NPHL Quick Reference Guide to Specimen Collection of Suspected Agents of Bioterrorism & Emerging Infectious Diseases

Updated: 7/20/2015

All potential agents, if not ruled out must be referred to NPHL - including original specimen and all isolates

Call 24/7 pager (402) 888-5588 or notify NPHL via STATPack™

DISEASE/ AGENT	SPECIMEN SELECTION		Time & Temp		SPECIMEN PLATING AND PROCESSING				
			Transport	Storage	SBA	CHOC	MAC	Stain	Other
Anthrax *(Bacillus anthracis)*	Cutaneous	**Vesicular Stage:** collect fluid from intact vesicles on sterile swab(s). The organism is best demonstrated in this stage.	≤2 h RT	≤24 h RT	X	X	X	Gram Stain	India Ink and slide motility NOT recommended due to safety considerations
		Eschar Stage: without removing eschar, insert swab beneath the edge of eschar, rotate and collect lesion material.	≤2 h RT	≤24 h RT	X	X	X	Gram Stain	India Ink and slide motility NOT recommended due to safety considerations
	Gastro-Intestinal	**Stool:** collect 5-10 g in a clean, sterile, leakproof container.	≤1 h RT	≤24 h 4°C	Inoculate rountine stool plating media plus CAN or PEA				Minimal Recovery
		Blood: collect per institution's procedure for routine blood cultures.	≤2 h RT	Δ	Blood Culture Bottles				Postiive in late stages of disease
	Inhalation	**Sputum:** collect expectorated specimen into a sterile, leakproof container.	≤2 h RT	≤24 h RT	X	X	X		Minimal Recovery
		Blood: collect per institution's procedure for routine blood cultures.	≤2 h RT	Δ	Blood Culture Bottles				Postiive in late stages of disease
Brucellosis *(Brucella sp)*	Acute, Subacute or chronic	**Serum:** collect 10-12 cc acute phase specimen as soon as possible after disease onset. Followed by a convalescent specimen, obtained 14-21 days	~2 h RT	-20°C	Specimen should be stored and shipped frozen @ -20°C				Serologic diagnosis: 1. Single titer:≥ 1:160 2. 4-fold rise 3. IgM
		Blood: collect per institution's procedure for routine blood culture.	≤2 h RT	Δ	Blood Culture Bottles			Gram Stain	Blood culture isolation rates vary from 15-70% depending on methods and length of incubation
		Bone Marrow: collect per institution's surgical/pathology procedure	≤15 min RT	≤24 h 4°C	X	X	X	Gram Stain	Inoculate to blood culture bottles or enrichment broth
					Hold cultures for at least 7 days				
		Spleen or Liver: Submit in sterile container, May add 1-2 drops of saline to keep moist		Δ	X	X	X	Gram Stain	
Plague *(Yersinia pestis)*	Pneumonic	**Sputum/throat:** collect routine throat culture using a swab or expectorated sputum collected into a sterile, leakproof container.	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	Minimal recovery.
		Bronchial/tracheal wash: collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/ "bubble".	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	
		Blood: collect per institution's procedure for routine blood cultures.	≤2 h RT	Δ	Blood Culture Bottles				Patients with negative cultures having a single titer, ≥1:10, specific to F1 antigen by agglutination would meet presumptive criteria
	Bubonic	**Tissue or aspirate:** Submit in sterile container, May add 1-2 drops of saline to keep moist	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	

Abbreviations: Δ , delayed entry depends on instrument; A, autopsy; BCYE, buffered charcoal-yeast extract agar; C, centigrade; CA, chocolate agar; CAN, colistin-nalidixic acid agar; g, grams; h, hours; MAC, MacConkey agar; PEA, phenylethyl alcohol blood agar; RT, room temperature

DISEASE/ AGENT	SPECIMEN SELECTION		Time & Temp		SPECIMEN PLATING AND PROCESSING				
			Transport	Storage	SBA	CHOC	MAC	Stain	Other
Tularemia *(Francisella tulerensis)*	Pneumonic	**Sputum/throat:** collect routine throat culture using a swab or expectorated sputum collected into a sterile, leakproof container.	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	Minimal recovery. Add BCYE plate
		Bronchial/tracheal wash: collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/ "bubble".	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	Add BCYE plate
		Blood: collect per institution's procedure for routine blood cultures.	≤2 h RT	Δ	Blood Culture Bottles			Gram Stain	Delayed entry may depend on instrument
		Biopsy, tissue, scrapings, aspirate or swab: Submit in sterile container. For small tissue samples add several drops of sterile normal saline to keep tissue moist. Swabs are collected by obtaining firm sample of advancing margin of the lesion. Place swab in transport package to keep swab moist with the transport medium inside packet.	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	
Glanders & Melioidosis *Burkholderia mallei & pseudomallei*		**Blood or Bone Marrow:** collect using standard automated blood culture system per institution's procedure for routine blood culture.	≤2 h RT	Δ	Blood Culture Bottles			Gram Stain	Delayed entry may depend on instrument
		Sputum/Bronchial: collect into sterile leakproof container	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	
		Abscess material and wounds: tissue aspirate, tissue fluid preferred to swab alternative	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	
		Urine:	≤2 h RT	≤24 h 4°C	X	X	X	Gram Stain	
		Serum: collect (≥1 ml) acute phase specimen as soon as possible after disease onset. Followed by a convalescent specimen, obtained 14-21 days. Specimens should be collected if serologic diagnosis is available in the United States.			Specimen should be stored and shipped frozen @ -20°C				Serologic diagnosis: 1. Single titer:≥ 1:160 2. 4-fold rise 3. IgM

Abbreviations: Δ , delayed entry depends on instrument; A, autopsy; BCYE, buffered charcoal-yeast extract agar; C, centigrade; CA, chocolate agar; CAN,colistin-nalidixic acid agar; g, grams; h, hours; MAC, MacConkey agar; PEA, phenylethyl alcohol blood agar; RT, room temperature

NPHL Quick Reference Guide to Specimen Collection of Unknown Virus

DISEASE/ AGENT	SPECIMEN SELECTION		Time & Temp		SPECIMEN PLATING AND PROCESSING
			Transport	Storage	
Q fever *Coxiella burnettii*		Serum: Collect serum as soon as possible after onset of symptoms (acute) and with a follow up specimen (convalescent) at ≥ 14 days for serological testing.	< 2 hr RT	≤ 4°C	Note: Sentinel laboratories should not accept environmental or animal samples: such specimens should be forwarded directly to the Nebraska Public Health Environmental Laboratory (NPHEL).
		Blood: Collect EDTA (lavender) or sodium citrate (blue) for PCR testing. If possible, collect specimens prior to antimicrobial therapy.	4° C	4 °C	
		Tissue, Body Fluids and Other including cell culture & cell supernatants. Arrange for immediate shipment at 2-8 °C to an appropriate higher-level LRN laboratory.	< 24 hr < 4 °C	-70°C or on dry ice	
Smallpox (Variola virus)	Rash	Biopsy specimens: aseptically place two to four portions of tissue into a sterile, leakproof, freezable container.	~6 h 4°C	-20°C to -70°C	1. A suspected case of smallpox should be reported immediately to the respective state health department for review 2. And if, after review, smallpox is still suspected, CDC's Poxvirus Section @ 404-639-2184 should be contacted for approval to send 3. At this time review the packaging/shipping requirements with CDC and request assistance in coordinating a carrier for transport/shipment
		Scabs: aseptically place scrapings/material into a sterile, leakproof, freezable container.	~6 h 4°C	-20°C to -70°C	
		Vesicular fluid: collect fluid from separate lesions onto separate sterile swabs. Be sure to include cellular material from the base of each respective vesicle.	~6 h RT	-20°C to -70°C	
Viral Hemorrhagic fever (VHR)		Serum: collect 10-12 cc of serum. Laboratory tests used to diagnose VHF include: antigencapture ELISA, IgG ELISA, PCR, and virus isolation.	~2 h RT	-4°C	Specific handling conditions are currently under development.

Abbreviations: A, autopsy; C, centigrade; g, grams; h, hours; RT, room temperature; cc, cubic centimeter (ml)

Disease/ Agent	Specimen Selection					Specimen Handling		Comments
	Specimen Type	Clinical Syndrome				Specimen volume	Transport temp	Specimen(s) of choice for confirming botulism: a. Serum b. Wound/tissue c. Stool and incriminated food
		Foodbourne	Infant	Wound	Intentional Release			
Botulism *(Clostridium botulinum)*	Enema Fluid	X	X	X	X	20 cc	4 ° C	Purge with a minimal amount of sterile nonbacteriostatic water to minimize dilution of toxin
	Food Sample	X	X		X	10-50 g	4 ° C	Foods that support C. botulinum growth will have a pH of 3.5-7.0, most common pH is 5.5-6.5. Submit food in original container, placing individually in leak proof sealed transport devices.
	Gastric Fluid	X,A	A			20 cc	4 ° C	Collect up to 20 cc
	Intestinal Fluid	A	A				RT	Autopsy: intestinal contents from various areas of the small and large intestines should be provided
	Nasal swab				X			For aerosolized botulinum toxin exposure, obtain nasal cultures for C. botulinum and serum for mouse toxicity testing
	Serum	X,A		X	X		4 ° C	Serum should be obtained as soon as possible after the onset of symptoms and before antitioxin is given. A minimum of 10 cc of serum (20 cc of whole blood) is required for mouse toxicity testing. In infants, serum is generally, not useful, since the toxin is quickly absorbed before serum can be obtained.
	Stool	X	X	X	X		4 ° C	Botulism has been confirmed in infants with only "pea-sized" stools. Please note: anticholinesterase given orally, as in patients with myasthenia gravis, has been shown to interfer with toxin testing
	Vomitus	X					4 ° C	Collect up to 20 cc
	Wound/tissue			X			RT	Exudate, tissue or swabs must be collected and transported in an anaerobic transport system. Samples from an enema or feces should also be submitted since the wound may not be the source of botulinum-toxin
	Environmental sample		X		X		RT	Environmental swabs

Abbreviations: A, autopsy; C, centigrade; g, grams; h, hours; RT, room temperature; cc, cubic centimeter (ml)

NPHL Quick Reference Guide to Specimen Collection for Staphylococcal Enterotoxin B

Disease/ Agent	Specimen Selection	Specimen Handling	Comments
Staphylcoccal Enterotoxin B	NOTE: Sentinel laboratories should not accept environmental (including food samples) or animal specimens for testing; such specimens should be forwarded directly to the Nebraska Public Health Environmental Laboratory. Exposure to SEB as a result of a bioterrorist event may include exposure to both the organism S. aureus and the enterotoxin or exposure to the enterotoxin only. Specimens may be tested for both the presence of enterotoxin and the bacterium.	Ship Immediately at 2-8° C	Foods should be left in their original containers if possible or placed in sterile unbreakable containers. Place containers individually in leakproof containers (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected contaminated foods can be examined. Environmental samples such as paper, powder, swabs, wipes, water, and soil can be sent to NPHEL for SEB testing.
	Serum is the preferred specimen for testing for inhalation SEB intoxication by detecting antibodies to SEB. Use a red-top or serum separator-type (SST) tube to obtain serum. Samples should be obtained as soon as possible after the onset of symptoms to detect the toxin. Serum should also be collected 7 to 14 days after onset of illness to compare acute- and convalescent-phase antibody titers. Do not send whole blood, since hemolysis during transit will compromise the quality of the specimen.	Ship Immediately at 2-8° C	The tube must be free of anticoagulants. Approximately 10 ml of blood should be drawn to provide 5 ml of serum.
	Nasal swab: Rub dry, sterile swab (Dacron or rayon) on the mucosa of the anterior nares. Place in protective transport tube.	Ship Immediately at 2-8° C	Collect a nasal swab within 24 h of exposure
	Induced Respiratory Sections: Sputum induced by instilling 10 to 25 ml of sterile saline into the nasal passages should be collected into a sterile screw-top container.	Ship Immediately at 2-8° C	
	Urine: A 20- to 30-ml urine sample should be collected from the patient into a sterile screw-top container as soon as possible.	Ship Immediately at 2-8° C	
	Stool/gastricaspirate: A 10- to 50-g sample of stool should be placed in a sterile leakproof container with a screw-top lid.	Ship Immediately at 2-8° C	
	Postmortem: Obtain specimens of the intestinal contents from different levels of the small and large bowel. Place 10 g of specimen into a sterile unbreakable container.	Ship Immediately at 2-8° C	
	Culture isolate: If an isolate of S. aureus is recovered from a specimen, it may be sent for toxin testing on an appropriate agar slant that supports its growth or a transport swab.	Ship at room temperature	