

Patient Information:

Name; _____

Age; _____

Address; _____

CNIC NO: _____

Sex: _____

Referred By; _____

Sample Collection Date: _____

Hospital/Lab ID#: _____

SPECIMEN CULTURES (RAW SPECIMEN)

Bacterial Cultures

☐

Culture test for Cholera

☐

Culture test for Diptheria

☐

Culture test for Salmonella

☐

Culture test for Acute/Bloody Diarrhea

☐

Culture test for Shigellosis

☐

Culture test for Typhoid fever/ XDR Typhoid

☐

Culture test for Pertussis

☐

Culture test for Brucellosis

List of Current Medications: _____



Relevant Lab Tests: _____

Suspected Diagnosis: _____

Special Instructions: _____

Family History

(Required for Interpretation)

	Public Health Reference Laboratory (PHRL)		
	SALMONELLA DISEASE		
Page 2 of 15	Doc# KMU/PHRL/SOP-23	Effective Date: 07-06-2022	Version: 01

SALMONELLA DISEASE:

The gram-negative bacteria *Salmonella* typically cause diarrhea and sometimes cause a more serious infection, typhoid fever.

- People are usually infected when they eat contaminated food, such as undercooked chicken or eggs.
- The bacteria usually infect the digestive tract but can travel through the bloodstream and infect other parts of the body.
- People have nausea and crampy abdominal pain, followed by watery diarrhea, fever, and vomiting.
- Identifying the bacteria in a sample, usually of stool, confirms the diagnosis.
Lost fluids are replaced.
- Antibiotics are usually not helpful for people who have *Salmonella* intestinal infections, but antibiotics are helpful for people who are at risk of or have bacteremia.

Salmonella bacteria cause several types of infection. Most often, these bacteria cause **gastroenteritis**, but they sometimes cause **typhoid fever**, a more serious infection.

There are over 2,500 different types of *Salmonella* bacteria.

Some *Salmonella* reside only in people. Other species of *Salmonella* normally reside in the digestive tract of many wild and domestic animals, such as cattle, sheep, pigs, fowl, and reptiles (including snakes, lizards, and turtles). Many of these can cause infections in people.

Salmonella bacteria are excreted in the feces of infected animals and people, leading to contamination. In the United States during the 1970s, many infections were spread by pet turtles, so their sale was prohibited, resulting in fewer infections. Recently, the legal and illegal sale of pet reptiles has increased. Up to 90% of pet reptiles and amphibians, such as aquatic frogs, are infected with *Salmonella*.

People are infected usually by eating undercooked poultry or eggs but sometimes by eating undercooked beef and pork, unpasteurized dairy products, or contaminated seafood or fresh produce. *Salmonella* bacteria can infect the ovaries of hens and thus infect the egg before the egg is laid. Other foods may be contaminated by animal feces (for example, in slaughterhouses) or by infected food handlers who do not adequately wash their hands after using a toilet. People can also become infected if they drink contaminated water. Other reported sources of infection include infected pet turtles and reptiles and contaminated marijuana.

Because stomach acid tends to destroy *Salmonella*, a large number of these bacteria must be consumed for infection to develop, unless people have a deficiency of stomach acid. Such a deficiency may occur in

- Children under 1 year old
- Older people
- People taking antacids or drugs that inhibit stomach acid production, including histamine-2 (H2) blockers (such as **famotidine**) or proton pump inhibitors (such as **omeprazole**)

Salmonella bacteria cause inflammation of the intestine (**gastroenteritis**) and thus are a common cause of diarrhea.

Symptoms of *Salmonella* Infections

When the intestine is infected, symptoms usually start 12 to 48 hours after the bacteria are ingested. Nausea and crampy abdominal pain occur, soon followed by watery diarrhea, fever, and vomiting. *Salmonella* symptoms resolve in 1 to 4 days. Occasionally, symptoms are more severe and last a long time.

Long after symptoms are gone, a few people continue to excrete the bacteria in their stool. Such people are called carriers.

About 10 to 30% of adults develop **reactive arthritis** weeks to months after diarrhea stops. This disorder causes pain and swelling, usually in the hips, knees, and Achilles tendon (which connects the heel bone and calf muscle).



Other symptoms may develop if bacteremia develops and infection spreads. For example, if a bone is infected, the area over it is often tender or painful. If a heart valve is infected, people may feel short of breath. If the aorta is infected, the back and abdomen may be painful.

People usually recover well. Exceptions are people who had a disorder, particularly one that weakens the immune system, before the *Salmonella* infection or who have a complication due to the infection.

Diagnosis of *Salmonella* Infections

Culture of stool, pus, blood, or a sample from the rectum

To diagnose a *Salmonella* infection, doctors take a sample of stool, pus, or blood or use a swab to obtain a sample from the rectum. The sample is sent to a laboratory where bacteria, if present, can be grown (cultured). Identifying the bacteria in the sample confirms the diagnosis. Bacteria are also tested to see which antibiotics are effective (a process called **susceptibility testing**).

	Public Health Reference Laboratory (PHRL)			 Public Health Reference Laboratory
	Title: STNADARD OPERATING PROCEDURE FOR SALMONELLA SAMPLE COLLECTION			
Page 5 of 15	Doc# PHRL/CSC/L2-056	Eff. Date: 12-05-2022	Rev. Date: 11-05-2024	Rev:00

PROCEDURE FOR SAMPLE COLLECTION

Criteria	PHRL Microbiology Management System
Document Size	04 Pages

Prepared by

Designation

Lab Manager / Lab Incharge

Reviewed & Approved by

Designation

Director

DOCUMENT CONTROL BLOCK (REVISION HISTORY)

[illegible]

1.0 Purpose

To describe the procedure for collection of Salmonella sample.

2.0 Responsibility

Lab Manager & Incharge

3.0 Scope

This SOP is applicable for early detection, confirmation, and appropriately respond to Salmonella epidemics.

Accountability

Lab Manager & Incharge

4.0 Procedure

4.1 Specimen Requirements

Collection of blood for blood culture (adapted from WHO manual):

Infection can be transmitted from patient to staff and from staff to patient during the blood-taking procedure. Viral agents pose the greatest hazard and in some instances are potentially lethal. Of particular importance are the hepatitis viruses and the human immunodeficiency virus (HIV; the virus causing acquired immunodeficiency syndrome [AIDS]). To decrease the risk of transmission of these viral agents, the following recommendations should be practiced:

- a) Wear latex or vinyl gloves impermeable to liquids.
- b) Change gloves between patients.
- c) Inoculate blood into blood-culture media immediately to prevent the blood from clotting in the syringe. Syringes and needles should be disposed of in a puncture-resistant, autoclavable container. No attempt should be made to recap the needle. A new syringe and needle must be used for each patient.
- d) Wipe the surface of the blood-culture bottle and the gloves with a disinfectant.
- e) Label the bottle.
- f) For the transport to the microbiology laboratory, place the blood-culture medium in a container that can be securely sealed.



- g) Specimen containers should be individually and conspicuously labeled. Any containers with blood on the outside should be wiped thoroughly. Such containers should be transported in individual, sealed plastic envelopes.
- h) Remove gloves and discard in an autoclavable container.
- i) Wash hands with soap and water immediately after removing gloves.
- j) Transport the specimen to the microbiology laboratory or, if that facility is closed, store the specimen in an approved location.
- k) In the event of a needle-stick injury or other skin puncture or wound, wash the wound thoroughly with soap and water, encouraging bleeding. Report any contamination of the hands or body with blood, or any puncture wound, or any cut to the supervisor and the health service for treatment, as appropriate.

Venipuncture

- a) Gather everything needed to complete the blood collection process: gloves, syringe, needle, tourniquet, gauze squares, cotton balls, adhesive bandage, puncture resistant container, culture medium and antiseptic; iodine tincture (100 ml of 70% isopropyl alcohol to 1 g of iodine) or povidone iodine is preferred, but 70% alcohol is an acceptable alternative. The size of the needle will depend on the collection site and the size of the vein. A 23-gauge needle that is 20 – 25 mm in length or a butterfly needle is generally used for children. Collecting a large amount of blood from a child can be difficult: 1 – 3 ml is usually sufficient, but volume of blood is directly related to culture yield. Blood cultures from young children should be diluted to 1 – 2 ml of blood in 20 ml of broth (1:10 to 1:20). Blood cultures from adults should be diluted to 5 – 10 ml of blood in 50 ml of broth (1:5 to 1:10).
- b) Select an arm and apply a tourniquet to restrict the flow of venous blood. The most prominent vein is usually chosen for venipuncture.
- c) Vigorously wipe the skin with the 70% alcohol, and swab with the iodine tincture or povidone-iodine. Rub over the selected area. Allow to dry. If the vein is palpated again, repeat the skin disinfection.
- d) After the disinfectant has dried, insert the needle into the vein with the bevel of the needle face-up. Once the vein is entered, withdraw the blood by pulling back the barrel of the syringe in a slow, steady manner. Air must not be pumped into a vein. After the

desired amount of blood is obtained, release the tourniquet and place a sterile cotton ball over the insertion site while holding the needle in place. Withdraw the needle and have the patient hold the cotton ball firmly in place until the wound has stopped bleeding. Inoculate the culture medium. Put the adhesive bandage on the wound.

e) Use vacutainer tubes for blood collection, if they are available. Specimens should be put into a bloodculture bottle immediately and placed in an incubator as soon as possible; if incubation is not feasible, the blood culture bottle can be kept at room temperature (20° – 25°C) for up to 8 hours. Ideally, the blood samples should be processed in a bacteriology laboratory as soon as possible after collection (i.e., within 2 hours).

	Public Health Reference Laboratory (PHRL)			 Public Health Reference Laboratory
	Title: STNADARD OPERATING PROCEDURE FOR SALMONELLA BLOOD CULTURING			
Page 10 of 15	Doc# PHRL/SBC/L2-060	Eff. Date: 12-05-2022	Rev. Date: 11-05-2024	Rev:00

PROCEDURE FOR SALMONELLA BLOOD CULTURING

Criteria	PHRL Microbiology Management System
Document Size	07 Pages

Prepared by

Designation

Lab Manager / Lab Incharge

Reviewed & Approved by

Designation

Director

DOCUMENT CONTROL BLOCK (REVISION HISTORY)

[illegible]

2.0 Purpose/ Background

1.1 The blood of healthy individuals is usually sterile. Blood cultures are a standard laboratory tool for detecting invasive bacterial/fungal disease. The BACTEC is an automated blood culture system which incubates and continuously monitors blood cultures. A positive result is signaled immediately upon detection of carbon dioxide production in the blood culture bottle.

1.2 The quality of blood collection greatly affects the sensitivity and reliability of any blood culture system. Critical factors are: Timing of blood collection. Before administration of antibiotics. Volume of blood collected. Sensitivity increases with volume; bacteremias can be missed by relying upon small blood volumes, which is a particular issue in children. Skin disinfection. Contamination of blood cultures with bacteria that commonly reside on the skin can cause false positive results for bacteremia, may prevent identification of a true pathogen, and complicate the interpretation of our laboratory results. Thorough disinfection of the venipuncture site can significantly reduce the number of contaminated blood cultures.

1.3 The quality of blood collection greatly affects the sensitivity and reliability of any blood culture system. Critical factors are:

Timing of blood collection. Before administration of antibiotics.

Volume of blood collected. Sensitivity increases with volume; bacteremias can be missed by relying upon small blood volumes, which is a particular issue in children.

Skin disinfection. Contamination of blood cultures with bacteria that commonly reside on the skin can cause false positive results for bacteremia, may prevent identification of a true pathogen, and complicate the interpretation of our laboratory results. Thorough disinfection of the venipuncture site can significantly reduce the number of contaminated blood cultures.

1.4 The purpose of this SOP is to give guidance on isolation of organisms found in blood specimens.

3.0 Scope

This SOP is applicable to all trained laboratory technicians/technologists/scientists working in the microbiology laboratory.

4.0 Specimen

Blood received in the laboratory in BACTEC Paeds PLUS blood culture bottles

5.0 Prerequisites / Supplies Needed

4.1. Equipment

4.1.1 CO2 Incubators or Candle jar

4.1.2 Aerobic incubator

4.1.3 BD BACTEC machine

4.2. Media

4.2.1 5% Sheep Blood agar

4.2.2 Chocolate agar

4.2.3 MacConkey agar

4.3. Materials

4.3.1 Alcohol

4.3.2 Antimicrobial susceptibility discs.

4.3.3 Biochemical reagents

4.3.4 Paediatric blood culture bottles BACTEC Paeds Plus

4.3.5 Cotton wool/gauze

4.3.6 Gram stain reagents

4.3.7 Syringes and needles

4.3.8 Sterile gloves

4.3.9 Tourniquet

4.3.10 Microscope Slides

4.3.11 Sharps container

5.0. Safety/Risk Assessment

5.1 Wear Personal Protective Equipment at ALL times when processing blood culture samples. Process all blood cultures in a biosafety cabinet.

5.2 Waste disposal: Discard all sharps in sharp boxes. Autoclave all culture plates and other clinical wastes before taking them for incineration. Reusable material must be autoclaved before washing.

6.0 Procedural Steps

6.1. Pre-processing specimen handling

Blood culture bottles should be placed in the BACTEC blood culture instrument as soon as possible after arrival in the laboratory. Store at air-conditioned room temperature if unable to process immediately. [This will need to be modified based on specific manufacturers' instructions, as some systems allow incubation in a standard incubator before placement on automated instrument].

6.2 Initial processing of blood culture bottles

Each blood culture bottle should be weighed and the value subtracted from the uninoculated weight of the bottle. The result is the weight of the inoculated blood and is recorded on the CRF.

6.3 . Protocol for positive blood cultures (BD Bactec 9050)

Day 1

6.3.1 Unload positive blood culture bottle(s) according to BD Bactec 9050 instrument operation protocol.

6.3.2 In the Biohazard cabinet, sterilise the rubber top of the bottle with 70% alcohol. Using a BD vent needle inoculate the following:

- 5% Blood Agar (aerobic)
- Chocolate Agar
- MacConkey Agar
- Slide for Gram stain
- Spread plates and incubate aerobic plates at 35-37°C with 5% CO₂

6.3.3 Leave Gram to air dry then stain and examine.

6.3.4 Once the Gram stain reaction and morphology is known, notify the clinician.

Day 2

6.3.5 Examine plates and identify isolates according to standard microbiological methods

6.3.6 Perform antibiotic susceptibility testing

6.3.7 Reincubate plates for a further 24 hours.

Day 3

6.3.8 Re-examine plates. Identify and perform antibiotic susceptibility testing on any further isolates that may have grown.

6.3.9 Report confirmed sensitivities and final identification if available.

Notes:

- All positive blood cultures are initially regarded as significant.
- All clinically significant isolates should be frozen at -80°C.