

Appendix D: Collection and Submission of Clinical Samples

- 1) Collection and Packaging of Enteric Pathogens**
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Collection and Packaging of Enteric Pathogens



Kentucky Public Health
Prevent. Promote. Protect.

Supplies Needed for Enteric Pathogens



Cary Blair Transport Media with Indicator
Zorb Sheet
Plastic Zippered Bag



Aluminum Can with
Biohazard label
*Ensure rubber gasket is in lid



Outer Can with Lid



Biological Substance, Category B Box
with 95kPa bag and Air Pillows



Laboratory Form 219
or
Outreach Form

Collection of Specimen - DO NOT DISCARD LIQUID IN VIAL

1. Store Cary Blair media at room temperature.
2. Check expiration date of specimen vial.
3. Make sure two identifiers or lab label is on specimen vial.
4. Specimen should be sent to the laboratory as soon as possible. This medium is intended for use as a transport medium and should not be used as a storage or enrichment medium.

Stool Specimens

1. Collect stool specimen into clean container.
DO NOT mix urine or water with sample.
2. Open vial carefully. Using the collection spoon attached to the cap, add enough specimen until the liquid reaches the arrow on the label. Approximately one gram.
3. Replace cap tightly and agitate vial.

Swab Specimens

1. Remove cap and immerse swab into medium.
2. Break swab shaft evenly with the lip of the vial.
3. Replace cap and tightly.

Packaging and Shipping

1. Place sample vial into zippered bag with Zorb sheet.
2. Place zippered bag inside the aluminum can and tighten lid.
3. Wrap lab form around outside of aluminum can and place inside the outer can. Address label should be on the outside of the outer can.

Specimen must be mailed to KY Public Health Lab on the day of collection.

When sending by FedEx, place completed canister specimen container inside FedEx UN3373 Pak.



1. Place sample vial into zippered bag with Zorb sheet.
2. Place zippered bag into 95kPa bag and seal.
3. Place air pillows inside box and place lab form on top.
4. Close box and place label on top of box.

Instructions for closure are on the flap of box.



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Collection and Packaging of Norovirus

Supplies Needed for Norovirus



- Kit Components:
- Cold Pack
 - 95kPa Bag with absorbent
 - Vial in zip bag with Zorb Sheet
 - Outreach/Lab Form 275

Collection of Specimen

1. Check expiration date of specimen vial.
2. Make sure two identifiers or lab label is on specimen vial.
3. Fill out lab or Outreach form completely.

- Stool and Vomitus Specimens
1. Collect stool/vomitus specimen into clean container.
DO NOT mix urine or water with sample.
 2. Open vial carefully. Using the collection spoon attached to the cap, fill vial with specimen until half full.
 3. Replace cap tightly.

- Swab Specimens
1. Remove cap and place into clean vial.
 2. Break swab shaft evenly with the lip of the vial.
 3. Replace cap and tightly.

If larger specimen collection containers are used, contact KY DLS for additional instructions.

Packaging and Shipping



Place sample vial into zip bag with Zorb sheet



Place sample/samples into 95kPa bag



Place sample bag on top of frozen freezer block and replace styrofoam lid



Place Outreach or Lab Form 275 on top of styrofoam lid



Close box and place appropriate label on top of box

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KY Division of Laboratory Services 100 Sower Blvd., North Loading Dock, P.O. Box 2020 Frankfort, Kentucky 40602-2020 Phone: 502/564-4446 Fax: 502/564-7019 Jeremy Hart, MD, FCAP, Director	 Kentucky Public Health <small>Prevent. Promote. Protect.</small> <h2 style="margin: 0;">Special Microbiology</h2>
<i>Please complete a separate form for each specimen.</i>	
PATIENT INFORMATION:	
Name (Last, First, MI) _____	
Social Security # _____ Sex _____ Race _____ Age _____ DOB _____	
Home Address _____	
City _____ State _____ Zip Code _____ County _____	
Send Report To:	
Submitter _____	
Street Address (PO BOX) _____	
City _____ State _____ Zip Code _____	
Specimen Information:	
Purpose of Exam _____	<input type="checkbox"/> Clinical Specimen
Specimen Source _____	<input type="checkbox"/> Referred Culture
Date of Collection _____	Bloody Diarrhea <input type="checkbox"/> Yes <input type="checkbox"/> No
Examination Requested: <i>(Please mark one)</i>	
<input type="checkbox"/> Enteric Pathogens	<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;"> <u>Organism Suspected:</u> </div>
<input type="checkbox"/> *Miscellaneous Bacterial Culture	
<input type="checkbox"/> Intestinal Parasites	
<input type="checkbox"/> Pinworm	
<input type="checkbox"/> Other _____	
Other pertinent Medical Data: <i>*Please complete this section when submitting Miscellaneous Bacterial Cultures</i>	
FOR LABORATORY USE ONLY:	
Date Received:	Laboratory Number:

Please Use "L" Label or Fill in Completely

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Specimen Submission: Select appropriate specimens for the clinical symptoms present, collect at proper intervals during illness, and handle as indicated below.

Specimen Required (without preservatives)	Preparation	Shipping
Serum, 3 ml or Whole Blood, 6 ml	For antibody and/or immune status: submit a single serum For diagnostic determination: * by IgM tests - a single serum (a 2 nd serum may be requested later) by IgG tests -paired sera: <u>acute</u> phase collect within 7 days of onset of illness. <u>convalescent</u> phase collect 10 to 21 days later.	Ambient or refrigerated temperature
Throat Washings	Use 5 - 10 ml sterile Hank's Balanced Salt Solution or sterile saline. 3 or 4 washings from the patient may be pooled in a sterile screw-cap container. Seal tightly.	
Throat Swabs, Vaginal / cervical Swabs	A swab collection kit is provided by the State Lab but any <u>Viral</u> transport medium is acceptable.	
Spinal Fluid	Submit in a sterile screw-cap tube and seal tightly.	Specimens arriving within 24 hours of collection may be shipped refrigerated. If there will be longer storage or shipping times freezing is best. **
Vesicle Fluid, or Pustule Crusts,	Collect fluids on swabs and place in 1 ml of liquid (sterile Hank's or saline) in screw-cap container and seal tightly.	
Autopsy Tissues	Place each tissue in a separate sterile screw-cap container and seal tightly. Label each organ.	

* A significant rise in antibody ratios or titers may determine a recent infection. It is important that there is enough time between acute and convalescent sera to allow for antibody rise. A history of transfusion within 6 weeks of serum collection will invalidate serologic test results.

** When it is necessary to ship clinical samples frozen, use enough dry ice to keep sample frozen throughout shipment..

If the agent suspected is Respiratory Syncytial Virus (RSV) or Cytomegalovirus (CMV) the specimen should be refrigerated but do not freeze. It should arrive at the laboratory within 4 hours of collection.

A completed electronic OUTREACH or #275 form must be enclosed for each patient !
Dates of specimen collection, and when appropriate, date of onset of illness are required!



REQUISITION FOR LABORATORY SUPPLIES

Email to: DPHlabKits@ky.gov or Fax to: (502)564-7019

Facility:

Delivery address

Phone:

Date Requested:

Requested By:

Gonorrhea/Chlamydia (Genprobe kits) # of kits	Swabs	Urine	Norovirus Kit	
			Viral Isolation Swab Kit (Includes shipper)	
Enteric Pathogen Kit (Cary Blair)			M4RT Viral Media (Swab/ Media; used for Virus & Flu)	
TB Sputum Kit				
Newborn Screening Filter Paper				
Mailing Labels				
Purple (All samples) Qty			Red Stopper Tubes	
			PPT Tubes (HCV testing)	
			SST Tubes	
Shippers				
			Multi-Shippers (Ship CT/GC, Syphilis, HIV, etc)	
Forms are available online at http://chfs.ky.gov/dph/info/lab			Multi-Shippers with Cold Pack (Ship Flu, Herpes)	
			Tube Shuttles	
OTHER				
			Rabies Collection Kit	
			Water Bacteriology Kit	
			Food Collection Kit	

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Reference List of Tests

BACILLUS CEREUS

DLS Department:	Bacteriology Environmental Microbiology
OUTREACH Test Code:	MEP
Includes:	
Methodology:	Culture/ Isolation/ PCR
Availability:	M-F
Turn-around-Time:	Clinical 4-7 days/ Food 4-15 days
Specimen Requirements:	Clinical- Feces Isolate- Pure culture isolate on agar slant: Heart Infusion, trypticase soy, blood or chocolate agar slant Food- By request of Epidemiology or Food Safety Branch/ ONLY for investigation of foodborne disease outbreaks
Collection Kit/Container:	Clinical – Cary Blair Transport Media
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	Clinical- Mail immediately after collection Isolation- Provide fresh growth slant; mail immediately Food - Specimen must be shipped overnight in cooler with cold packs. Call laboratory before sending.
Unacceptable Conditions:	≥ 7 days from collection
Reference Range:	Clinical – No B. cereus isolated Food - <100 cfu/g
CPT Code:	Stool 87046; Vomitus 87070 PCR 87153
Additional Comments:	Collect during acute stage (Days 1-3) of diarrheal disease and before initiation of anti-microbial or drug therapy.

Reference List of Tests

BOTULISM	
DLS Department:	Preparedness
OUTREACH Test Code:	MEP
Includes:	Clostridium Botulinum Identification and Confirmation
Methodology:	ELISA, Mouse Bioassay, Culture
Availability:	
Turn-around-Time:	Food: 24-96 hours toxin test, 5-12 days culture Stool: 24-96 hours toxin test, 5-12 days culture Serum: 24-96 hours
Specimen Requirements:	Patient's physician must consult the Division of Epidemiology to verify the need to perform appropriate tests
Collection Kit/Container:	Consult with DLS for specific information of collecting and submitting specimens
Patient Preparation:	
Collection and Packaging Instructions:	Serum: 10ml Stool: 10-50g Enema Wash: 20ml Infants- Stool or Enema Wash
Specimen Handling and Transport:	
Reference Range:	ELISA: "No botulinum toxin A, B, E or F detected. Assay does not detect botulinum toxins C, D and G". Mouse Bioassay: No C. botulinum toxin detected by mouse bioassay Culture: No C. botulinum isolated
CPT Code:	87003; 87158; 87076
Additional Comments:	Refer to the Sentinel Guidelines on asm.org for more information

Reference List of Tests

CAMPYLOBACTER	
DLS Department:	Bacteriology (clinical) Environmental Microbiology (Food)
OUTREACH Test Code:	CAMP
Includes:	
Methodology:	Culture
Availability:	M-F
Turn-around-Time:	Clinical specimen- 4-7 days Pure culture isolate- 3-5 days Food- 5-15 days
Specimen Requirements:	Clinical- Feces Isolate- swab from culture in Cary Blair, Campy thio or Campy blood plate in bag with Campy gas or in semi-solid motility type media. Chocolate slant is also acceptable.
Collection Kit/Container:	Clinical: Cary Blair Transport Media Isolate: Submitter packaging
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/ronlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	Feces- mail immediately after collection on cold pack. Isolate-Provide fresh growth slant; mail immediately; avoid extreme temperatures Food- Call laboratory; ship overnight in cooler with cold packs
Unacceptable Conditions:	Stool 7 days or older
Reference Range:	NA
CPT Code:	87040; stool 87046
Additional Comments:	Food- By request of Epidemiology or Food Safety Branch ONLY for investigation of foodborne disease outbreaks

Reference List of Tests

ESCHERICHIA COLI

DLS Department:	Bacteriology Environmental Microbiology
OUTREACH Test Code:	ECO
Includes:	Shiga-toxin producing E.coli
Methodology:	Isolation/Identification and Antigenic typing/ Food Culture
Availability:	M-F
Turn-around-Time:	Clinical 4-5 days/ Food 48 hrs-15 days
Specimen Requirements:	Clinical- Feces Identification and Antigenic typing- Agar slant: Heart infusion, trypticase soy, blood or chocolate Food- By request of Epidemiology or Food Safety Branch ONLY for investigation of foodborne disease outbreaks
Collection Kit/Container:	Clinical- Enteric pathogens kit with Cary Blair or Amies transport media Isolate – Pure fresh growth on agar slant Food- Food Kit
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	Clinical- Mail immediately after collection to be rec'd in 24 hrs Isolation- Provide fresh growth slant; mail immediately Food - Food specimens - Ship overnight in cooler with cold packs. Call laboratory before sending specimens.
Unacceptable Conditions:	Stools 7 days or older
Reference Range:	NA
CPT Code:	Stool 87046, 87147 PCR 87150
Additional Comments:	Collect during acute stage (Days 1-3) of diarrheal disease and before initiation of anti-microbial or drug therapy.

Reference List of Tests

HEPATITIS A

DLS Department:	Virology
OUTREACH Test Code:	HAV
Includes:	
Methodology:	EIA (IgM only)
Availability:	M-F
Turn-around-Time:	48 hours
Specimen Requirements:	Serum or whole blood in 7 ml red stoppered tube
Collection Kit/Container:	
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/B2DC7607-7525-4749-A34F-CDD906D2C37C/0/Multishipper2017web.pdf http://chfs.ky.gov/NR/rdonlyres/9D5D708A-5491-4375-9A52-70A25799C665/0/MultishipperwithcoldpackBlood.pdf
Specimen Handling and Transport:	Specimen should be stored at 2-8°C prior to shipping. Ship ASAP. If possible, ship on cold pack.
Unacceptable Conditions:	
Reference Range:	Non-reactive
CPT Code:	86709
Additional Comments:	

Reference List of Tests

LISTERIA MONOCYTOGENES

DLS Department:	Bacteriology (clinical) Environmental Microbiology (Food)
OUTREACH Test Code:	MC (clinical)
Includes:	Environmental Microbiology identifies Listeria species
Methodology:	Identification/Culture/Molecular
Availability:	M-F
Turn-around-Time:	4-6 days
Specimen Requirements:	Clinical- Pure culture TSA or blood agar slant Environmental- Food
Collection Kit/Container:	
Collection and Packaging Instructions:	Submitter packaging for clinical isolates Food: http://chfs.ky.gov/NR/rdonlyres/60E2687E-19FA-4DC9-8B33-AC798D70167A/0/RevisedFoodSampleCollection.pdf
Specimen Handling and Transport:	Clinical- Provide fresh growth on slant. Mail immediately to be received within 24 hours. Avoid extreme temperatures Food specimens - Ship overnight in cooler with cold packs. Call laboratory before sending specimens.
Unacceptable Conditions:	
Reference Range:	NA
CPT Code:	Clinical- 87077, 87153
Additional Comments:	Clinical – Call the laboratory if Listeria is suspected and when specimen is shipped. Food- By request of Epidemiology or Food Safety Branch ONLY for investigation of foodborne disease outbreaks.

Reference List of Tests

NOROVIRUS	
DLS Department:	Virology
OUTREACH Test Code:	NORO
Includes:	
Methodology:	PCR
Availability:	M-F
Turn-around-Time:	24-48 hours
Specimen Requirements:	Raw stool, Emesis
Collection Kit/Container:	Sterile container without preservative. Ship with cold pack.
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/0C2947A7-D115-4846-B133-B565DFDEBA57/0/Norovirus2017web.pdf
Specimen Handling and Transport:	Specimen should be collected in sterile screw top container within 72 hours of onset of symptoms and refrigerated prior to shipping. DO NOT FREEZE. Ship with cold pack ASAP
Unacceptable Conditions:	
Reference Range:	Negative
CPT Code:	87798
Additional Comments:	Call Epidemiology prior to sending specimens.

Reference List of Tests

SALMONELLA

DLS Department:	Bacteriology/ Environmental Microbiology
OUTREACH Test Code:	Stools- SGT Culture isolate- SGT
Includes:	All Sero-Types
Methodology:	Isolation Identification Antigenic Testing
Availability:	M-F
Turn-around-Time:	Clinical-8-10 days Food- 5-15 days
Specimen Requirements:	Isolation- Stool Identification and Antigenic Typing- Pure culture isolate. Agar slant: Heart infusion, trypticase soy, blood or chocolate agar
Collection Kit/Container:	Stools - Cary Blair
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	Food specimens - Ship overnight in cooler with cold packs. Call laboratory before sending specimens.
Unacceptable Conditions:	Stools 7 days or older
Reference Range:	
CPT Code:	Stool- 87045 Identification and Antigenic Typing- 87147
Additional Comments:	Stool-Mail immediately after collection; to be received within 24 hours. Provide fresh grown on slants and mail immediately at room temperature. Food- By request of Epidemiology or Food Safety Branch/ONLY for investigation of foodborne disease outbreaks

Reference List of Tests

SHIGELLA

DLS Department:	Bacteriology/ Environmental Microbiology
OUTREACH Test Code:	Stools- SHGR Culture isolate- SHGR
Includes:	All Sero-Types
Methodology:	Isolation Identification and antigenic typing
Availability:	M-F
Turn-around-Time:	Clinical-8-10 days Food- 5-15 days
Specimen Requirements:	Isolation- Stool Identification and Antigenic Typing- Pure culture isolate. Agar slant: Heart infusion, trypticase soy, blood or chocolate agar
Collection Kit/Container:	Stools - Cary Blair
Collection and Packaging Instructions:	http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	Food specimens - Ship overnight in cooler with cold packs. Call laboratory before sending specimens.
Unacceptable Conditions:	Stools 7 days or older
Reference Range:	
CPT Code:	Stool- 87045 Identification and Antigenic Typing- 87147
Additional Comments:	Stool-Mail immediately after collection; to be received within 24 hours. Provide fresh grown on slants and mail immediately at room temperature. Food- By request of Epidemiology or Food Safety Branch/ONLY for investigation of foodborne disease outbreaks

Reference List of Tests

STAPHYLOCCUS AUREUS

DLS Department:	Bacteriology
OUTREACH Test Code:	Stool - MEP Pure culture isolate on agar slant- MC
Includes:	
Methodology:	Isolation and Identification
Availability:	M-F
Turn-around-Time:	Stool-3-7 days Pure culture isolate- 24hrs- 7days Food- 5-15 days
Specimen Requirements:	Clinical- Stool Isolation- Pure culture isolate on agar slant: Heart Infusion, trypticase soy, blood or chocolate agar slant Food- By request of Epidemiology or Food Safety Branch/ ONLY for investigation of foodborne disease outbreaks
Collection Kit/Container:	Clinical – Stool -Cary Blair Transport Media
Collection and Packaging Instructions:	Clinical: http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf Food: http://chfs.ky.gov/NR/rdonlyres/60E2687E-19FA-4DC9-8B33-AC798D70167A/0/RevisedFoodSampleCollection.pdf
Specimen Handling and Transport:	Food specimens - Ship overnight in cooler with cold packs. Call laboratory before sending specimens.
Unacceptable Conditions:	Stools 7 days or older.
Reference Range:	NA
CPT Code:	87046, 87153
Additional Comments:	Clinical testing- Only by request of the Division of Epidemiology. Stool-Mail immediately after collection; to be received within 24 hours. Isolate - Provide fresh grown on slants and mail immediately at room temperature. Food- By request of Epidemiology or Food Safety Branch. ONLY for investigation of foodborne disease outbreaks.

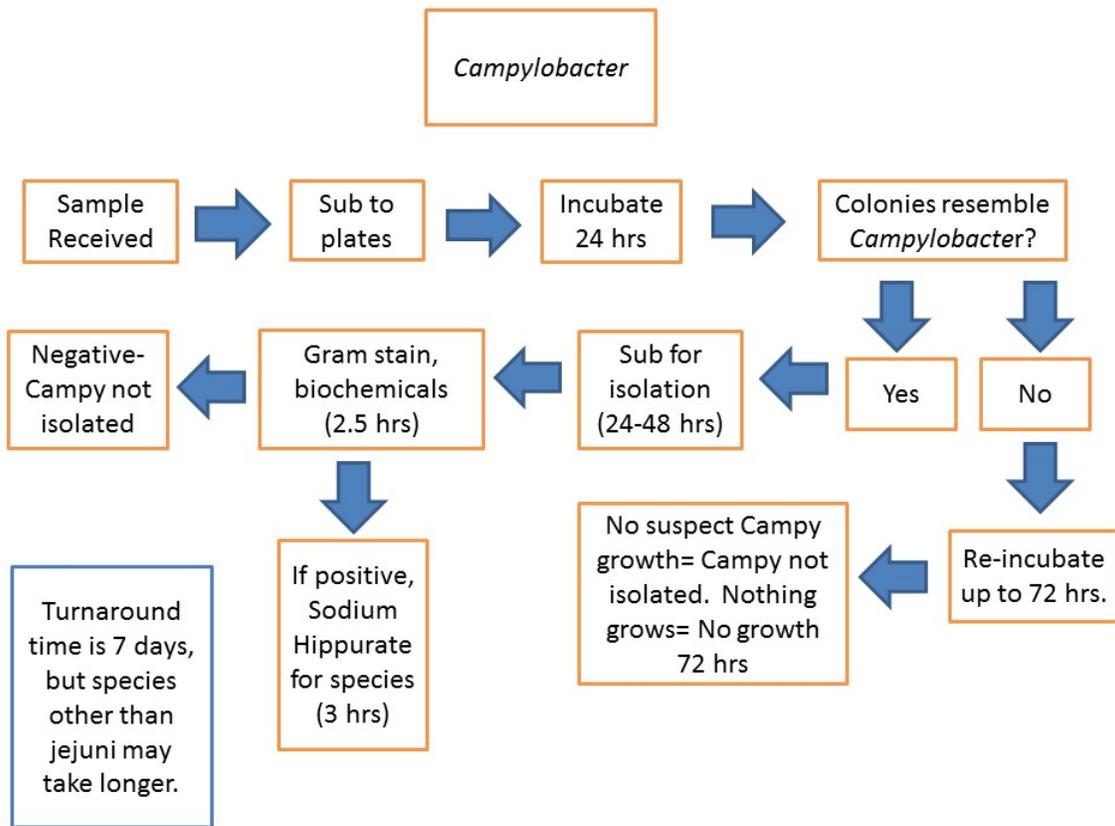
Reference List of Tests

VIBRIO CHOLERAЕ	
DLS Department:	Bacteriology
OUTREACH Test Code:	VIB
Includes:	Vibrio cholera and other vibrio species
Methodology:	Identification and Typing
Availability:	M-F
Turn-around-Time:	48 hours- 7 days
Specimen Requirements:	Clinical - Stool Identification and Typing- Pure culture isolate
Collection Kit/Container:	Stool – Cary Blair Culture Isolate: Submitter packaging
Collection and Packaging Instructions:	Clinical: http://chfs.ky.gov/NR/rdonlyres/73A1085E-AD36-44C5-8EF8-EDED9E062BB9/0/Enterics2017web.pdf
Specimen Handling and Transport:	
Unacceptable Conditions:	Stool 7 days or older
Reference Range:	
CPT Code:	87046
Additional Comments:	Call the laboratory if Vibrio is suspected and before shipping specimen.

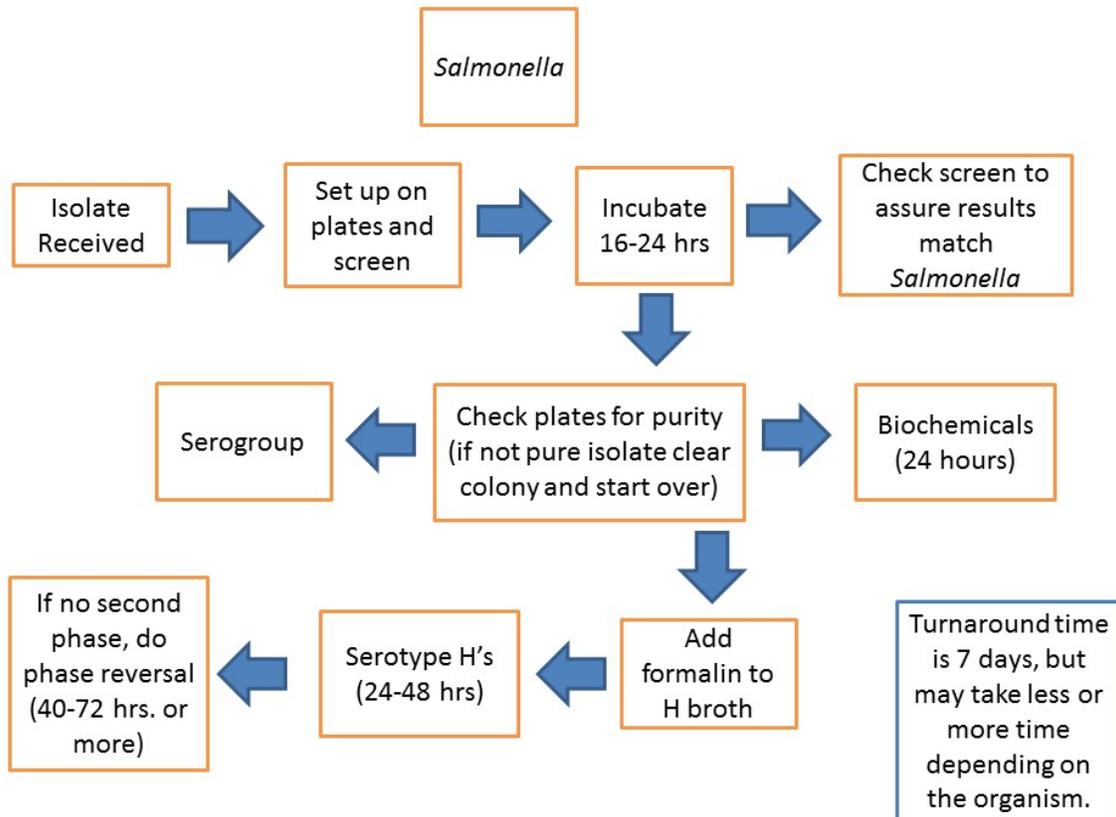
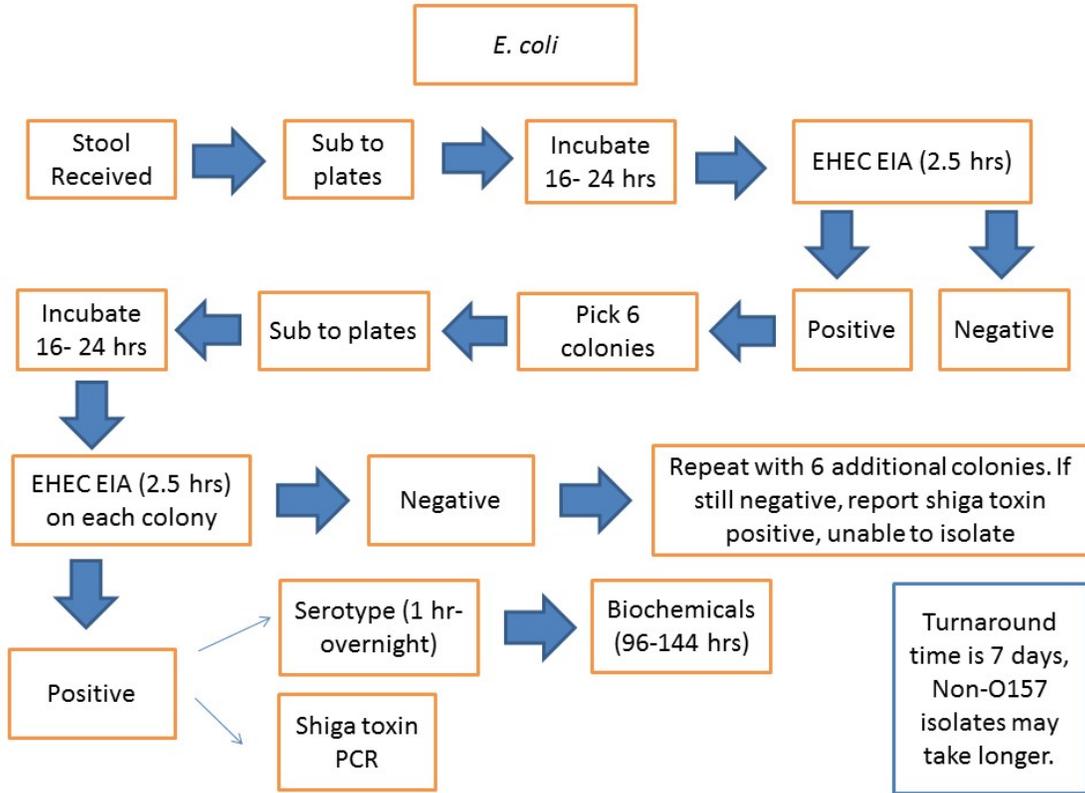
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Bacteriology Flow Charts

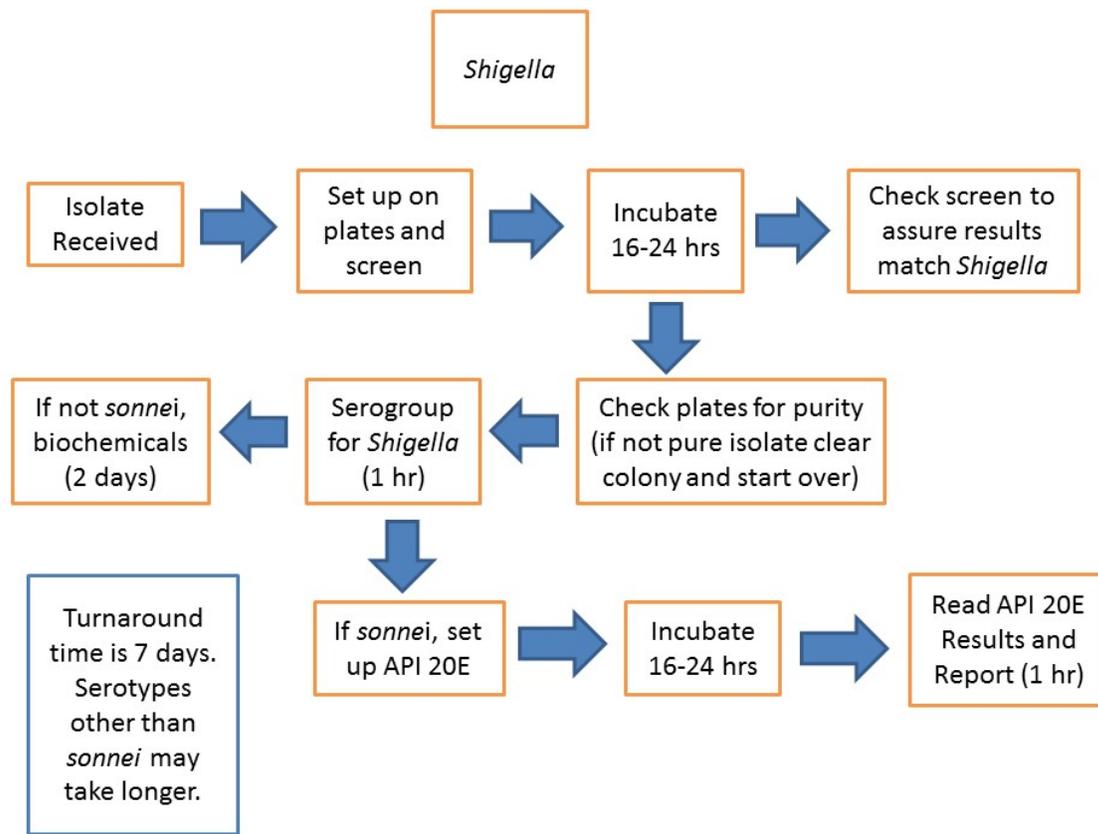
Please note that the following flow charts do not reflect the entire work process for testing of laboratory specimens. Rather, they have been simplified to provide a basic description of specimen flow through the lab and the testing process. These flow charts are intended to provide a general idea of the steps and time required for testing.



COLLECTION AND SUBMISSION OF CLINICAL SAMPLES



APPENDIX D



PFGE Flow Chart

PFGE Flow Chart

