of collection to avoid having the red blood cells hemolyze and "spill" into the upper portion of the tube.

4. Acute serum that is held until the collection of a convalescent serum should be separated from the blood clot and stored frozen until collection of the convalescent serum. Convalescent specimens may be run as stand alone specimens in limited situations. Consultation before the convalescent serum will be tested singly.

#### **Specimen Identification**

1. **Complete all the information on the form.** Include pertinent clinical information with each specimen. Be specific about why the specimen is being submitted to the laboratory.

For rubella, measles (rubeola), and mumps, indicate whether the specimen is for diagnosis of a current infection or for immunity screening.

2. Using indelible ink, label each specimen with the patient's first and last name and the date of collection. Unlabeled specimens or specimens containing information that does not match the information on the accompanying test request form will not be tested.

#### **Shipment of Specimens**

 Place the specimen in a plastic bag. Pack it in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb fluid if breakage or leakage should occur. Place the test request form in the outer container and secure the cap with tape. Ship at ambient temperatures.

- 2. Place the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label on the container.
- 3. Ship specimens to the South Dakota Public Health Laboratory in Pierre
- 4. Use first-class postage on US mail.

# **Reporting Procedure and Interpretation**

An interpretation of the results is given with each report. For specimens sent to the CDC, the CDC will provide interpretation of test results.

**Paired acute and convalescent sera** – When paired (acute and convalescent) sera are tested, the demonstration of a 4-fold increase in antibody titer from the acute to the convalescent serum strongly suggests recent infection with the agent for which the test was performed.

#### **Final Reporting**

The results of all specimen requests are reported to the provider who submitted the specimen.

If the result of the specimen is positive for a notifiable disease, this result is also reported to the Office of Disease Prevention.

# **Criteria for Unacceptable Specimens**

- 1. The specimen is not properly identified with the patient's name or identifier and the date of collection.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen is broken or leaked in transit.
- 4. The specimen is extensively hemolyzed, lipemic (chylous), extremely turbid, or grossly contaminated with bacteria.
- 5. Whole, clotted blood was collected more than 7 days prior to receipt by the laboratory.
- 6. The quantity of the specimen received is not sufficient to allow accurate completion of test(s) requested. (QNS-Quantity Not Sufficient).
- 7. The convalescent serum was collected sooner than 10 days from the date of collection of the acute serum. (The provider will be notified and asked to provide a more appropriately timed convalescent serum.)
- 8. No test request form was received with the specimen or no specimen was received with the form.

#### **Human Immunodeficiency Virus Serology**

#### Introduction

Serologic assays are available for the detection of antibodies to the human immunodeficiency virus (HIV-1/2). An enzyme immunoassay (EIA) is used as a screening test for antibodies to HIV. All reactive EIA's are repeated in duplicate to verify the initially reactive test result. All repeatably reactive EIA tests (two or more reactive EIA's) are confirmed by the Western Blot (WB) Assay.

#### Specimen Collection/Labeling/Requisition Form

Serum/Plasma Specimen

Using Universal Precautions, and standard venipuncture technique collect approximately five milliliters of whole blood (or serum) in a red top tube (no additive), labeled with patient's identifier, date, and name of the submitter, use a marker that will not fade, smear, or run during transportation. Plasma specimens are acceptable in tubes with the following anticoagulants: EDTA, sodium and lithium heparin, sodium citrate, CPD, CPDA-1 and ACD.

Oral Fluid

Using Universal Precaution, follow instructions provided with the Orasure collection kit to collect specimen properly.

# **Requisition Form**

Complete the SDPHL requisition form providing:

- 1. Name or unique patient identifier
- 2. Test(s) requested
- 3. Date specimen collected
- 4. Submitter's name, address, and code
- 5. Any information submitter needs for patient identification, e.g., chart number, address, physician name, contact person and phone number
- 6. Race, sex and age/DOB

# **Shipment of Specimens**

Specimens may be mailed, shipped by common carrier, or delivered to the laboratory by courier. Place the biohazard bag with its contents inside the cardboard outer can. Please place only one or two specimens in the cardboard can so they can be removed without mishap. If a screw-cap mailed is shipped by the Postal Service, the cap must be secured by tape, or the Postal Service will return them for taping. Be sure to use the proper mailing label for the final specimen destination.

# Reporting and Interpretation of Results

The following chart provides information regarding turn-around times (the time the specimen is received to the time the test is completed) and interpretations.

Description	Test Procedure	Turnaround Time
HIV 1/2 + O Antibody Screening	EIA	Working days 2-5
HIV-1 Antibody Confirmation	Western Blot	Working days 7-14

HIV-2 confirmation will be sent to the CDC at physician's request.

When EIA results are negative, the test results will be reported as "Negative" and no further testing will be performed on this sample. Repeat sampling at a later date may be necessary in order to detect developing antibodies.

The SD Public Health Laboratory uses the APHL/CDC criteria shown below for the interpretation of the HIV-1 Western Blot.

Interpretation	Criteria		
Negative	The absence of any and all bands-not just viral bands.		
Indeterminate	The presence of any viral or non-viral band or bands that fail to meet		
	the positive criteria.		
Positive	The presence of any two of the following bands:		
	• P24		
	• Gp41		
	• Gp120/gp160		

Reference: Centers for Disease Control. Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections. MMWR 1989;38:1-7.

The following recommendations are made regarding follow-up specimens:

- 1. If the result of a Western Blot is indeterminate, submit another specimen for testing within a month. If the second specimen is also indeterminate, the patient should be tested again at three and six months.
- 2. When a patient receives his/her first positive test result and has not identified a high risk behavior, collect a verification specimen at the time the patient is given the results of the first test.

# **Unacceptable Specimens**

- 1. ID on form and specimen do not match (ID mismatch)
- 2. No ID on specimen
- 3. Specimen over 14 days old
- 4. Specimen transport tube broken in transit
- 5. Insufficient quantity for testing (QNS)
- 6. No sample received with form

#### Syphilis Serology

#### Introduction

Syphilis is a disease caused by infection with the spirochete *Treponema pallidum*. Serological tests greatly aid in the diagnosis of syphilis. Serologic assays used to screen patients for syphilis are non-treponemal tests. The non-treponemal test performed by the South Dakota Public Health Laboratory is the Rapid Plasma Reagin test (RPR). Quantitative RPR results may be used to monitor therapy for *T. Pallidum* infections.

Confirmation of reactive screening test results (RPR) is obtained with specific treponemal tests for syphilis. The *Treponema pallidum*-Particle Agglutination test (TP-PA) is the South Dakota Public Health Laboratory's primary confirmatory test for *T. Pallidum*-specific antibody. Suspected biologically false-positive results sometimes produced in the RPR test may be investigated with a TP-PA test. The Fluorescent Treponema Antibody Stain (FTA) also detects *T. Pallidum*-specific antibody. The TP-PA is **not a screening procedure** and is only performed when required for proper patient management.

# **Specimen Acceptance Policy**

Testing for syphilis, non-treponemal and treponemal-specific, is available to all health care providers.

South Dakota does not require premarital testing for syphilis.

#### Type of Specimen Required

Refer to Chart V-2.

# Chart V – 2 Serological Tests for Syphilis

	Test	Specimen Required	Application of Test
Nontreponemal	RPR	Whole, clotted blood, serum, or plasma *	Screening (for example, prenatal or STD clinics), monitoring treatment.
Tests			
Treponemal Antibody	TP-PA	Whole, clotted blood or serum	Detection of false-positive RPR results, monitoring of infants for possible congenital syphilis.
Tests**			

\* Plasma can be tested with the RPR test, but plasma is not the preferred specimen. Serum is preferred because it is required for subsequent treponemal antibody tests that may need to be performed after the RPR test is completed. Also, plasma must be tested within 48 hours of collection or the risk of false RPR results is greatly increased.

\*\* Treponemal antibody tests will not routinely be performed on specimens that produce negative results on the screening test (RPR). An exception is that the TP-PA will be performed at the provider's request on specimens that may produce negative RPR results but are from patients (birth to 15-months-old) who may have congenital syphilis.

#### **Specimen Collection**

Draw one blood tube on each patient, even for those requiring a confirmatory test. Additional tubes are unnecessary.

# WHOLE, CLOTTED BLOOD OR SERUM

Draw at least 5 to 7 ml of blood into a red-stoppered vacuum tube allowing the tube to fill completely. Allow the tube to stand at room temperature to ensure complete clotting of blood. Blood should not be taken for 1 hour after a meal to avoid chylous serum.

Store the specimen in a refrigerator (2 to 8°C) until it is sent to the laboratory. If serum is to be sent, separate the serum from the blood clot by centrifuging the whole, clotted blood at 1,500 to 2,000 rpms at room temperature for 10 minutes. Pipette the serum into a new red-stoppered vacuum tube or plastic screw-capped vial. Submit at least 2 ml of serum.

#### **PLASMA**

Plasma is not a recommended specimen for syphilis testing. It may be submitted (1 to 2 ml) for the screening procedure for syphilis (RPR), but is not a suitable specimen for subsequent TP-PA procedure. Plasma must be tested within 48 hours from the time of collection to produce reliable RPR results.

# **Shipment of Specimens**

- 1. Place the specimen in a plastic bag. Pack it in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb fluid if breakage or leakage should occur. Place the form in the outer container and secure the cap with tape. Ship it at ambient temperatures.
- 2. Place the mailing label, return address, and specimen label on the container.
- 3. Ship blood or serum specimens to South Dakota Public Health Laboratory in Pierre

#### **Interpretation of Laboratory Results**

# Screening (RPR)

Normal: Non-reactive Reactive

#### Confirmatory(TP-PA)

Normal: Non-reactive, if no prior infection

Reactive, if documented previous infection

Abnormal: Reactive

Positive reactions will occur within 10 to 90 days following exposure or 7 to 10 days after onset of primary lesion.

<u>Biologial false-positive readings</u> may occur normally or may also indicate the presence of serious disease other than syphilis. Possible causes for biological false-positive RPR results:

Narcotic addiction

Aaina

Terminal malignancy

Viral diseases, e.g., chickenpox, measles,

Infectious mononucleosis, pneumonia, etc.

Malaria

**Hepatitis** 

Leprosy

Pregnancy

Rheumatoid arthritis

Systemic lupus erythematosus

# **Reporting Procedure and Interpretation**

Results of the non-treponemal tests for syphilis and the TP-PA performed on serum is available within 3 working days of receipt of the specimen.

Results of Tests for Syphilis are Reported
Reactive
Non-reactive

Patients with primary syphilis may have a non-reactive RPR and/or TP-PA. However, these tests will usually soon become reactive. Most patients treated for primary syphilis will have a reversion of the nontreponemal tests to non-reactive within two to three years. The TP-PA test will usually remain reactive after treatment. Non-reactive serologic tests and normal clinical evaluations do not exclude incubating syphilis.

The results of all specimen requests are reported to the provider who submitted the specimen. In addition, the Office of Disease Prevention and the STD control coordinator are sent reports on positive specimens.

#### **Criteria for Unacceptable Specimens**

- 1. The specimen is not properly identified with the patient's name.
- 2. The patient identifier on the specimen does not match that on test request form.
- 3. The specimen is broken or leaked in transit.
- 4. The specimen is extensively hemolyzed, lipemic (chylous), extremely turbid, or grossly contaminated with bacteria.
- 5. Whole, clotted blood collected more than 7 days prior to receipt by the laboratory.
- 6. Plasma collected more than 48 hours prior to receipt by the laboratory.
- 7. The quantity of the specimen received is not sufficient to allow accurate completion of test requested. (QNS-Quantity Not Sufficient.)



8.

#### Rabies Virus

#### Introduction

The SDPHL tests for the presence of rabies viral antigen in suspect animal tissue. Testing is performed on fresh brain tissue utilizing the direct fluorescent antibody procedure and is performed on suspect animals where there has been either human or domestic pet exposure. This testing service is available to State rabies control personnel, veterinarians licensed in the state, Game Fish and Parks officers, city animal control officers, and any health care provider licensed by and practicing in South Dakota.

#### **Animal Submission**

- Dogs and cats are the only animals that should be kept alive and held 10 days for observation following a bite. Observation is of value because the period of time the virus can be excreted in the saliva prior to onset of signs can be predicted. It is known that dogs and cats can excrete rabies virus up to five days prior to onset of signs. The ten day observation period for dogs and cats is twice that predicted time, allowing a margin of safety. If a dog or cat shows no clinical signs of rabies after 10 days of observation, it is safe to assume that the animal was not shedding rabies virus at the time of the bite. Conversely, if a dog or cat exhibits signs of rabies, it should be tested. Euthanize the animal and submit only the head to the laboratory for testing.
- 2. A wild or stray animal that bites a human or another animal must always be killed immediately and sent to the laboratory for examination. Only the head should be sent to the laboratory for rabies virus detection. Do not destroy the brain.
- 3. Bats should be caught, euthanized, and submitted whole to the laboratory for rabies virus detection. Do not destroy the brain.
- 4. Large animals (over 100 lbs.) needing to be tested for rabies must first have the brain removed from the skull, and then only the brain should be submitted. This includes testing on equine, bovine and large dogs.

#### **Specimen Collection**

- Animals should be euthanized in a manner that will not destroy the brain. The animal's neck should be severed at the midpoint between the base of the skull and the shoulders.
- 2. Rabies testing is performed on intact brain tissue. Care must be exercised to avoid damaging the brain when destroying animals for such testing. Extensive damage to the head of the animal, such as that sustained by a gun shot or bludgeoning, may render the specimen unsuitable for testing.
- 3. Wrap the specimen (animal head) in an absorbent, padding material such as newspaper. The specimen must be sealed in two heavy plastic bags, each individually sealed. The double bagged head should then be placed in another bag containing several hard frozen ice packs. The bag is placed in an inner Styrofoam box with a wax treated outer cardboard box. Specimens must not be frozen, fixed on formalin, or shipped on dry ice. If submitting brain only, place the brain in a plastic bag and submit as above.

- 4. The name and phone number of the submitter should be attached to the shipping container. Place the test request form in an envelope and place it on the outside of the primary shipping container.
- 5. Care should be taken to refrigerate, not freeze, the specimen. Brain tissue should be transported to the laboratory with the least possible delay.
- 6. Laboratory staff are available to accept rabies specimens during regular work hours: 8 a.m. to 5 p.m., CT, Monday through Friday. In the event that a specimen is transported and arrives after regular hours, or on a weekend or holiday, persons transporting such specimens should be directed to take the shipment to Capitol Security, located inside the north door of the Capitol Building, unless prior arrangements have been made with the SDPH Laboratory staff.
- 7. The laboratory must be notified of all impending shipments of animal specimens **before** transport. This communication helps to ensure the proper transport and delivery of specimens and in the event of a failure of delivery aids in the tracing and locating of a missing specimen.

#### **Specimen Acceptance Policy**

- In all cases, there should have been an exposure of human or domestic animals to the suspected rabid animal. Exposure is defined as a bite or contamination of scratches, abrasions, open wounds, or mucous membranes with infectious saliva. Specimens not involving a human or domestic pet exposure will result in a charge to the submitter.
- 2. Only whole brain specimens received in good condition with at least two identifiable principal brain parts are approved for reporting test results. Brain parts must include the cerebellum and the brain stem.
- 3. The Virology Laboratory is not equipped to handle whole carcasses, therefore, only the HEAD is accepted. Small animals such as bats may be sent intact, if recently expired. Unless circumstances surrounding the exposure suggest rabies infection, caged rodents such as hamsters, rabbits, etc., should not be submitted for testing.
- 4. Only the brain (not the entire head) of very large animals (e.g. cows/horses/goats/large dogs) will be accepted, as the laboratory is not equipped to handle these large heads due to limited hood and sterilizer space. Veterinarians should be requested to remove the brain from the cranium and submit the brain only.

#### **Specimen Identification**

A laboratory submission form containing the name and phone number of submitter, name and birthdate of person exposed, type of exposure, and type of animal must be submitted with the specimen. Under the heading "Symptoms exhibited \_\_\_\_\_\_" the details of the exposure as well as the symptoms exhibited by the animal must be listed. Please include animal vaccination history. Please indicate if the animal is a stray or wild. Failure to provide such information will cause delay in reporting test results to the submitter.

#### Reporting a rabies exposure

- 1. To report possible exposure and for guidance in submission of animals or for medical consultation, telephone 1-800-592-1861 or 1-605-773-6769 prior to destroying the animal, during regular work hours (8 a.m. 5 p.m. CT, Monday through Friday).
- 2. For emergencies after regular hours, or on weekends and holidays, call 280-4810.

#### **Reporting Procedures and Interpretation**

- 1. Positive rabies test results will be reported immediately by telephone to the submitter of the specimen and to the State Health Department, Office of Disease Prevention.
- 2. Unsatisfactory and negative specimens will be reported to the submitter by telephone.
- 3. All test results will be mailed to the provider. Results are available within 24-36 hours.

### Criteria for rejection

- 1. Whole animals larger than 2 lbs., non-mammalian species, decomposed specimens, whole head of very large animal, skull crushed, specimen preserved in formalin, alcohol, etc.
- 2. Whole carcass animals (larger than 2 lbs.) will be shipped to a local veterinarian to have the head removed. Veterinarian fees and a \$25 handling fee will be billed back to the submitter.

#### **Virus Culture**

#### Introduction

The Virology Laboratory is responsible for culturing and identifying viruses from clinical specimens. Virus culture provides a mechanism for the detection and identification of many human viruses capable of causing a wide variety of common illnesses. Specimens for culture of human viruses will be accepted from both public and private health care providers.

Virus culturing and identification that is not performed in the South Dakota Public Health Laboratory may be available at the Centers for Disease Control and Prevention (CDC) in Atlanta. **Consult with the Virology Lab prior to submitting specimens for testing.** According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory.

#### **Specimen Collection**

Collect specimens for virus isolation during the early, acute, febrile phase of illness. Specimens collected more than one week after onset of symptoms usually do not yield live viruses. The source of the specimen collected must be carefully matched with the virus suspected. The virus isolation services available and the specimen of choice for each virus is described in Chart V – 3 VIRUSES FOR WHICH ROUTINE CULTURING IS AVAILABLE.

Collect the specimen aseptically and place it in one of the following environments immediately.

- 1. Refrigerate at 2 to 8<sup>o</sup>C if the specimen will be delivered to the laboratory within 48 hours.
- 2. If receipt of the specimen by the laboratory will be longer than 48 hours from the time of collection, freeze the specimen at –70°C or at the lowest temperature possible and ship to remain frozen during transport.

#### NOTE: DO NOT FREEZE THE FOLLOWING (Ship on cool packs):

Specimens for isolation of respiratory syncytial virus (RSV). Specimens for isolation of cytomegalovirus (CMV). Blood specimens for virus isolation.

<u>Autopsy or Biopsy</u>: Collect fresh, unfixed tissue from the probable sites involved using a separate sterile instrument for each sample. Place each specimen into a separate small sterile vial of virus transport medium.

Consult with the Virology Laboratory prior to submitting autopsy or biopsy specimens for virus isolation.

Cerebrospinal Fluid (CSF): Aseptically collect 2 to 3 ml of CSF and transfer it to a sterile vial.

**Feces**: Place a piece of feces 4 to 8 grams (about the size of a grape) into a sterile container.

**Rectal Swab**: Generally, rectal swabs are less satisfactory than feces for the isolation of viruses. If used, obtain a rectal swab by inserting a dry cotton swab at least 5 cm into the anal orifice, rotating the stick, and then withdrawing it. Some fecal material must be visible on the cotton. Break the tip off into a vial of viral transport medium.

**Throat Swab**: Vigorously rub both tonsils and the posterior wall of the pharynx with a dry , sterile, cotton swab. The swab should not touch the tongue or buccal mucosa. Break off the swab tip into a vial of virus transport medium.

# **Bone Marrow:**

### Chart V - 3

Viruses for Which Routine Culturing is Available
Consult with the SDPH Laboratory for additional tests
which may be available at CDC.

Virus	TEST METHOD	SPECIMEN SOURCE/TYPE
Adenovirus (Types 1-41)	Cell culture IFA	Throat washing or swab, nasopharyngeal wash or swab, conjunctival swab, feces, urine
Coxsackie virus (A & B)	Cell culture	Throat swab, feces, CSF, pericardial fluid
Cytomegalovirus	Cell culture IFA	Urine, throat swab, buffy coat, lung tissue, lung aspirate
Echovirus (Types 1-33)	Cell culture	Throat swab, feces, CSF, pericardial fluid
Enterovirus (Types 68-71)	Cell culture	Throat swab, feces, CSF, pericardial fluid, vesicle scraping
Herpes simplex virus (Types 1 & 2)	Cell culture DFA	Vesicle scraping, brain biopsy, conjunctival swab, urogenital swab
Influenza virus (A & B)	Cell culture IFA	Throat washing or swab, nasopharyngeal washing or swab
Measles virus (Rubeola)	Cell culture IFA	Throat washing or swab, Nasopharyngeal washing or swab, conjunctival secretions
Mumps virus	Cell culture IFA	Throat washing or swab, urine, CSF
Parainfluenza virus (Types 1,2,& 3)	Cell culture IFA	Throat washing or swab, nasopharyngeal washing or swab
Poliovirus (Types 1,2,& 3)	Cell culture IFA	Throat washing or swab, feces, nasopharyngeal washing or swab, rectal swab
Respiratory Syncytial Virus	Cell culture IFA	Nasopharyngeal washing or swab
Rubella virus	Cell culture IFA	Nasopharyngeal washing or swab, CSF, urine
Varicella-zoster virus (Chickenpox-shingles)	Cell culture DFA	Vesicle scraping, throat washing

# **Abbreviations**

IFA – Indirect fluorescent antibody

DFA- Direct fluorescent antibody

<u>Nasal Swab</u>: Insert a dry cotton or polyester (not alginate) swab into the nostril parallel to the palate and leave in place for a few seconds. Slowly withdraw it with a rotating motion. Obtain specimens from both nostrils with the same swab. Break off the tip of the swab into a tube containing approximately 1.5 ml of viral transport medium.

<u>Nasal Washing</u>: Place the patient in a comfortable position with the head slightly tilted backward. Advise him to keep the pharynx closed by saying "K" while the washing fluid is applied to the nostril. With a transfer pipette, apply 1 to 1.5 ml of washing fluid to one nostril at a time. Ask the patient to tilt his head forward and let the washing fluid flow into a sterile beaker or petri dish. Repeat the process alternately with both nostrils until approximately 8 ml of the washing fluid has been used. Transfer the washings from the sterile catch container (the sterile beaker or petri dish) to a sterile container with a leak-proof top for transport to the laboratory.

**Nasopharyngeal Swab**: Take nasal and throat swabs as described above and place into the same vial of transport medium.

<u>Nasopharyngeal Aspirate</u>: collected by respiratory therapy, place in sterile container or vial of viral transport medium.

**<u>Urine</u>**: Collect clean-catch urine, preferably the first voided morning urine, in a sterile container.

<u>Vesicle</u>: Using a sterile instrument, **OPEN** the fluid-filled vesicle. Using firm pressure, absorb the fluid with a sterile cotton swab and scrape the perimeter of the lesion obtaining cellular material on the swab tip. Avoid causing excessive bleeding. Break off the swab tip into a vial of virus transport medium.

<u>Tissue Culture Isolates</u>: The Virology Laboratory provides reference services for laboratories that perform viral isolation. Viral isolates should be observed microscopically at the initial laboratory until 50% or more of the available cell sheet is exhibiting viral cytopathic effect (CPE). Once the cell sheet is exhibiting 50% CPE, send a tube of the infected cell culture (frozen or unfrozen) to the SDPH Laboratory for identification and/or typing of the virus. If the specimen is to be transported at ambient temperature, the tube of infected cell culture should be filled with a cell-culture-maintenance medium. If the specimen is frozen for transport no more than 1 ml of maintenance medium should be in the tube. Indicate the type of cell culture and the number of times the virus was passed through culture on the specimen tube. Indicate the suspected virus on the form.

#### **Specimen Identification**

- 1. Complete <u>all</u> the information on the SDPHL Requisition Form. Include pertinent clinical information with each specimen.
- Using indelible ink, label each specimen with the patient's first and last name, the specimen source, and the date of collection. Unlabeled specimens or specimens that contain information that is not compatible with the information on the test request form will not be tested.

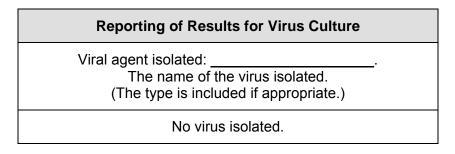
#### **Shipment of Specimens**

- Specimens that are to be hand-carried to the laboratory should be wrapped in paper toweling and placed in a leak-proof container with or commercial refrigerant packs. Be careful not to contaminate the specimen with any of the moisture produced by the refrigerant and be careful not to break the specimen container by crushing or colliding with the refrigerant.
- 2. Specimens that will arrive at the testing laboratory more than 48 hours after collection should be refrigerated after collection as soon as possible. If these specimens are to be sent to the laboratory by the US Postal Service or commercial courier, wrap them in paper toweling or some other absorbent material and place them in a leak-proof plastic bag. Pack them in a leak-proof insulated shipping container with enough ice packs to last 48 hours longer than the expected time required for transport of the specimen to the laboratory. Place the form in or on the container so that the test request form cannot be contaminated by the specimen even if breakage of the primary specimen container should occur.

Do not freeze specimens for isolation of respiratory syncytial virus (RSV) or cytomegalovirus (CMV) or blood specimens. Ship these specimens refrigerated.

- 3. Place the mailing label, return address, infectious substance (etiologic agent) or clinical (diagnostic) specimen label, and dry ice label on the shipping container.
- 4. Ship the specimen to the South Dakota Public Health Laboratory in Pierre.
- 5. Use first-class postage on US mail.

#### **Reporting Procedures and Interpretation**



Virus identification is consistent with the American Society of Microbiology's *Manual of Clinical Microbiology*.

Turn-around time for negative cultures varies from 1 to 4 weeks. Cultures yielding virus isolates may require more time for identification of the virus depending upon the isolate involved. Failure to isolate a virus may be the result of a number of factors, including improperly collected specimens, specimens collected at a period in the disease when the patient is not shedding virus, improperly transported specimens, low viral load, or a lack of sensitivity in the system being used for isolation. Failure to isolate virus should not rule out the virus as a cause of the clinical illness. Conversely, since people may asymptomatically carry a variety of viruses, viruses may be isolated which are unrelated to the current clinical illness.

The results of all specimen requests are reported to the provider who submitted the specimen. In addition Office of Disease Prevention is sent reports on all positive results.

#### **Criteria for Unacceptable Specimens**

- 1. The specimen is not properly identified with the patient's name.
- 2. The patient identifiers on the specimen do not match those on the test request form.
- 3. The specimen is broken or leaked in transit.
- 4. The specimen is inappropriate for virus isolation.
- 5. The quantity of specimen received is not sufficient to perform the requested testing. (QNS Quantity Not Sufficient.)
- 6. The specimen is received in a compromising condition (i.e., warm, delayed in transit) situation testing may or may not be initiated depending on circumstances.
- 7. Viral transport media expired.

#### Influenza Real-time RT-PCR

#### Introduction

The Virology Laboratory is responsible for culturing and identifying viruses from clinical specimens. Virus culture provides a mechanism for the detection and identification of many human viruses capable of causing a wide variety of common illnesses. Specimens for culture of human viruses will be accepted from both public and private health care providers.

Virus culturing and identification that is not performed in the South Dakota Public Health Laboratory may be available at the Centers for Disease Control and Prevention (CDC) in Atlanta. **Consult with the Virology Lab prior to submitting specimens for testing.** According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory.

#### **Specimen Collection**

Collect specimens for virus isolation during the early, acute, febrile phase of illness. Specimens collected more than one week after onset of symptoms usually do not yield live viruses. The source of the specimen collected must be carefully matched with the virus suspected. The virus isolation services available and the specimen of choice for each virus is described in Chart V – 3 VIRUSES FOR WHICH ROUTINE CULTURING IS AVAILABLE.

Collect the specimen aseptically and place it in one of the following environments immediately.

- 1. Refrigerate at 2 to 8°C if the specimen will be delivered to the laboratory within 48 hours.
- 2. If receipt of the specimen by the laboratory will be longer than 48 hours from the time of collection, freeze the specimen at –70°C or at the lowest temperature possible and ship to remain frozen during transport.

NOTE: DO NOT FREEZE THE FOLLOWING (Ship on cool packs):

#### **Blood Lead**

#### Introduction

Atomic Absorption Spectrometry is the method of testing in the SDPHL. Blood lead levels above 10 micrograms/deciliter are targeted for follow-up and treatment if necessary.

#### Specimen Collection/Labeling/Requisition Form

- I. Micro or Fingerstick
  - A. Procedure for Finger Preparation
    - Select examination gloves and rinse to remove powder from the gloves if present. This will help avoid contamination of the specimen.
    - 2. Thoroughly wash the patient's hands with soap and water, then dry using appropriate toweling (low-lint). If water is not available, foam type soap is acceptable. Again, this step is necessary to avoid contamination.
    - 3. Once washed, the finger to be used must not be allowed to come into contact with any surface, including the patient's other fingers.
    - 4. The finger to be punctured (often the middle finger) must be free of any visible infection or wound.
    - 5. Grasp the finger that has been selected for puncture between your thumb and index finger with the palm of the patient's hand facing up.
    - 6. If not done during washing, massage the fleshy portion of the finger gently.
    - 7. Clean the ball or pad of the finger to be punctured with an alcohol swab. Dry the fingertip using a sterile gauze.
    - 8. Puncturing the fingers of infants less than one year of age is not recommended. Puncturing the heel is more suitable for these children (NCCLS, 1986). From age birth to one year use the big toe or exterior lower lateral side of the heel, and over age one year use the middle or ring finger.
  - B. Puncturing of the Finger and Forming Drops of Blood
    - 1. Grasp the finger and quickly puncture it with a sterile lancet in a position slightly lateral of the center of the fingertip.
    - 2. Wipe off the first droplet of blood with the sterile gauze. (This drop contains tissue fluids that will produce inaccurate results).
    - 3. If blood flow is inadequate, gently massage the proximal portion of the finger and then press firmly on the digital joint of the finger. A well beaded drop of blood should form at the puncture site.

4. Do not let the blood run down the finger or onto the fingernail. (This blood is unsuitable).

#### C. Filling the Collection Container

- 1. Make sure that the microcontainer you are using contains only EDTA anticoagulant. These tubes have purple or lavender tops and are provided for use by the SDPHL.
- 2. Continue to grasp the finger, touch the tip of the collection container to the beaded drop of blood.
- 3. Draw the blood into the container maintaining a continuous flow of blood.
- 4. Fill the microcontainer to the collection line. Place the cap on the microcontainer.
- 5. Holding the tube with your thumb and forefinger, immediately invert the tube several times to mix the blood and anticoagulant thoroughly and to avoid the formation of clots. The specimen will be reported unsatisfactory if any clots are noted, or if the quantity of blood is insufficient for testing.
- 6. When you have finished filling and mixing the container, put a gauze pad on the finger and have the patient or mother hold until the bleeding has stopped. If bleeding continues after 3-5 minutes of applying pressure, consult a physician.
- 7. Properly label the microcontainer with patient's first and last name. Use a label and marker that will be legible when the specimen reaches the name listed on the requisition form.

#### II. Venous Specimens

- A. Select examination gloves and rinse to remove any powder from the gloves. This will avoid contamination of the specimen.
- B. Clean the puncture site with an alcohol swab or sponge, apply tourniquet, and perform venipuncture using butterfly needles appropriate for size.

  Make sure the vacutainer tube you are using contains only EDTA anticoagulant (purple or lavender top).
- C. Fill the tube at least one-half and preferable three-fourths full. Immediately invert several times to insure proper mixing of the blood and anticoagulant, and prevent clotting.
- D. Properly label the tube with a patient's first and last name exactly as it appears on the requisition form. Use a marker that will be legible when the specimen reaches the laboratory.

#### Requisition Form

- 1. Fill out the form completely. Important elements to be filled in on the form for the collection of data are:
  - a. Submitter name, address and phone number
  - b. Patient name
  - c. County of residence
  - d. Birthdate
  - e. Race, ethnicity, gender
  - f. Date collected
  - g. Method of collection
  - h. Test reason
- 2. Make sure the name on the form exactly matches the name on the specimen.

#### **Blood Lead Screening Schedule**

MEDICAID HEALTH KIDS KLUB PERIODICITY SCHEDULE (American Academy of Pediatrics recommendation)

BIRTH	1	2	4	6	9	12	15	18	24	3	4	5	Annually
	MO.	YR.	YR.	YR.	Through Age 20								

#### **Assessment of Risk**

The child's risk for high-dose exposure should be assessed using the developed questionnaire.

1. When a child is considered at low-risk:

BLOOD LEAD TEST IS REQUIRED AT 12 MONTHS AND 24 MONTHS OF AGE.

2. When a child is considered high-risk:

#### BLOOD LEAD TEST BEGINNING AT 6 MONTHS OF AGE.

a. If the initial blood test is <10 micrograms/deciliter a screening blood lead test is required at every visit prescribed by the periodicity schedule through 72 months of age, while the child remains at high risk unless the child has already received a blood lead test within the last 6 months of the periodic visit.

## **Shipment of Specimens**

- 1. Specimens should be refrigerated and sent to the SDPHL as soon as possible. Specimens drawn on Monday through Thursday should be sent on the same day. Those drawn on Friday or Saturday should be held until Monday to be sent.
- 2. To prepare for sending, the labeled specimen should be placed in the biobag with the Blood Lead Analysis requisition form in the outside pouch. The biobag is then placed in the secondary container and this is placed in the outer mailing container. The outside should have a clearly labeled address and biohazard sticker attached.

#### **Reporting and Interpretation of Results**

Lead levels above 10 micrograms/deciliter are considered abnormal, and case managers should refer to the guidelines for recommendations on follow-up screening and treatment. All venous specimen levels greater than 10 mg/dl are immediately telephoned to the submitter for prompt action.

#### **Unacceptable Specimens**

Specimens will be reported "no test – unsatisfactory" for the following reasons:

- 1. No patient identification on specimen (first and last names must be legible)
- 2. Discrepancy between patient identification on specimen and requisition form
- 3. Insufficient quantity for testing
- 4. Specimen broken or leaked in transit
- 5. Specimen age exceeds 14 days
- 6. Specimen clotted
- 7. Wrong anticoagulant

#### Norovirus (Norwalk-Like Virus)

#### Introduction

Noroviruses (genus *Norovirus*, family *Calicivirdae*) are a group of related, single stranded RNA, non-enveloped viruses that cause acute gastroenteritis in humans. *Norovirus* was recently approved as the official genus name for the group of viruses provisionally described as "Norwalk-like viruses" or NLV. This group has also been referred to as caliciviruses (because of their family name) and as "small round structed viruses, SRSV" because of their morphological features. Noroviruses are named after the original strain "Norwalk virus" which caused an outbreak of gastroenteritis in a school in Norwalk, Ohio in 1968. Currently there are at least four norovirus genogroups, which are in turn divided into at least 20 genetic clusters.

The incubation period for norovirus-associated gastroenteritis in humans is usually between 24 and 48 hours, but cases can occur within 12 hours of exposure. Norovirus infection usually present as acute-onset vomiting, watery non-bloody diarrhea with abdominal cramps, and nausea. Low-grade fever also occasionally occurs, and vomiting is more common in children. Dehydration is the most common complication especially among the young and elderly, and may require medical attention. Symptoms usually last 24-60 hours. Recovery is usually complete with no evidence of serious, long-term sequelae. Noroviruses are primarily transmitted through the fecal-oral route, either by consumption of fecally contaminated food or water or by direct person-person spread. Good evidence exists for transmission due to acrosolization of vomitus. Noroviruses are highly contagious and it is thought that an inoculum of as few as 10 viral particles may be sufficient to infect an individual.

Norovirus is detected by performing two separate RT-PCR reactions, one for Genogroup GI and one for Genogroup GII.

#### **Specimen Collection**

#### **Stool Specimens**

Identification of the virus is best made from stool specimens taken within 48-72 hours after onset of symptoms. Positive results may be achieved from specimens taken as along as 5 days after symptom onset. Stool specimens should be submitted in clean containers without transport media or preservatives. The samples should be sent on cool packs, not frozen.

#### **Emesis (Vomitus) Specimens**

Identification of the virus may be possible from emesis specimens. Specimens should be submitted in clean containers without transport media or preservatives. The samples should be sent on cool packs, not frozen.

#### **Specimen Identification**

- 1. Complete **all** the provider and patient information areas on the SDPHL requisition form. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name (or a unique identifier). Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be used.

#### **Specimen Shipment**

- Place the specimen container inside a plastic bag with the form in the outer envelope. Place the bag in a double-walled shipping container or the equivalent. Package with absorbent material to prevent breakage and to absorb fluid if breakage or leakage should occur. Include cool packs.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label on the container.
- 3. Ship to the South Dakota Public Health Laboratory in Pierre by quickest way.
- 4. Use first-class postage if US Mail service is used.

#### **Reporting and Interpretation of Results**

Specimens are reported
Positive for Norovirus RNA*
Negative for Norovirus RNA

<sup>\*</sup>Positives are identified and Genogroup GI or GII on report.

Results are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the Office of Disease Prevention.

#### **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly labeled with the patient's name or a unique identifier.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen was not sent on cool packs.

#### Shiga Toxin

#### Introduction

Disease caused by shiga-toxin producing *Escherichia coli* ranges from self-limiting diarrhea to hemorrhagic colitis and hemolytic uremic syndrome. Serotype 0157:H7, the most frequently implicated serotype, has been isolated from large food-borne outbreaks as well as sporadic cases. However, 60 serotypes have been implicated in diarrheal disease and several non-0157:H7 serotypes have been identified in food-borne outbreaks and cases of HUS. *E. coli* 0157:H7 is easily distinguished from other *E. coli* because of its inability to rapidly ferment sorbitol. Use of MacConkey agar with sorbitol provides an quick and easy screening method for *E. coli* 0157:H7. Non-0157:H7 STEC are phenotypically similar to commensal non-pathogenic *E. coli* and are not detected with sorbitol MacConkey agar. To detect these non-0157:H7 STEC, non-culture methods are used (Enzyme immunoassay or PCR). The South Dakota Public Health Laboratory uses a microwell format enzyme immunoassay for the detection of Shiga-like toxins I and II (Verotoxins) in stool specimens.

#### **Specimen Collection**

Stool samples (collected in clean containers) or rectal swabs should be placed into enteric transport media and transported to the laboratory on cool packs.

#### Specimen Identification

- 1. Complete **all** the provider and patient information areas on the SDPHL requisition form. Include pertinent clinical information with each specimen.
- 2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name (or a unique identifier). Unlabeled specimens or specimens where the patient identifier on the specimen does not match the identifier on the form will not be tested.

#### **Specimen Shipment**

- 1. Place the specimen container inside a plastic bag with the form in the outer envelope. Place the bag in a double-walled shipping container or the equivalent. Package with absorbent material to prevent breakage and to absorb fluid if breakage or leakage should occur. Include cool packs.
- 2. Affix the mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label on the container.
- 3. Ship to the South Dakota Public Health Laboratory in Pierre by quickest way.
- 4. Use first-class postage if US Mail service is used.

### **Reporting and Interpretation of Results**

Specimens are reported					
**Positive					
Negative					

Results are reported on the health care provider who submitted the specimen. In addition, positive results are reported to the Office of Disease Prevention.

# **Criteria for Unacceptable Specimens**

- 1. The specimen was not properly labeled with the patient's name or a unique identifier.
- 2. The patient identifier on the specimen does not match the identifier on the form.
- 3. The specimen was not sent on cool packs.

# IV. APPENDIX



**Program Use Only** 

Outbreak

**Patient Information:** Patient Name: (Last)

Patient's Address

**Patient Data** Principal Symptoms

Diagnostic Code

Serum

☐ Whole Blood (Red Top) ☐ Whole Blood (EDTA)

City

CD Billing Code\_
Flu Surveillance

Public Health Investigation

Specimen Data: Specimen Collect Date

#### **South Dakota Public Health Laboratory** 615 E. Fourth Street Pierre, SD 57501

Phone 605-773-3368 Fax 605-773-6129 www.state.sd.us/doh/Lab/index.htm

Date of Birth

State

alth Laboratory Street 7501 x 605-773-6129 ab/index.htm	Lab Use Or	nly				
Facility	······································				_	
Address					_	
•					_	
Phone					_	
	Clinician Name	ð				
UP	IN#					
	(First)				(MI)	
Birth		Sex		Race/Ethnicity		
		Zip Code		Medicaid/Medica	are Number	
D: 0 . 1			D (	of Onset		
Disease Suspected			Date	of Onset		
Fever (Over 100 F)			Rash	? Y/ N		
Immunization Date			Screening Y/N			
Specimen Sourc	e:					
□Bronch Wash □Cervical □Eye □Joint Fluid □Lesion	□NP Aspira □NP Swab □Oral Fluid □Pleural □Rectal Sw	□Sputum l □Stool □Throat	iid	□Urine □Vaginal □Fluid, other □Tissue sour		
COLOGY OI Adenovirus Cul OI Cytomegalovirus OI Enteric Virus C	us Culture	□BO	P Ov	LOGY va & Parasite Exa yptosporidium	m	

Venous / Capillary							
SEROLOG	GY						
SBR	Brucella Ab						
□CMG	Cytomegalovirus IgG Ab						
$\Box$ CMM	·						
$\square$ STU	Francisella tularensis Ab						
<b>HPS</b>	Hantavirus IgG/IgM Ab						
<b>HAM</b>	Hepatitis A IgM Ab						
<b>HAP</b>	Hepatitis A Total Ab						
	Hepatitis B Acute Profile						
$\square$ HBC	Hepatitis B Chronic Profile						
□VHC	Hepatitis B Core Total Ab						
<b>□</b> VCM	Hepatitis B Core IgM Ab						
<b>□VHG</b>	Hepatitis B Surface Ab						
∐VSG	Hepatitis B Post Vac. Screen						
<b>□</b> VSB	Hepatitis B Surface Ag						
∐HCV	Hepatitis C Ab						
□HSQ	Herpes Simplex Ab						
	,						
□VRO	Measles IgG (Rubeola) Ab						
	Measles IgM (Rubeola) Ab						
	1 8 -						
□VUM	Mumps IgM Ab						
□VQS	Q Fever IgG Ab						
	Rickettsial Ab Panel						
□VSF	Rocky Mt. Spotted Fever IgG Ab						
<b>□</b> VRE	Rubella IgG Ab						
	St. Louis Enceph. IgM Ab						
	Typhus IgG Ab						
	West Nile Virus IgM Ab						
	West Nile Virus IgG Ab						
□VNZ	Varicella Zoster IgG Ab						

=	Cervical Eve	□NP Swab □Oral Fluid
	oint Fluid	Pleural
	esion	Rectal Swab
_		_
VIROLO	OGY	
	Adenovirus Cu	
□VCI	Cytomegalovir	us Culture
□VEI	Enteric Virus C	Cultrue
	Herpes Virus C	
□VRI	Influenza Virus	Culture
	Influenza A/B I	
NORO	Norovirus PCR	2
	Respiratory Vi	
	Varicella Zoste	r Culture
□VOI	Other	
BLOOD BLT	<b>LEAD</b> Blood Lead	
MYCOR	BACTERIOLO	GY
_	Mycobacteria	· ·
	Mycobacteria 1	D
	M. tuberculosis	
_		
STD/SC	REENING	
□GPB	Chlamydia/Goi	norrhoeae
	GP1 C. trachome	atis only
	<b>GP2</b> N. gonorrha	peae only
$\square$ HIV		
	HIV (Orasure)	
SSR	Syphilis Ab RI	PR

hral	
	TOLOGY
	Ova & Parasite Exam
	Cryptosporidium
□BCS	Cyclospora
BACTE	RIOLOGY
$\square$ BMD	Bacillus culture/ID
☐ PPR	B pertussis PCR
BPC	B pertussis culture
$\square$ BMD	Brucella culture/ID
$\Box$ CAM	Campylobacter ID
□BSD	Corynebacterium diphtheriae
BEE	E. coli 0157 confirmation
	Francisella tularensis
HFLU	Haemophilus influenzae typing
□BGR	Neisseria gonorrhoeae culture
NMEN	Neisseria meningitidis serotyping
	Salmonella serotyping
	Shigella serotyping
<b>□STX</b>	
	Enteric Stool Culture
<b>□</b> BVC	Vibrio culture/ID
_	Yersinia culture/ID
□BMI	Yeast/Fungus ID
	ER



#### South Dakota Public Health Laboratory 615 E. Fourth Street Pierre, SD 57501 Phone 605-773-3368 Fax 605-773-6129

of Health	Pierre, SD : Phone 605-773-3368 F www.state.sd.us/doh/	ax 605-773-6129				
Submitting Facility						
Address						
City/State			_			
Phone	After ho	ours phone		-		
Veterinarian						
Patient Information: Patient Name: (L	ast)		(First)		(MI	
Patient's Address	Da	ate of Birth		Sex	Date Bitten/Exposed:	
City	St	ate		Zip Code	Phone Number	
Animal Death Da	te:	Animal Subm	itted:			
//	_	dog	☐ cat	□skunk	$\square$ cow	
		horse	☐ bat	raccoon		

Lab Use Only

Animai Death Date.	Allilliai Sub	mitteu.		
/	dog	cat	□skunk	$\square$ cow
	horse	☐ bat	raccoon	
	other			
Symptoms Exhibited by animal:				
1				

#### Appendix B

#### Packaging and Shipping Diagnostic Specimens And Infectious Substances (Etiologic Agents)

#### **General Considerations:**

- 1. Actively growing cultures of organisms should be submitted in tubes with screw-cap closures and on media appropriate for the organism. The cap should additionally be sealed with waterproof tape.
- 2. Cultures on Petri dishes are not an acceptable form of transport media.
- 3. All specimen containers should be closed tightly and sealed in order to prevent leakage and contamination.
- 4. The following definitions and classification criteria are taken from a 29 CFR, Section 1910.1030; IATA (International Air Transport Association) Dangerous Goods Regulations; ICAO (International Civil Aviation Organization) Technical Instructions for the Safe Transport of Dangerous Goods and US Postal Regulations (Domestic) and are current as of the time of this printing. It is the responsibility of the shipper to make sure that specimens and packages are sent under the proper guidelines and that proper training has been undertaken in order to understand the rules for proper shipping.

Definitions and Classification Criteria – 49 CFR 173.134, Class 6, Division 6.2:

- Division 6.2 (Infectious Substance): A material known to contain or suspected of containing a pathogen. A pathogen is a virus, or microorganism (including its viruses, plasmids, or other genetic elements, if any) or a proteinaceous particle (prion) that has the potential to cause disease in humans or animals. An infectious substance must be assigned the identification number UN 2814, UN 2900, UN 3373 or Un 3291 as appropriate, and must be assigned one of the following categories:
  - a. Category A: An infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. A Category A infectious substance must be assigned to identification number UN 2814 or UN 2900 as appropriate. Assignment to either of these numbers must be based on the known medical history or symptoms of the source patient or animal, endemic local conditions, or professional judgment concerning the individual circumstances for the source human or animal.
  - b. Category B: An infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances transported for diagnostic or investigational purposes. A Category B infectious substance must be described as "Biological substance, Category B" and assigned identification number UN 3733.

#### General Packing Requirements

#### Diagnostic Specimens:

- 1. Watertight primary receptacle(s). (Maximum quantity must not exceed 500 ml).
- 2. Watertight secondary packaging (maximum per outer package must not exceed 4 Liter).
- 3. Absorbent material placed between the primary receptacle and the secondary package sufficient to absorb all the specimen.
- 4. Outer packaging of adequate strength for its capacity, weight and intended use. Packaging must be manufactured and assembled so as to be capable of successfully passing the prescribed tests and conforming to the requirements of 42 CFR, part 173.24.
- 5. Packages must be at least 4 inches in the smallest overall external dimension.
- 6. If sent by air, the primary receptacle OR the secondary packaging must be capable of withstanding without leakage, an internal pressure which produces a pressure differential of not less than 95kPa in the range of 40 C to +55 C.
- 7. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.
- 8. The package should be marked "Diagnostic Specimen".
- 9. A biohazard label and/or an etiologic agent label must be permanently affixed to outer package.
- 10. For detailed instructions refer to 49 CFR, IATA/IACO and/or 42 CFR.

#### Infectious Substances:

- 1. Watertight primary receptacle(s). (Total volume not to exceed 50 ml or 50g).
- 2. Multiple primary receptacles placed in a single secondary package must be wrapped individually, separated and/or supported to ensure that contact between them is prevented.
- 3. Watertight secondary packaging.
- 4. Absorbent material of sufficient quantity to absorb the contents of all the primary receptacle(s) located between the primary and secondary package.
- 5. The outer package must be of adequate strength for its capacity, weight and intended use. Packaging must be manufactured and assembled so as to be capable of successfully passing the prescribed tests and conforming to the requirements of 42 CFR, part 173.24. The outer package must bear the Specification Markings for shipment of infectious substances. Packages must be at least 4 inches in the smallest overall external dimension.
- 6. If sent by air, the primary receptacle OR the secondary packing must be capable of withstanding without leakage an internal pressure which produces a pressure differential of not less than 95 kPa in the range of 40 C to +55 C.
- 7. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

- 8. All packages containing infectious substances must be marked durably and legibly on the outside of the package with the Name and Telephone Number of a person responsible for the shipment.
- 9. Shipments of infectious substances require the shipper to make advance arrangements with the receiver.
- 10. Appropriate labels including 6.2 Infectious Substances label must be applied to the outer packaging.
- 11. For detailed instructions refer to 49 CFR, ICAO/IATA and/or 42 CFR.

#### Use of Dry Ice When Shipping Specimens:

Dry Ice (carbon dioxide, solid) is prohibited in international mail. Dry ice is permitted in domestic mail or surface transportation when used as a refrigerant to cool the contents of a mail piece. The dry ice must be packed in a container that is designed to permit the release of carbon dioxide gas, preventing build up of pressure and possible rupture of the package. Do NOT place inside the primary, or secondary packaging.

For air transportation, each package may not contain more than 5 pounds of dry ice. The address side of the package must be clearly marked "Carbon Dioxide Solid, UN 1845" or "Dry Ice, UN 1845" along with the net weight of the dry ice and the identity of the contents being cooled. A shipper's declaration prepared in triplicate and a DOT Class 9 warning label for miscellaneous hazardous materials must be affixed to the outer packaging.

For surface transportation the address side of the package must be clearly marked "Carbon Dioxide Solid, UN 1845" or "Dry Ice, UN 1845" along with the net weight of the dry ice (which may exceed 5 pounds for surface transportation) and the identity of the contents being cooled.

#### Shipper's Declaration:

IATA requires a particular form to be used as the dangerous goods declaration shipping paper. This dangerous goods declaration is in addition to the air waybill (regular air cargo shipping form) and must accompany the air waybill. IATA requires that the Shipper's Declaration be signed by the **shipper**, not the shipper's agent.

#### Shipment of Etiologic Agents from

Interstate Shipment of Etiologic Agents, 42 CFR Part 72, Federal Register, Vol. 45, No. 141-Monday, July 21, 1980, Centers for Disease Control and Prevention, Office of Health and Safety, Biosafety Branch, (Last Modified: 5/20/97)

www.cdc.qov/od.ohs/biosfty/shipreqs.htm

#### Etiologic Agents from 42 CFR Part §72.3

"Etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease. The following agents are considered etiologic agents:

#### **Bacterial Agents**

Acinetobacter calcoaceticus. Leptospira interrogans-all serovars.

Actinobacillus – all species. Listeria – all species. Actinomycetacease – all members. Mimae polymorhpa.

Aeromonas hydrophilia.

Arachnia propionica.

Arizona hinshawii – all serotypes.

Mindo polymernipa.

Moraxella – all species.

Mycobacterium – all species.

Mycoplasma – all species.

Bacillus anthracis. Neisseria gonorrhoeae,

N. meningitidis
Bacteroides spp.
Bartonella – all species.
Bordetella – all species.
Borrelia recurrentis, B. vincenti.

N. meningitidis
Nocardia asteroides.
Pasteurella – all species.
Plesiomonas shigelloides.
Proteus – all species.

Brucella – all species.

Brucella – all species.

Proteus – all species.

Pseudomonas mallei.

Campylobacter (Vibrio) foetus, C. (Vibrio) Pseudomonas pseudomallei.

jejuni.

Chlamydia psittaci, C. Trachomatis. Salmonella – all species and all serotypes.

Clostridium botulinum, Cl. Chauvoei, Cl. Shigella – all species and all serotypes. Haemolyticum, Cl. Histolyticum, Cl novyi, Cl.

Septicum, CI. tetani.

Corynebacterium diphtheriae, C. equi, C. Sphaerophorus necrophorus. haemolyticum, C. pseudotuberculosis, C.

pyogenes, C. renale.

Edwarsiella tarda.

Erysipelothrix insidiosa.

Staphylococcus aureus.

Streptobacillus moniliformis.

Escherichia coli, all enteropathogenic Streptococcus pneumoniae. serotypes.

Francisella [Pasteurella] Tularensis. Streptococcus pyogenes.

Haemophilus ducreyi, H. Influenzae. Treponema careteum, T. Pallidum, and T.

pertenue.

Klebsiella – all species and all serotypes. Vibrio cholerae, V. Parahaemolyticus Legionella – all species and all Legionella-like Versinia (Pasteurella) pestis, Y.

organisms. enterocolitica.

**Fungal Agents** 

Blastomyces dermatitidis. Histoplasma capsulatum. Coccidioides immitis. Paracoccidioides brasiliensis.

Cryptococcus neoformans.

Viral and Rickettsial Agents

Adenoviruses - human - all types.

Arboviruses – all types.

Coxiella burnetii.

Coxsackie A and B viruses – all types.

Creutzfeldt – Jacob agent.

Cytomegaloviruses.

Dengue viruses - all types.

Ebola viruses.

Echoviruses – all types. Encephalomyocarditis virus.

Hemorrhagic fever agents including, but not limited to, Crimean hemorrhagic fever (Congo), Junin, Machupo viruses, and

Korean hemorrhagic fever viruses.

Hepatitis associated materials (hepatitis A,

hepatitis B, hepatitis nonA-nonB).

Herpesvirus – all members. Infectious bronchitis – like virus.

Influenza viruses – all types.

Kuru agent.

Lassa virus. Lymphocytic choriomeningitis virus.

Marburg virus.

Measles virus.
Mumps virus.

Parainfluenza viruses – all types.

Polioviruses – all types. Rabies virus – all strains. Reoviruses – all types. Respiratory syncytial virus. Rhinoviruses – all types. Rickettsia – all species. Rocha limaea quintana.

Rotaviruses – all types.

Rubella virus. Simian virus 40.

Tick – borne encephalitis virus complex,

including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central

European encephalitis viruses.

Vaccinia virus. Varicella virus.

Variola major and Variola minor

/iruses

Vesicular stomatis viruses – all types

White pox viruses. Yellow fever virus.<sup>2</sup>

#### 42 CFR Part §72.3 (c) Dry ice.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

#### 42 CFR Part §72.3 (e) Damaged packages.

The carrier shall promptly, upon discovery of evidence of leakage or any other damage to packages bearing Etiologic Agents/Biomedical Material label, isolate the package and notify the Director, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, Georgia 30333, by telephone (404) 633-5313. The carrier shall also notify the sender.

<sup>&</sup>lt;sup>2</sup> This list may be revised from time to time by Notice published in the Federal Register to identify additional agents which must be packaged in accordance with the requirements contained in this part.

#### 42 CFR Part §72.3 (f) Registered mail or equivalent system

Transportation of the following etiologic agents all be by registered mail or an equivalent system, which requires or provides for the sending notification of receipt to the sender immediately upon delivery:

- Coccidioides immitis.
- Ebola virus.
- Francisella (Pasteurella) tularensis.
- Hemorrhagic fever agents including, but not limited to, Crimean hemorrhagic fever (Congo), Junin, Machupo viruses, and Korean hemorrhagic fever viruses.
- Herpesvirus simiae [B virus].
- Histoplasma capsulatum.
- Lassa virus.
- Marburg virus.
- Pseudomonas mallei.
- Pseudomonas pseudomallei.
- Trick-borne encephalitis virus complex including but not limited to Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses.
- Variola major and Variola minor and White pox viruses.
- Yersinia (Pasteurella) pestis

#### 42 CFR Part §72.4 Notice of delivery; failure to receive

When notice of delivery of materials known to contain etiologic agents listed in 72.3[f] is not received by the sender within 5 days following the anticipated delivery of the package, the sender shall notify the Director, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, Georgia 30333, by telephone (404) 633-5313. This section does not apply to select agents and toxins that are subject to requirements under the provisions of 42 CFR 73.16 concerning transfer of select agents and toxins.

A complete listing of select agents along with other information can be found at <a href="http://www.selectagents.gov/index.html">http://www.selectagents.gov/index.html</a>.

# Example

Sample IATA Shipper's Declaration for Dangerous Goods form for a shipment that includes an infectious substance and dry ice in the same package. Special provision A81 is being used to go over the normal 50-ml package limit for passenger aircraft.

Two completed and signed copies of this Declaration must be handed to the operator				WARNING				
TRANSPORT DETAILS		,		Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder or an IATA cargo agent.				
This shipment is within the limitations prescribed for (delete non-applicable)  PASSENGER CARGO AND CARGO AIRCRAFT ONLY	·	of Departi	ure					
Airport of Destination: Miami, FL			Shipm	ent type (delete nor	n-applicable	)		
				NON-F	RADIOACTIVE	RADIOAC1	IVE	
NATURE AND QUANTITY	OF DAN	IGEROUS	GOODS	S				
Dangerous Good	s Identif	ication	Т		T			
Proper Shipping Name	Class or Divi- sion	UN or ID No.	Pack- ing Group	Subsi- diary Risk	Quantity and type of packing	Packing Inst.	Authorization	
Infectious Substances, Affecting Humans (Ebola Virus)  Dry Ice	<ul><li>6.2</li><li>9</li></ul>	UN2814 UN1845	III		1000 ml	620 954	A81	
Additional Handling Information  Emergency Response Phone number: 888-555-1111							mada	
	•				ods Regulations 1.3.3.		mauc.	
I hereby declare that the contents of this consignment a and accurately described above by the proper shipping and are classified, packaged, marked and labeled/placa and are in all respects in proper condition for transport according to applicable international and national governmental regulations.				name, Ann Thracks, Lab Mgr.				

# V. FEE SCHEDULE FOR LABORATORY SERVICES

# South Dakota Public Health Laboratory Fees Current as of: February 2012 TEST NAME TEST CODE FEE

# MEDICAL MICROBIOLOGY

Adenovirus, Direct Antigen, F.A.	VAF	\$21.50
Adenovirus, IgG Antibody, Quantitative (requires paired specimens)	VAD	\$21.50
Blood Lead (see Lead, Blood)		
Bordetella pertussis Culture	BPC	No Charge
Bordetella pertussis PCR	PPR	
		No Charge
Borrelia Burgdorferi Antibody (see Lyme Disease)		
Brucella Antibody, Quantitative (paired specimens preferred)	SBR	\$36.50
Chlamydia – Amplified DNA Detection	GP1	\$15.00
Chlamydia/Gonorrhoae Panel – Amplified DNA Detection	GPB	\$20.00
Cryptosporidium	BCP	\$36.00
Cytomegalovirus IgG Antibody, Qualitative	CMG	\$15.50
Cytomegalovirus Culture (Virus Isolation)	VCI	\$45.00
Cytomegalovirus, IgG Antibody, Quantitative (paired specimens)	CMV	\$19.50
Cytomegalovirus, IgM Antibody	СММ	\$23.50
Diphtheria Culture	BSD	\$81.00
E. Coli 0157:H7, Isolation and Identification	BEE	No Charge
Enteric Bacterial Reference Culture	BER	No Charge
Environmental Mold Culture	MISC	\$76.00

# South Dakota Public Health Laboratory Fees Current as of: February 2012

TEST NAME TEST CODE FEE

BEP	\$55.00
VEI	\$74.00
BGP	\$30.00
BGR	No Charge
GP2	\$15.00
HPS	\$43.00
HAM	\$16.50
HAV	\$16.00
VSB	\$23.50
VHG	\$26.50
VCM	\$31.50
VHC	\$23.50
HBV	\$41.50
VSG	\$26.50
HAP	\$81.50
HBC	\$60.50
HBD	\$67.50
HCV	\$27.50
VHI	\$43.00
	VEI BGP BGR GP2 HPS HAM HAV VSB VHG VCM VHC HBV VSG HAP HBC HBD

# South Dakota Public Health Laboratory Fees Current as of: February 2012

TEST NAME TEST CODE FEE

,		
Herpes Simplex, IgG Antibody, Qualitative	HSQ	\$31.00
Herpes Simplex, IgG Antibody, Quantitative (paired specimens)	VHS	\$26.50
HIV Antibody (if reactive, reflex to confirmatory test) - serum	HIV	\$16.00
HIV Antibody (if reactive, reflex to confirmatory test) – oral fluid	ORA	\$19.00
Influenza type A, Direct Antigen, F.A.	VFA	\$21.50
Influenza type B, Direct Antigen, F.A.	VFB	\$21.50
Influenza RT-PCR	IAB	\$60.00
Legionella, IgG Antibody, Quantitative (paired specimens)	VLS	\$21.50
Lead, Blood	BLT	\$15.00
Lyme Disease, Antibody (if reactive, reflex to Western Blot)	VLM	\$23.50
TP-PA	TPA	\$18.00*
Miscellaneous Bacterial Reference Culture	BMD	No Charge
Mumps, IgG Antibody, Qualitative – immunity screen	VMS	\$15.50
Mumps, IgG Antibody, Quantitative (requires paired specimens)	VMA	\$21.50
Mumps, IgM Antibody	VUM	\$22.00
Mumps RT-PCR	MPCR	\$60.00
Mycobacteria Primary Culture	TTB	No Charge
Mycobacteria Reference Culture (Isolates only)	ТОТ	No Charge
Mycology Reference Culture	ВМІ	No Charge
Norovirus (PCR)	NORO	\$60.00
Ova and Parasites, Intestinal	ВОР	\$30.00

<sup>\*</sup>No Charge if reflexed from positive RPR.

# South Dakota Public Health Laboratory Fees Current as of: February 2012 TEST NAME TEST CODE FEE

VPD	\$46.50
	\$12.00
VQS	\$37.00
VRT	\$47.50
VRB	****No Charge
	\$15.00
VRD	\$65.00
VR2	\$19.50
VRI	\$64.00**
VRK	\$53.50
VSF	\$21.50
RPR	\$13.50
VRM	\$31.50
VRE	\$13.50
VRO	\$15.50
VMD	\$21.50
	VQS VRT VRB  VRD  VR2 VRI VRK  VSF  RPR VRM VRE VRO

<sup>\*\*</sup>No Charge for Sentinel Sites.

<sup>\*\*\*\*</sup>Fee is \$78.00 for specimens not approved by Communicable Disease Office.

# South Dakota Public Health Laboratory Fees Current as of: February 2012 TEST NAME TEST CODE FEE

Rubeola, IgM Antibody	VMM	\$27.50
Shigatoxin	STX	\$20.00
Sin Nombre Virus Antibody (see Hantavirus)		
St. Louis Encephalitis, IgM Antibody	SLM	\$22.00
St. Louis Encephalitis, IgG Antibody, Quantitative (paired specimens)	VSL	\$22.00
Syphilis (see RPR, TP-PA)		
Tularemia, Antibody, Quantitative (paired specimens)	STU	\$39.00
Typhus, IgG Antibody, Quantitative (paired specimens)	VTY	\$21.50
Varicella-Zoster, IgG Antibody, Qualitative	VNZ	\$15.50
Varicella-Zoster, IgG Antibody, Quantitative (paired specimens)	VND	\$21.50
Virus Culture, Miscellaneous	VOI	\$65.00
Vibrio Culture	BVC	\$60.00
West Nile Virus, IgM Antibody	WNM	\$22.00***
West Nile Virus, IqG Antibody	WNG	\$22.00
Yersinia Culture	BYC	\$60.00
Additional test not listed		\$50.00

<sup>\*\*\*</sup>May be free of charge.