

COAST PROVINCIAL GENERAL HOSPITAL ANTIRETROVIRAL THERAPY (ART) PROGRAMME

Standard Operating Procedures for Laboratory Services

March 1, 2005

In collaboration with

Laboratory Department
Coast Provincial General Hospital
Ministry of Health, Kenya

Prepared with assistance from



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This document was made possible through support provided by the U.S. Agency for International Development, under the terms of Cooperative Agreement Number HRN-A-00-00-00016-00. The opinions expressed herein are those of the author(s) and do not necessarily reflect the views of the U.S. Agency for International Development.

About RPM Plus

The Rational Pharmaceutical Management Plus (RPM Plus) Program, funded by the U.S. Agency for International Development, works in more than 20 developing countries to provide technical assistance to strengthen drug and health commodity management systems. The program offers technical guidance and assists in strategy development and program implementation in improving the availability of health commodities—pharmaceuticals, vaccines, supplies, and basic medical equipment—of assured quality for maternal and child health, HIV/AIDS, infectious diseases, and family planning, as well as in promoting the appropriate use of health commodities in the public and private sectors.

Recommended Citation

Rational Pharmaceutical Management Plus Program, in collaboration with the Laboratory Department, Coast Provincial General Hospital, and Ministry of Health, Kenya. 2004. *Standard Operating Procedures for Laboratory Services*. Prepared for Antiretroviral Therapy (ART) Programme, Coast Provincial General Hospital. Submitted to the U.S. Agency for International Development. Arlington, VA, USA: Management Sciences for Health.

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ACKNOWLEDGEMENTS

The dedication and hard work of the following people made the preparation of these Standard Operating Procedures possible:

- Dr Kishor Mandaliyia, Pathologist, CPGH
- Mr Douglas Denje, Laboratory Supervisor, CPGH
- Dr Ephantus Njagi, RPM Plus Consultant
- Javan Musera, Laboratory Technologist
- Douglas Kalu, Laboratory Technologist
- Arnold Jana, Laboratory Technologist
- Margastone Kitunga, Laboratory Technologist
- Pantaleo Mwamburi, Laboratory Technologist
- Sandra Fernandes, Laboratory Technologist
- Esther Mpenzwe, Laboratory Technician
- Edward Bejah, Laboratory Technician
- Dickson Kenga, Laboratory Technician

ACRONYMS

ACD	acid citrate dextrose (anticoagulant)
AIDS	acquired immunodeficiency syndrome
ALT/SGPT	alanine aminotransferase
AML/CML	acute myeloid leukaemia/chronic myeloid leukaemia
ART	antiretroviral therapy
AST/SGOT	aspartate aminotransferase
BUN	blood urea nitrogen
CNP	2-chloro-4-nitrophenol
CNPG3	2-chloro-4-nitrophenyl-maltotrioxide
CPGH	Coast Provincial General Hospital
CV	coefficient of variation
DMSO	dimethyl sulfoxide
EDTA	ethylene diamine tetra-acetic acid
FCS	forward scattered [light]
GT	glutamyl transferase
HbsAG	hepatitis B surface antigen
HBV	hepatitis B virus
HCT	hematocrit
HCV	hepatitis C virus
HDL	high density lipoprotein
HGB	hemoglobin test
HIV	human immunodeficiency virus
IP	inpatient
ISE	ion-selective electrode
KBS	Kenya Bureau of Standards
LDH	lactate dehydrogenase
LDL	low density lipoprotein
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDH	malate dehydrogenase
MSH	Management Sciences for Health
MSO	methyl sulfoxide
MWP	micro well plate
NASBA	nucleic acid sequence–based amplification
NHP	negative human plasma
NRBC	nucleated red blood cell
OD	optical density
OP	outpatient
OPD	outpatient department

PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEP	postexposure prophylaxis
PPE	personal protective equipment
QC	quality control
QS	quantitation standard
RBC	red blood cell
RPM Plus	Rational Pharmaceutical Management Plus Program
RT	room temperature
SD	standard deviation
SOP	Standard Operating Procedure
SSC	side scattered [light]
U/L	units per litre
VLDL	very low density lipoprotein
WBC	white blood cell

**Coast Provincial General Hospital
Antiretroviral Programme**

**General
Standard Operating Procedures**

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Processing Haematology and Clinical Chemistry Specimens	
Lab SOP No.: 101	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

This SOP defines the handling of a specimen from the time it is received until the time a report is released from both the Haematology and Chemistry Sections. The specimen may be tracked for efficiency in reporting results in a timely fashion, as well as for identifying those individuals responsible for processing the specimen and issuing a final report.

II. SPECIMEN

The specimen received must correspond to the test requested. If it does not, take appropriate action. (See SOP 102: Criteria for Rejecting Laboratory Specimens.)

III. QUALITY CONTROL

Depending upon the assay, quality control (QC) is performed either daily or with each test/batch of an assay requested. Determine which QC is to be performed with the requested assay.

IV. PROCEDURE

- A. Ensure the laboratory request form accompanying any sample received in the laboratory has been properly “clocked in.”
- B. Ensure all laboratory requests contain the following minimum information: patient’s name, age, and gender; attending physician; ward or clinic; tests requested; date and time specimen was collected; and pertinent clinical information.
- C. Determine if the specimen should be rejected. See SOP 102: Criteria for Rejecting Laboratory Specimens.
- D. Enter the sample(s) on the register, indicating patient’s name, IP/OP number, sex, clinic, diagnosis, and all tests requested for that sample.
- E. Note the specimen condition on the requisition slip according to the following:
 - 1. Haemolysed—Red-tinged
 - 2. Lipemic—Lactescent or “milky”

3. Icteric—Yellow-green or “jaundiced”
 4. Clotted samples
- F. Perform analysis of assays on the appropriate analyser. Run QC accordingly.
 - G. All reports generated will be reviewed and initialled by the technologist performing the analysis. Date stamp when report is completed prior to release of results.
 - H. Repeat or dilute any assay deemed necessary according to the review guidelines of the procedure. Always repeat or dilute a specimen from the original tube (not the sample cup) if quantity is sufficient.
 - I. Ensure all repeats are included with laboratory report.
 - J. Notify the medical officer in-charge of CCC of any result that is a panic value. On the report indicate the name of the individual accepting the results, time, and initials of the technician who made the notification.
 - K. All final reports are reviewed at the end of each day by the section supervisor for clerical errors, assays requested vs. assays performed, and reliability of reported results.
- V. PROCEDURAL NOTES
- A. In order to avoid technical and clerical errors, the same technician should run the specimen and enter the patient identification numbers into the analyser. The report should include the condition of the sample and any other pertinent information found on the original request form.
 - B. Reference values will be indicated on all reports released.
 - C. When reviewing laboratory results, look for:
 1. Acceptable quality control
 2. Panic values
 3. Results that are inconsistent with other chemistries or haematology requested

VI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Criteria for Rejecting Laboratory Specimens	
Lab SOP No.: 102	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

The guidelines established in this SOP ensure the integrity of the specimen and the reliability of the results generated for the physician.

II. PROCEDURE

A. Specimens may be rejected in the following situations:

1. Mismatched specimens and/or lab forms from clinics, wards, and lab processing: Processing personnel should contact appropriate location and give them an opportunity to correct the problem within 2 hours for specimen to be processed. A note indicating that correction has been done for processing of the specimen has to be made in case of any discrepancy. If the processor is unable to contact the submitter, the forms and/or specimens can be sent back with a specimen rejection form stating the problem. Ultimately, repeat offenders must be monitored closely with copies of the mismatched forms or specimens provided to the pathologist for follow-up.
2. Unlabelled specimens: The ward/clinic will be notified and requested to identify the sample or to submit another specimen. Otherwise, if no confirmation is forthcoming, the unlabelled specimen(s) will be discarded.
3. Incomplete label: All specimens must have the patient's full details. Contact the collector to correct any problems.
4. Requests accompanied by lipemic, icteric, or haemolysed specimens will have the condition noted on the lab result. In addition, when the results are reported by telephone, the condition of the submitted specimen (i.e., lipemic or haemolysed) will be conveyed to the individual receiving the results.
TELEPHONE REPORTING WILL FOLLOW A SPECIFIED PROTOCOL AS SET OUT BY THE LABORATORY AND CCC.
5. Contaminated specimen or request form: The ward or clinic will be called and provided with the opportunity to submit a new specimen/form.
6. Improper specimen container for requested assay: Technicians will not perform a test if the specimen is not in the acceptable container. See individual SOPs for proper specimen containers.

- a. Samples for urea and electrolyte assays that are NOT collected in serum tubes (red tops) or lithium heparin.
 - b. Samples for complete blood count that are NOT collected on heparin-containing purple tops.
 - c. Samples for CD4 T-cell counts that are NOT collected on K3 EDTA-containing purple tops.
 - d. Samples for liver function tests that are NOT collected on red tops.
 - e. Samples for viral loads by RT-PCR that are NOT collected on ACD/EDTA-containing purple tops.
 - f. Samples for PBMC that are NOT collected on yellow tops.
7. Insufficient quantity of specimen submitted for the testing requested. Contact the physician, ward, or clinic and have the patient's blood redrawn or have the physician prioritize tests requested for analysis; however, this should be highly discouraged.
- a. 2 ml for complete blood count
 - b. 3 ml for CD4 cell count
 - c. 5 ml for liver function tests
 - d. 4 ml for urea and electrolytes
 - e. 4 ml for viral load
 - f. 30 ml for PBMCs separation
- B. In the above situation, notify the ward or clinic of the problem, exhibiting a professional, courteous attitude. Whenever possible, cooperate with the physician to determine alternate testing in the case of "hard to draw" patients, especially infants and burn patients.

III. PROCEDURAL NOTES

The following is a summary of corrective actions for improperly collected or handled specimens.

- A. Take appropriate action to correct the problem. If necessary, have the specimen re-collected as soon as possible.
- B. Document all actions on the request forms or specimen rejection form, indicating the reason for rejection. Include name of the person contacted, date and time of notification, action taken, and initials of person making the notification.
- C. Notify the physician if a delay in performing the analysis will occur; this allows him or her to make appropriate decisions.
- D. The supervisor will review all rejected request forms/specimen for each day.

IV. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Correcting Erroneous Reports	
Lab SOP No.: 103	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

It is essential that false information or misinformation presented to a physician is corrected in a manner that does not compromise patient care. Clerical discrepancies, tests reported vs. tests requested, and reporting erroneous test results are areas that necessitate a corrected report.

II. SCOPE

Immediately following the discovery of an erroneous assay result, notify the physician of the error. If unable to contact the physician, inform the lab supervisor/pathologist and the head of ward or head nurse.

III. PROCEDURE

- A. Upon discovering an error, make a new request form with the correct results and annotate "Corrected Report" on the request. Initial the report and indicate the date and time.
- B. For reports, indicate in the comment section: "Corrected Report for Spec # X, drawn at date and time."
- C. If the physician was contacted, annotate on the report whom you spoke with, along with the date and time.
- D. Send the corrected report to the ward or clinic.
- E. Cross out the error from the initial report by drawing a single line indicating error. Attach the laboratory copy of the corrected report to the initial laboratory slip.
- F. ***DO NOT DESTROY THE INITIAL LABORATORY SLIP THAT CONTAINS THE ERROR. IT IS A LEGAL DOCUMENT.***

IV. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Laboratory Critical/Panic Values	
Lab SOP No.: 104	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

The purpose of this SOP is to identify clinical laboratory quantitative values above or below an established reference range, to which a clinician would respond by taking some further action to assess the patient's condition.

II. PRINCIPLE

Quantitative clinical laboratory values provided by the laboratories.

III. PROCEDURE

A. Verify the result to ensure it is a true panic value.

NOTE: All values falling within the critical range must be repeated. Whenever possible, critical values should be reviewed by a supervisor before notifying the physician.

B. Immediately notify the requesting ward, clinic, or physician of the critical value. If unable to make contact, you may need to send a runner. Document each call or course of action.

1. If there is no requester annotated on the lab slip, contact the casualty unit to determine if the patient is an inpatient and on which ward he/she is located.
2. If necessary, send slip to the ward by runner and enter the information into the log (if applicable). Retain verification on file copy slip.

C. If the requester cannot be identified or there is no answer on the ward, notify the pathologist, lab head, or supervisor of your section.

NOTE: Legibly annotate on the lab slip all steps taken; include date and time.

IV. PANIC/CRITICAL VALUES:

	<u>Less Than:</u>	<u>Greater Than:</u>
SGPT	(40% difference of prior value)	
Amylase	(25% difference of prior value)	150 U/L
SGOT	(40% difference of prior value)	
Chloride	85 mmol/L	115 mmol/L
CK	(40% difference of prior value)	1,000 U/L
Creatinine	26 µmol/L	663 µmol/L
Glucose (adult, fasting)	2 mmol/L	2 mmol/L
Potassium	2.5 mmol/L	6.0 mmol/L
Sodium	120 mmol/L	160 mmol/L
Total Bilirubin (adult)	(50% difference of prior value)	
Total Protein	40.0 g/L	90.0 g/L
Urea Nitrogen (BUN)	1.0 mmol/L	54 mmol/L

V. REFERENCE RANGES—ROUTINE CHEMISTRIES:

Normal Range Units

Glucose: fasting	3–6 mmol/L
Urea Nitrogen (BUN)	2.9–8.2 mmol/L
Creatinine	53–106 mmol/L
SGPT	7.0–56 U/L
Amylase	30–110 U/L
SGOT	5.0–35 U/L
Chloride	98–107 mmol/L
CK	30–170 U/L
Conjugated Bilirubin (direct)	0.0– < 5 µmol/L
Potassium	3.6–5.0 mmol/L
Sodium	137–145 mmol/L
Total Bilirubin	2–21 µmol/L
Total Protein	63–82 g/L
Unconjugated Bilirubin (indirect)	0.0–14 µmol/L

VI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Sample Collection, Labelling, Handling and Shipment	
Lab SOP No.: 105	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

This standard operating procedure defines the specimens to be collected, labelling to be done, and handling and transporting of specimens.

II. SPECIMEN COLLECTION

- A. 2 ml of venous blood will be collected for complete blood count using heparin-containing purple tops, per predetermined patient schedule. Blood will be well mixed and placed on a roller to prevent clotting and will be processed within 24 hours. Where necessary, differential count will be done using the same sample.
- B. 3 ml of venous blood will be collected for CD4 T cell enumeration using K3 EDTA-containing purple tops. All specimens will be gently mixed to prevent clot formation and placed on a roller before processing. Blood samples should not be obtained from patients with acute infections such as tuberculosis, herpes virus infections, bacterial sepsis, and respiratory infections because these may affect the level of the CD4 cell count. Such patients will be allowed to recover from such conditions before CD4 cell enumeration.
- C. 5 ml of venous blood will be collected for liver function tests using red tops. All necessary clinical chemistry parameters will be performed using this sample.
- D. 4 ml of venous blood will be collected for urea and electrolyte tests using red tops. All necessary renal function parameters will be performed using this sample.
- E. 4 ml of venous blood will be collected from predetermined patients for viral load. This specimen will be centrifuged to separate plasma. Four aliquots of plasma will be stored at -80°C and transported to a predetermined laboratory at an appropriate time to maintain the agreed cold chain for viral load determination. Such samples will not be obtained from those patients who have just been immunized with influenza vaccine, pneumococcal vaccine, or hepatitis B vaccine or who are suffering from tuberculosis, herpes virus infection, or other infections. Such patients will be given 2–4 weeks before viral load samples are obtained from them.
- F. 30 ml of venous blood will be collected from some predetermined patients for viral resistance studies using green tops. PBMCs will be collected from such samples and stored at -80°C for future use. PBMCs separation will be done according to protocol.
- G. Other samples will be collected as indicated by the protocol.

III. SPECIMEN LABELLING

- A. Each container should be labelled with name, type of specimen, and date of collection. Included should be a brief description of the illness; place, date, and time of collection; requesting clinician; and clinic or ward. Samples for viral load and resistance analysis should be kept as cold as possible, preferably frozen.
- B. Routine laboratory request forms should accompany each sample. Data on laboratory request forms should include number of days since onset of symptoms and the reason that samples were obtained as well as name, age, and sex of patient, and date and time of specimen removal.

IV. SPECIMEN HANDLING AND SHIPMENT

- A. All specimens should be submitted through the main reception area for processing. Samples must be in a clearly marked CCC container.
- B. It is the responsibility of the lab coordinator in concert with the CCC physician to ensure that specimens are submitted correctly and expeditiously to an appropriate diagnostic laboratory.

V. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Blood Collection by Venipuncture in Adults and Children	
Lab SOP No.: 106	Version No.: 1.0
Date prepared:	Date adopted:

I. PURPOSE

To obtain peripheral blood samples from adults and children needed for laboratory testing, with proper specimen identification and handling, while ensuring patient and staff safety.

II. BACKGROUND

- A. Laboratory investigations are a critical part of patient management . Strict adherence to SOP requirements ensures quality laboratory results. Obtaining sufficient volume of blood in the proper collection tubes is the responsibility of the phlebotomist.
- B. It is also the duty of the phlebotomist to insure that the specimens collected are identified properly and labelled in a legible manner for the laboratory. Mislabeled specimens can jeopardize the outcome of the laboratory results, causing incorrect patient management or the discarding and thus loss of specimens with ambiguities that cannot be resolved.
- C. It is the duty of the laboratory receiving the specimens to notify the study staff if inadequate specimens are received, so that corrective action may be taken if possible. Such specimens include those in poorly labelled tubes; of insufficient specimen volume; or inadequate specimens due to clotting, spilling, and the like.

III. MATERIALS AND EQUIPMENT REQUIRED

- A. 10% household bleach (Jik) or 4% chlorhexidine solution
- B. Disposable latex gloves
- C. Alcohol swabs, isopropyl alcohol
- D. Tourniquet (latex rubber)
- E. Vacutainer holders
- F. Appropriate size sterile disposable needles or butterfly needle set
- G. Vacutainer specimen tubes
- H. Cotton balls
- I. Sharps disposal container

IV. PROCEDURES

A. Adult Phlebotomy

1. All required materials for blood drawing should be assembled before the procedure.
2. Sterile, single-use needles (or butterflies) and Vacutainer tubes are to be used for each blood draw, and after completion needles are to be properly disposed of in a puncture resistant container. They are never to be cleaned and reused for any purpose.
3. Check the study specific Lab Requisition Form to confirm the quantity of blood to be drawn and which kind of Vacutainer tubes to use. Make sure you have selected the right additive if one is necessary.
4. Vacutainer tubes or other specimen containers should be labelled by patient identification number.
5. Be sure to verify the **identity of the client** and **clinic number** before labelling tubes.
6. Do not prepare tubes for more than one subject at a time.
7. The phlebotomist should wear latex gloves and use aseptic technique during phlebotomy. Gloves should always be worn when handling or transporting specimens if there is any possibility of direct contact with blood or other body secretion.
8. Explain the blood drawing procedure to the client and reassure him/her.
9. Seat ambulatory patients in a comfortable chair with the extremity from which blood will be drawn supported on a sturdy table or other support. The preferred sites for phlebotomy are the median antecubital and basilic veins of the upper extremity. Veins on the dorsum on the hand and other forearm veins are possible alternative sites. A tourniquet may be used to transiently distend veins prior to blood drawing. Do not leave the tourniquet on the arm for longer than necessary as this is uncomfortable for the client and may alter the results of certain laboratory tests, such as serum potassium measurements and some enzyme measurements.
10. Using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture.
11. Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.

12. Using aseptic technique, insert the needle (or butterfly) of the Vacutainer device into the vein. It is often helpful to make counter-traction distally over the vein to stabilize it for needle puncture. If possible, always allow the full amount of blood to be drawn by each evacuated tube when using the Vacutainer system and tubes. After drawing, mix the blood in tubes containing additives by inverting the tubes several times.
13. After drawing the required blood samples, release the tourniquet (if used). Remove the needle from the vein, cover the puncture site with a cotton swab, and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.
14. Subjects occasionally feel dizzy or faint during phlebotomy. If the subject complains of dizziness or feels faint, stop the phlebotomy procedure, secure the vessel puncture site and help the subject lie down or sit with his/her head between the knees. Do not allow subjects to stand until they have fully recovered. Summon medical assistance from other clinic staff if necessary.

B. Paediatric Phlebotomy

1. Preparation

- a. Drawing blood from infants and small children may be physically traumatic for the infant and emotionally traumatic for the mothers. Therefore, it should be done by persons experienced in paediatric blood drawing to minimize the discomfort of both the mother and the child.
- b. As obtaining blood from newborns and young infants is sometimes difficult, if sufficient blood is not obtained on the first attempt, a second attempt may be made. If the second attempt also fails and an additional trained person is available, a second person may attempt to obtain the specimen. No more than three attempts should be made for any child at a time. If blood is not obtained after three attempts, the child may be scheduled to return on a later date for additional attempts by the most experienced staff member.
- c. All supplies required for the blood draw should be assembled in advance so that blood obtained can immediately be placed in the proper tubes. This preparation is especially important for specimens that must be anti-coagulated. Delay in filling the tubes containing the anticoagulant may result in an inadequate clotted specimen, which would require an additional blood draw.
- d. Sterile, single use needles and tubes are to be used for each blood draw and after completion should be properly disposed of in a puncture resistant container. They are never to be cleaned and reused for any purpose.
- e. Tubes should be either pre-labelled with the patient's study identification number or labelled immediately after the specimen is obtained. A pre-printed label should be used so as to avoid transcription errors in specimen

identification. The number should be double-checked against the patient file to be sure the specimen is labelled properly prior to the release of the patient. Only one patient and the tubes for one patient should be present at any time so as not to mix up patient specimens.

2. Procedures

- a. The phlebotomist should wear latex gloves and use aseptic technique during phlebotomy. Gloves should be worn when handling or transporting specimens if there is any possibility of direct contact with blood or other body secretion.
- b. The key to successful blood drawing on children is in the positioning and holding of the child. One should not attempt to draw blood alone, without the aid of a second person to restrain the child. A trained staff member is the ideal person to assist with the blood drawing. If no staff members are available to assist in the holding, then the mother may be instructed in how to hold the child in the manner that makes the drawing the easiest for the blood drawer. If the mother does not want to be present for the blood drawing, her wishes should be respected and the blood should be obtained by two staff members.
- c. Study blood should be obtained from peripheral veins such as antecubital veins, veins on the dorsum of the hand, and veins on the dorsum of the foot. Femoral, jugular, or arterial punctures can be used where necessary if specimens are required for the health and medical care of the child. The risks associated with these procedures do not justify their use for other purposes.
- d. A tourniquet may be used to transiently distend veins prior to blood drawing. Do not leave the tourniquet on the child's arm for longer than necessary as this is uncomfortable for the infant and may alter the results of certain laboratory tests, such as serum potassium measurements and some enzyme measurements.
- e. Using the tip of the index finger, examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture.
- f. Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
- g. Using aseptic technique, insert the needle (or butterfly) of the Vacutainer device into the vein. It is often helpful to make counter-traction distally over the vein to stabilize it for needle puncture.
- h. If possible, always allow the full amount of blood to be drawn by each evacuated tube when using the Vacutainer system and tubes. After drawing,

mix the blood in tubes containing additives by inverting the tubes several times.

- i. After drawing the required blood samples, release the tourniquet (if used). Remove the needle from the vein, cover the puncture site with a cotton swab, and hold (or have the mother hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.
- j. As obtaining blood from newborns and young infants is sometimes difficult, using a butterfly needle combined with a syringe will sometimes work better than Vacutainer tubes. A 23 or 25 gauge butterfly needle should be used with 2 ml or 5 ml syringe.
- k. As soon as sufficient blood is obtained, or it is determined that the blood is no longer flowing, the tourniquet should be released, the needle should be removed, and the blood in the syringe placed immediately in tubes, followed by repeated tube inversion to prevent clotting (if necessary). Cover the puncture site with a cotton swab, and hold (or have the mother hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.

C. Finger Prick and Heel Stick

In cases when only a small quantity of blood is necessary or when venous access is limited, such as in newborns and infants, the most efficient way to obtain a blood specimen is to collect capillary blood from a finger prick or heel stick. Depending on which analysis one wishes to perform, blood can be collected in Microtainer tubes, in capillary tubes, or on filter-paper. For collection and handling of the different kinds of specimens refer to the appropriate SOPs.

1. Required Materials and Equipment

- a. Vinyl gloves
- b. Sharps disposal containers
- c. Alcohol swabs (70%)
- d. Sterile blood lancets
- e. Cotton wool
- f. 10% household bleach (Jik) or 4% chlorhexidine solution

For sample collection:

- a. Capillary tubes or disposable Pasteur pipettes
- b. Specimen collection filter papers or Microtainer tubes
- c. Funnels

2. Finger Prick Procedure

- a. The technician should wear vinyl gloves and use aseptic technique. (If latex gloves are used, they should be washed before wearing because powder coating on them can cause interference with some assays.)

- b. Establish a rapport with the client, for example by explaining carefully what you will be doing, if it's going to hurt, etc.
- c. Position the client: For this procedure, the client should sit in a chair and hyper-extend his/her arm.
- d. The best locations for a finger prick are the third and fourth fingers of the hand not used by the client for writing. Do not use the tip or the centre of the finger. Avoid the side of the finger where there is less soft tissue, where nerves are located, and where the bone is closer to the surface.
- e. Avoid puncturing a finger that is cold or blue, swollen, scarred, or covered with a rash.
- f. Warming the finger by wrapping a warm cloth around it will provide an optimal blood flow and more accurate samples.
- g. Clean the finger prick site with an alcohol swab. Allow the alcohol to air-dry completely before making the prick.
- h. Use a sterile lancet. Full skin penetration by the tip of the lancet should be accomplished in order to obtain adequate blood flow for collection. Shallow, inadequate penetration will require an additional puncture. If blood does not flow adequately from the initial puncture, a second puncture in a cross (X) pattern can increase blood flow significantly.
- i. Make the skin puncture just off the centre of the finger pad.
- j. With dry and clean cotton wool, wipe off the first drop of blood as this tends to contain excess tissue fluid.
- k. Gently massage the finger to allow a drop of blood to form at the punctured site. Collect sufficient quantities of blood for the technique in question, using the recommended equipment.
- l. Have the patient hold a small ball of dry cotton wool over the puncture site for a few minutes to stop the bleeding.

3. Heel Stick Procedure

Heel stick is the preferred method for obtaining capillary blood in an infant.

- a. Establish a rapport with the newborn/infant's mother (or any other person holding the newborn/ infant), for example by explaining carefully what you will be doing, if it's going to hurt, etc.
- b. Hold the newborn/infant in the mother's lap with the foot lowered relative to the rest of the body. **DO NOT** perform skin punctures on the fingers of newborns or infants.

- c. Collect blood from the newborn/infant's heel, using the lateral or medial side of the foot as illustrated in the figure below. **DO NOT** perform skin punctures for obtaining blood specimen on the heel pad of a newborn's or infant's foot as this may result in injury to the nerves, tendons, and cartilage.

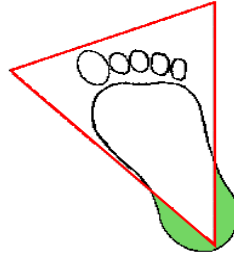


Figure 1: Heel stick site

The shaded regions in the figure indicate the recommended heel stick puncture site.

- d. Pre-warming the skin-puncture site provides optimal blood flow and more accurate samples. A warm, moist towel at a temperature of no more than 42°C may be used to cover the site.
- e. Clean the skin with an alcohol swab.
- f. Puncture the infant's heel with a sterile lancet. Full skin penetration by the tip of the lancet should be accomplished in order to obtain adequate blood flow for collection. Shallow, inadequate penetration will require the additional trauma of a repeated puncture. If blood does not flow adequately from the initial puncture, a second puncture in a cross (X) pattern can increase blood flow significantly.
- g. Hold the infant's heel loosely so as not to impede the flow of blood. If bleeding does not immediately occur, massage the lower portion of the leg in a downward direction. **DO NOT** squeeze the extremity as this may result in haemolysed blood and thereby jeopardize the result of the analysis.
- h. Using dry and clean cotton wool wipe off the first drop of blood, as this tends to contain excess tissue fluid.
- i. From the punctured site collect sufficient quantities of blood for the technique in question using the recommended equipment.
- j. Hold a small ball of dry cotton wool over the puncture site for a few minutes to stop the bleeding.

V. SAFETY PRECAUTIONS

- A. Always put on laboratory protective clothing and gloves and keep to the laboratory safety practices to avoid viral and other infectious disease transmissions.
- B. Needles should not be recapped, but should be placed in a proper needle disposal container immediately. Any blood-soiled materials should be placed in an infectious waste container for proper disposal.
- C. Discarded swabs, cotton, and other biohazard but non-sharp objects should be placed in a covered trash container lined with an autoclavable biohazard bag.
- D. Visibly soiled or splashed tourniquets and Vacutainer holders should be discarded in proper containers and new equipment used.
- E. Any blood spills or splashes should be immediately cleaned up with absorbent material using an approved disinfectant such as dilute 10% bleach or chlorhexidine solution. Counter surfaces in the phlebotomy work area should be disinfected at least 3 times a day or whenever visibly soiled.
- F. No food or drink is permitted in the phlebotomy work area.

VI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Postexposure Prophylaxis	
Lab SOP No.: 107	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

This standard operating procedure defines the procedures to be followed in the laboratory if an exposure to a potentially infectious material occurs.

II. UNIVERSAL PRECAUTIONS

- A. At all times, guidelines for good laboratory practices must be followed to ensure safety and to keep laboratory accidents to a minimum.
- B. White coats and gloves should be worn at all times to prevent bodily and personal clothing contamination.
- C. After any procedure, gloves should be removed and hands washed immediately at the designated sites.
- D. Testing of serum or plasma specimens should be performed in such a manner as to minimize any occupational risk.
- E. Contact of skin or mucous membranes with HIV-infected products should be avoided.
- F. Surfaces contaminated with HIV-infected products should be immediately disinfected.
- G. All contaminated waste should be safely disposed of.

III. PROCEDURE

- A. After exposure to a potentially infectious material, follow these procedures immediately:
 1. Wash the areas exposed to potentially infectious material with soap and water.
 2. Flush exposed mucous membranes with water. If saline solution is available, use it to flush eyes.
 3. Do not apply caustic agents, including antiseptics or disinfectants, to the exposed areas.

- B. Immediately inform the laboratory supervisor of the exposure and insist on completing the Occupational Exposure Incident Report in the applicable register. The following information should be provided:
1. Date and time of exposure
 2. Exposure site(s)
 3. Where and how the exposure occurred
 4. If a sharp object was involved, type and brand of device
 5. Type and amount of fluid exposed to
 6. Severity of exposure (e.g., depth of sharp puncture, intact skin, eyes)
 7. Exposure source
 8. Body fluids (e.g., blood products)
 9. Patient: If HIV patient, check for HIV status and document whether negative or positive, stage of disease, viral load, history of ART, etc.
- C. The laboratory supervisor will refer the exposed personnel to the medical officer in charge of postexposure prophylaxis (PEP) in CPGH. The medical officer will evaluate the laboratory health worker for potential exposure of HIV based on:
1. Type and amount of body fluid/tissue
 2. Type of exposure
 - a. Percutaneous injury
 - b. Mucous membrane exposure
 - c. Nonintact skin exposure
 - d. Bites resulting in blood exposure
 4. Infectious status of source
 - a. Presence of HIV antibody
 - b. Presence of HbsAG
 - c. Presence of HCV antibody
 5. Status of the source of infectious material
 - a. Presence of HIV antibody
 - b. Presence of HbsAG
 - c. Presence of HCV antibody
 6. Susceptibility of exposed person
 - a. Hepatitis B vaccine and response status
 - b. HIV, HBV, and HCV immune status

- D. The exposed health care worker will be offered pre-HIV test counselling based on informed consent, as well as ongoing counselling as desired. The confidentiality of the exposed health care worker will be maintained at all times.
1. If the HIV status of the source person is not known, the source person will be informed of the incident and consent obtained to perform HIV testing as soon as possible, maintaining confidentiality of the source person at all times.
 2. If the source person is negative for HIV, baseline testing or further follow-up of the exposed health care worker is not necessary.
 3. If the source person refuses to be tested for HIV, the attending medical officer will contact the senior administrator for authorization to perform HIV testing according to the Kenyan guidelines. The authorization of the senior administrator will also be sought when the source person is confused or in a coma, or in the case of a minor if a parent or guardian is not available.
 4. If the source person is not known, the exposure will be evaluated on the likelihood of high risk for infection: where and under what circumstances the exposure occurred.
- E. If the risk of exposure is determined to be there, PEP against HIV should be started immediately, within 1–2 hours of exposure if possible; however, if a delay occurs, initiate PEP regardless of the interval. PEP should be continued for 28 days, and HIV testing should be repeated after 2 weeks, then thereafter at 6 weeks, 3 months, 6 months, and 1 year.
- F. Risk definition
1. Low risk
 - a. Exposure to a small volume of blood or fluid contaminated with blood from asymptomatic HIV-positive patients with low viral load
 - b. Percutaneous exposure with a solid needle
 - c. Any superficial injury or mucocutaneous exposure
 2. High risk
 - a. Exposure to a large volume of blood or potentially infectious fluids
 - b. Exposure to blood or blood contaminated fluids from an HIV-infected patient with a high viral load
 - c. Injury with a hollow needle
 - d. Deep and extensive injuries
 - e. Confirmed ARV drug resistance in the source patient

G. Regimen for Risk Category

Risk Category	ARV Prophylaxis
Low	Retrovir (AZT) 300 mg twice a day X 28 days Epivir (3TC) 150 mg twice a day X 28 days (NOTE: Regimen may be dispensed as Combivir 1 tab twice a day.)
High	Retrovir (AZT) 300 mg twice a day X 28 days Epivir (3TC) 150 mg twice a day X 28 days Indinavir 800 mg three times a day X 28 days (NOTE: Combivir 1 tab twice a day may replace retrovir + epivir)

1. Toxicity of ARVs: Adverse symptoms with ARVs, such as headache, nausea, and diarrhoea, are common. Management without changing the PEP regimen is recommended (e.g., prescribing analgesic, antimotility, or anti-emetic agents). Please refer to the SOPs on Reporting ADRs.

IV. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Thermometer Quality Control	
Lab SOP No.: 108	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

Refrigeration of reagents is essential in ensuring shelf life and stability. Room temperature monitoring is also important for the proper functioning of equipment. Thermometers vary widely in their calibration accuracy. Therefore, it is essential that all thermometers are calibrated against a Kenya Bureau of Standards (KBS) thermometer for proper temperature control of freezers, refrigerators, incubators, and room temperature.

II. SCOPE

- A. Thermometers will be calibrated when they are brought into service.
- B. Existing thermometers will be calibrated yearly to assess accuracy. All thermometers can be calibrated during a single month or calibration can be staggered.

III. IDENTIFICATION

All thermometers will be uniquely identified. The identifier will be recorded in a logbook located in the laboratory, along with the initial and all future calibrations.

IV. PROCEDURE

- A. Visually inspect thermometer for any cracks in the capillary or bulb. Check the mercury or alcohol column for separations that may have occurred. If no defects are found, continue with this procedure.
- B. Immerse the KBS thermometer into the same glycerol/water bath as the thermometer that is being calibrated.

NOTE: Allow the system to equilibrate for one hour.

- C. Read and record the temperature on both thermometers.

V. RESULTS

Temperature readings must be within ± 1 degree of each other.

VI. CORRECTIVE ACTION

Discard any thermometer that is broken, does not read within ± 1 degree of the KBS thermometer, or contains bubbles. Notify section supervisor so that a new thermometer may be ordered.

VII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Refrigerator/Freezer Maintenance	
Lab SOP No.: 109	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

- A. Refrigerator. The general purpose laboratory refrigerator is normally an explosion-proof, insulated cabinet capable of reducing and holding temperatures to levels above the freezing point of water. There is usually an adjustable control for varying the internal temperature. This unit is used to store reagents, microbiological material, and blood products.
- B. Freezer. May or may not be attached to a refrigerator unit. Laboratory freezers have refrigeration units capable of reducing and holding temperatures below 0°C. They are used to store the same types of material stored in laboratory refrigerators.

II. FUNCTION VERIFICATION

- A. The internal temperature should be read and recorded daily. A thermometer should be installed in both units. The refrigerator thermometer should be mounted in a jar containing a solution of 50% glycerine and water. The freezer solution should be an antifreeze such as 50% glycerine/water. The acceptable range for refrigerators is 2–8°C and 0–⁻25°C or 0–⁻80°C for freezers.
- B. If the unit is equipped with a blower fan, check daily to see that it is operating.
- C. Check door gasket and seals every 6 months.
- D. Check unit every 6 months for level installation and to ensure door closes and seals properly.

III. INSTRUMENT MAINTENANCE

- A. Defrost unit and clean interior at least every 6 months. Wash interior with a solution of warm water and baking soda; rinse with clean water. Dry the interior. If the unit is self-defrosting, ensure drain tubes remain open.
- B. Clean the exterior with mild soap in water, rinse, and dry every 6 months.
- C. Clean and vacuum condenser and power units with a whisk broom every 6 months.
- D. If the gasket accumulates a black mould or fungus, apply a 50% solution of bleach with a small brush; rinse, and dry thoroughly.

- E. Check for level installation, ensuring the door closes properly and seals. Check levelling wheels or screws every 6 months.
- F. Check power cord monthly to ensure there is no fraying of wire covering.

IV. PRECAUTIONS

- A. Ensure the unit is properly grounded.
- B. Do not store explosive chemicals inside the unit if it is not explosion proof.
- C. Keep contents properly wrapped, covered, or stoppered to prevent frost build-up. Do not store food and drinks in a refrigerator that is used to hold reagents and chemicals.

V. CORRECTIVE ACTION

- A. Use wheels or screws to level the unit.
- B. If the temperature range is unacceptable, adjust and record.
- C. When adjustments fail to correct problem or gaskets and/or seals are faulty, notify supervisor and medical maintenance staff.
- D. Record all corrective action on maintenance chart.

VI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures Handling and Disposal of Biohazard Waste	
Lab SOP No.: 110	Version No.: 1.0
Date prepared:	Date adopted:

I. PURPOSE

The principal purpose of disposing biohazard wastes e.g. sharps, laboratory wastes, and microbiological specimens is to avoid contamination with infectious waste agents known to be infectious to humans.

II. BACKGROUND

Biomedical laboratories are special and unique work environments that may pose identifiable infectious disease risks to persons in or near them. Correct Biohazard Waste handling is therefore necessary to reduce or eliminate exposure to laboratory staff, other persons and the outside environment to potentially hazardous materials: such as blood or other body fluids, lab wastes and microbiology specimens which might be contaminated with agents known to be infectious to humans.

III. EQUIPMENT AND MATERIALS REQUIRED

- A. Incinerator
- B. Autoclave
- C. Waste bins
- D. Pipette baskets
- E. Bin liners for biohazard waste
- F. Unmarked waste bags
- G. Disposable sharps containers
- H. 10 % household bleach (JIK)
- I. Latex gloves
- J. Autoclavable tape

IV. KINDS OF BIOHAZARD WASTE

Infectious wastes include the following categories:

- A. Laboratory waste
 1. Laboratory equipment used to collect or test specimens, cultures or other material which may contain infectious pathogens.
 2. Disposable plastics such as pipettes, pipette tips, culture plates, micro plates, test tubes etc which has been used to test sample material
 3. Reusable glassware such as cylinders, flasks and beekers

B. Sharps

All implements used in the laboratory and which can break the skin are referred to as sharps:

1. Hypodermic needles
2. Syringes (with or without the attached needle)
3. Pasteur pipettes
4. Scalpel blades
5. Blood collection tubes
6. Needles with attached tubing
7. Glass culture dishes (regardless of presence of infectious agents)
8. Broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

C. Residual sample material

Specimens (samples) from the human body that the laboratory no longer needs for analysis or storage are referred to as residual body fluids. These include whole blood, plasma, serum, urine, stools, sputum, aspirates, breast milk, etc.

D. Chemical Wastes

Chemical wastes that are subject to the requirements of biohazard waste regulations include wastes from the following categories:

1. Laboratory reagents contaminated with infectious body fluids
2. Other chemicals that may be contaminated by infectious agents.

V. BIOHAZARD WASTE TREATMENT

There are three ways of treating biohazard wastes to render them harmless and biologically inert. These are:

- A. Incineration in an approved incinerator
- B. Steam sterilization for a sufficient time and at a sufficient temperature to destroy infectious agents in waste (autoclaving)
- C. Chemical disinfection in which contact time, concentration, and quantity of the chemical disinfectant are sufficient to destroy infectious agents in the waste

VI. DISPOSAL OF BIOHAZARD WASTE

A. General rules

1. Prior to any treatment, all biohazard wastes, including those to be incinerated, should be enclosed in a puncture-resistant, biohazard bag that is color-coded or labelled with the biological hazard symbol.
2. Untreated biohazard waste is not to be disposed of in the municipal waste (system) stream. All biohazard waste must be treated by chemical disinfection or autoclaving before in any way being disposed of in the municipal waste stream.
3. After disinfection, but before disposal in the municipal waste stream, all treated biohazard wastes (apart from liquids) should be enclosed in an unmarked outer bag that is not red or labelled with the biohazard symbol. Any biohazard waste that has been treated as described below and packaged such that it is clearly evident that the waste has been effectively treated, is not subject to be treated as biohazard waste and may be collected, transported for incineration or disposed of as municipal waste.
4. The person handling the emptying of waste bins, waste bottles or sharps containers must be careful not to touch anything without protective clothing and must use gloves to avoid contracting infections from the waste.
5. The waste bins, sharps containers, etc., must be clearly indicated/marked: **Biohazard wastes.**

B. Laboratory waste

1. Discarded swabs, cotton, sample containers and other biohazard but non-sharp objects used for sample collection should be placed in a covered trash container lined with an autoclavable biohazard bag. Visibly soiled or splashed tourniquets and Vacutainer holders should be discarded and new equipment used.
2. Disposable plastics such as tips, culture plates, micro plates, petri dishes, test tubes, etc., used for testing of samples should be disposed of in a covered waste bin lined with an autoclavable biohazard bag, autoclaved and transported for incineration.
3. Pipettes should be soaked in special pipette baskets in 10 % household bleach overnight, before being thrown in a waste bin lined with an autoclavable biohazard bag, autoclaved and incinerated.
4. Reusable glassware such as cylinders, flasks and beakers should be disinfected with 10 % household bleach over night before washed and autoclaved.

C. Sharps

1. All sharp implements used in the laboratory need to be handled carefully. They can easily break the skin and increase the risk of infection with infectious agents.
2. Broken glassware must also be handled as sharps. The laboratory should have a special box for broken glassware
3. If needles are used, they should never be recapped before disposal. Recapping needles may lead to somebody accidentally pricking him or herself.
4. Dispose of all sharps directly into a sharps disposal container with a secure perforated lid. The lid only allows the implements into the container but not out. The sharps container should be puncture-resistant, leak proof on the sides and bottom, and color-coded or labeled with a biohazard symbol.

Note: When selecting sharps containers, look for special safety features such as lids that lock tight for safe disposal and a container that can be sterilized by steam, gas, or chemicals. If sharps containers are not specifically constructed to be autoclaved, the resulting mass of melted plastic is extremely hazardous due to the needles that often protrude.

5. When full, seal top, autoclave, then arrange for incineration.
6. Decontamination is achieved by autoclaving the sharp implements. Incineration destroys the sharps completely hence eradicating the chances of accidental injury to people in the community.

D. Residual body fluids

1. Ensure that all tubes/containers containing residual body fluids are properly sealed to avoid spillage. These are then stored temporarily in waste bins lined with disposal bags.
2. Transfer the disposal bags, together with contents, into autoclaving bags stick a piece of autoclavable tape on the bag and then autoclave as required
3. After the autoclaving cycle is completed, transport and incinerate the whole package to completely destroy the tubes.

E. Chemical wastes

The following procedure is adopted for most chemicals used in low concentrations.

1. Collect all liquid chemical waste in properly labeled bottles with a little concentrated disinfectant (i.e., JIK).

2. Keep monitoring the rising level of waste in the trap bottles. Never fill the trap bottles to the very top to avoid spillage.
3. Empty the contents of the trap bottle down the drain, preferably a special sink in the laboratory, and wash down with more disinfectant, liquid soap and a large volume of water.
4. Minimum disinfection time of any liquid Biohazard Waste is 30 minutes.

Note: Some disinfectants react with organic compounds, acids, or bases. Consider this when selecting your disinfectant before commencing your work.

VII. SAFETY PRECAUTIONS

- A. Anybody handling Biohazard Wastes should always put on laboratory protective clothing and gloves and keep to the laboratory safety practices to avoid viral and other infectious disease transmissions.
- B. Any spills or splashes of infectious material should be immediately cleaned up with absorbent material using an approved disinfectant such as dilute 10% bleach or chlorhexidine solution.

VIII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

**Coast Provincial General Hospital
Antiretroviral Programme**

**Clinical Chemistry
Standard Operating Procedures**

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Alanine Aminotransferase (ALT/SGPT) Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 201	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Alanine aminotransferase (ALT or SGPT) is an enzyme found mainly in the liver. Elevated levels are seen in patients with hepatitis and mononucleosis.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump.

II. PRINCIPLE

A sample of 100 µl is mixed together with 1,000µl aspartate reagent solution in a test tube. The amino group of L-alanine is transferred to α -oxoglutarate in the presence of GPT to produce glutamate and pyruvate. The pyruvate formed in the deamination of L-alanine is converted by lactate dehydrogenase (LDH) in the presence of NADH, which is oxidated to NAD⁺. The rate of oxidation of NADH is monitored by reflectance spectrophotometry of 340 nm at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in international units per litre (U/L).

III. SPECIMEN

- A. Recommended specimen: 100 µl of serum or heparinised plasma or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Remove serum promptly from the clot. Haemolysed specimens should not be used because ALT contamination from red cells will occur. DO NOT freeze the sample; this will cause a loss of ALT activity.
- C. If concentration is greater than the analyser range, dilute with an equal volume of isotonic saline and reanalyse. Multiply the result by 2 to obtain the original ALT activity.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—see Operator’s Manual for additional information.

B. Reagents:

1. Buffer enzyme reagent: TRIS buffer pH 7.5, L-alanine 75 mmol/L, LDH \leq 1.2 kU/L
2. Substrate: 2-oxaloglutarate 90 mmol/L, NADH 0.9 mmol/L

Store reagents at temperatures between 2°C and 8°C.

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 μ l of distilled water into a sample cup and sip distilled water by pushing sip (press lever “P”). The sipping volume of 1,000 μ l is automatically measured in the flow-through cuvette.

D. The length of transportation of the air and water will be displayed on the screen.

E. Press [\leftarrow] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each day. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

A. Pipette 2 ml of substrate with 40 ml of the buffer and mix thoroughly. This working reagent is stable for 4 weeks at 2–8°C.

B. Label 2 tubes, tube 1 being the control and tube 2 being the sample test tube.

- C. In tube 1, pipette 1,000 µl of the reagent and 100 µl of control sera. In tube 2, pipette 1,000 µl of the reagent and 100 µl of sample.
- D. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- E. When the screen displays MEASURE BLANK, place cuvette with the control sera into cuvette holder and aspirate the solution by pushing lever [P].
- F. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample by pushing lever [P].
- G. Press [RESULT] and await possible delay while measuring the sample. The result will be displayed on top line.
- H. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 0–38 U/L
- B. Panic values: 40% difference from prior value
- C. Each laboratory should confirm these values

X. LIMITATIONS

- A. High total protein samples that are predominantly gamma globulins can increase ALT results. The sample should be diluted with an equal volume of isotonic saline and then reanalysed.
- B. In sera with very high activities, the initial absorbance may be very low because most of the NADH may have been consumed before the first readings. In this case, return the sample after dilution as described above.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Aspartate Aminotransferase (AST/SGOT) Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 202	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Aspartate aminotransferase (AST or SGOT) is an enzyme found mainly in the heart but also in other organs. Elevated levels may be associated with myocardial infarctions, various liver diseases, pulmonary emboli, and gangrene.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Determination of aspartate aminotransferase activity in 100 µl of serum or plasma is based on an enzyme-coupled oxidation of NADH to NAD⁺.

II. PRINCIPLE

- A. The 100 µl of patient sample is put in a test tube containing test reagent to rapidly activate the apoenzyme so that AST is fully active at the end of the lag phase without the need for a long preincubation.
- B. In the assay for AST, the amino group of L-aspartate is transferred to α-oxoglutarate in the presence of GOT to produce glutamate and oxaloacetate. The oxaloacetate formed in the deamination of the L-aspartate is converted to malate by malate dehydrogenase (MDH) in the presence of NADH, which is oxidized to NAD⁺. The rate of oxidation of NADH is monitored by reflectance spectrophotometry at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in international units per litre (U/L).

III. SPECIMEN

- A. Recommended specimen: 100 µl of serum or heparinised plasma or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Remove serum promptly from the clot. Haemolysed specimens should not be used because AST contamination from red blood cells will occur. Refrigerate specimens if analysis is not performed immediately. Freeze specimens if analysis is not performed within 48 hours.

- C. If concentration is greater than the analyser range, dilute with an equal volume of isotonic saline and reanalyse. Multiply the result by 2 to obtain the original AST activity.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator's Manual for additional information.
- B. Buffer/enzyme reagent: TRIS buffer 100 mmol/L; pH 7.8, L-aspartate 290 mmol/L, MDH ≥ 0.6 kU/L, LDH ≥ 0.6 kU/L
- C. Substrate: NADH 0.001 mmol/L, 2-oxaloglutarate 65 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 μ l of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 μ l is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [\leftarrow] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Pipette 2 ml of substrate with 40 ml of the buffer and mix thoroughly. This working reagent is stable for 4 weeks at 2–8°C.

- B. Label 2 tubes, tube 1 being the control and tube 2 being the sample test tube. In tube 1, pipette 1,000 µl of the reagent and 100 µl of control sera. In tube 2, pipette 1,000 µl of the reagent and 100 µl of sample.
- C. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE BLANK, place cuvette with the control sera into cuvette holder and aspirate the solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample. The result will be displayed on the top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 8–46 U/L
- B. Panic values: 40% difference from prior value
- C. Each laboratory should confirm these values.

X. LIMITATIONS

- A. High total protein samples that are predominantly gamma globulins can increase AST results. The sample should be diluted with an equal volume of isotonic saline and reanalysed. Highly active sera can have a very low initial absorbance because much of the NADH is already consumed prior to measurement. In such cases, dilute sample as specified.
- B. Analyser range: ≤ 150 U/L

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
γ-Glutamyl Transferase (γ-GT) Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 203	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. γ -Glutamyl transferase (GT) is an enzyme found mainly in the liver but also in other organs such as the kidneys, pancreas, and prostate. Elevated levels may be associated with alcoholic hepatitis or gross alcohol abuse or may be a result of drug induction or cholestatic liver damage diseases.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Determination of glutamyl transferase activity in 100 μ l of serum or plasma is based on kinetic colorimetric method in an enzyme-coupled reaction.

II. PRINCIPLE

- A. The 100 μ l of patient sample is put in a test tube containing test reagent containing glutamyl in the presence of GT, which transfers the glycyglycine to the glutamyl.
- B. In the assay for glutamyl transferase, the glycyglycine is transferred to glutamyl in the presence of gamma GT and 4-nitroanilide to produce glutamyl-glycyglycine and 5-amino-2-nitro-benzoate. The rate of the reaction is monitored by reflectance spectrophotometry at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in international units per litre (U/L).

III. SPECIMEN

- A. Recommended specimen: 100 μ l of serum or EDTA plasma. Collect specimens by standard venipuncture technique. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Haemolysed specimens should not be used because glutamyl transferase contamination from red blood cells will occur. There is no loss of enzyme activity within 7 days up to 25°C.
- C. If the absorbance per minute exceeds 0.200, dilute 0.1 ml of the sample with 0.5 ml of normal saline and repeat the assay using this dilution. Multiply the result by 6 to obtain the original glutamyl transferase activity.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—see Operator's Manual for additional information.

B. Buffer: TRIS buffer (pH 8.25) 100 mmol/L
 Glycylglycine 150 mmol/L

C. Substrate: L- γ -glutamyl-3 carboxy-4-nitroanilide 20 mmol/L

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 μ l of distilled water into a sample cup and sip distilled water by pushing sip, press lever [P]. The sipping volume of 1,000 μ l is automatically measured in the flow-through cuvette.

D. The length of transportation of the air and water will be displayed on the screen.

E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

A. Pipette 2 ml of substrate with 40 ml of the buffer and mix thoroughly. This working reagent is stable for 4 weeks at 2–8°C.

B. Label 2 tubes, tube 1 being the control and tube 2 being the sample test tube. In tube 1, pipette 1,000 μ l of the reagent and 100 μ l of control sera. In tube 2, pipette 1,000 μ l of the reagent and 100 μ l of sample.

- C. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE BLANK, place cuvette with the control sera into cuvette holder and aspirate the solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 9–60 U/L
- B. Panic values: 30% difference from prior value
- C. Each laboratory should confirm these values.

X. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Alkaline Phosphatase Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 204	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Alkaline phosphatase is an enzyme found mainly in the liver as well as in other organs such as the kidneys, pancreas, and prostate. Elevated levels may be associated with alcoholic hepatitis or gross alcohol abuse or may be a result of drug induction or cholestatic liver damage diseases.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Determination of alkaline phosphatase is based on measurement of increased absorbance against air.

II. PRINCIPLE

- A. The 20 µl of patient sample is put in a test tube containing test reagent containing the substrate p-nitrophenyl phosphate, which is hydrolysed in presence of alkaline phosphatase to phosphate and p-nitrophenol.
- B. In the assay for alkaline phosphatase, the p-nitrophenylphosphate is hydrolysed in presence of the enzymes to phosphate and p-nitrophenol. The hydrolysis is monitored by reflectance spectrophotometry at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in international units per litre (U/L).

III. SPECIMEN

- A. Recommended specimen: 20 µl of serum or heparinised plasma. Collect specimens by standard venipuncture technique. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Haemolysed specimens should not be used and samples more than 7 days old should not be used due to loss of the enzyme activity in 7 days.
- C. If absorbance change per minute exceeds 0.250, dilute 0.1 ml of the sample with 0.5 ml of normal saline and repeat the assay using this dilution. Multiply the results by 6 to obtain the alkaline phosphatase activity.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Buffer: Diethanolamine buffer (Ph 9.8).....1.0 mmol/L
Magnesium chloride.....0.5 mmol/L
- C. Substrate: p-Nitrophenyl phosphat55 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: *Chemistry Quality Control* for control procedures.

VII. PROCEDURE

- A. Pipette 2 ml of substrate with 40 ml of the buffer and mix thoroughly. This working reagent is stable for 4 weeks at 2–8°C.
- B. Label 2 tubes, tube 1 being the control and tube 2 being the sample test tube. In tube 1, pipette 1,000 µl of the reagent and 20 µl of control sera. In tube 2, pipette 1,000 µl of the reagent and 20 µl of sample. Mix well and read absorbance.

- C. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE BLANK, place cuvette with the control sera into cuvette holder and aspirate the solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 60–306 U/L
- B. Panic values: 20% difference from prior value
- C. Each laboratory should confirm these values.

X. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Amylase Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 205	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. High levels of amylase aid in the diagnosis of diseases of the pancreas. Analysis of amylase may be used to differentiate inflammation and haemorrhage of the pancreas and other disorders of the digestive system.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Necessary reagents are added in 10 µl of serum or plasma to determine amylase levels. The analysis is based on the reaction of the substrate 2-chloro-4-nitrophenyl-maltotrioxide (CNP3) and α-amylase to release 2-chloro-4-nitrophenol (CNP), which is a coloured dye. The intensity of the colour is proportional to the amount of α-amylase activity in the sample.

II. PRINCIPLE

This is a colorimetric method in which the substrate 2-chloro-4-nitrophenyl-maltotrioxide (CNP3) reacts directly with α-amylase to release 2-chloro-4-nitrophenol (CNP) from the substrate; the resulting absorbance increase per minute is directly related to the α-amylase activity in the sample.

III. SPECIMEN

- A. Recommended specimen: 10 µl of serum, heparinised plasma, or urine. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Refrigerate specimen at 4°C if analysis cannot be performed immediately. Allow specimen to reach room temperature prior to analysis.
- C. If concentration is greater than the analyser range, dilute with isotonic saline and reanalyse. Dilute 0.1 ml of the sample with 1.0 of 0.9% NaCl, repeat the assay, and multiply the results by 11.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Reagent: MES buffer (pH 6.0) 36 mmol/L, CNPG3 1.6 mmol/L, calcium acetate 3.6 mmol/L, sodium chloride 37 mmol/L, potassium thiocyanate 253 mmol/L, and sodium azide 0.095 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. The reagent is ready for use from manufacturer and is stable for 12 weeks at 2–8°C after opening.
- B. Label 2 tubes, tube 1 being the control and tube 2 being the sample test tube. In tube 1, pipette 1,000 µl of the reagent and 10 µl of control sera. In tube 2, pipette 1,000 µl of the reagent and 10 µl of sample. Mix well and incubate for 1 minute before reading absorbance.

- C. Press ZERO and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE CONTROL, place cuvette with the control sera into cuvette holder and aspirate by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: up to 200 U/L
- B. Panic values: 25% difference from prior value
- C. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Thoroughly rinse the adapter with distilled water after use. Do not pipette by mouth and avoid all contact with the reagent.
- B. Analyser range: $\leq 5,778$ U/L

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital	
Laboratory Standard Operating Procedures	
Total Bilirubin Analysis by Photometer 5010	
(Boehringer Mannheim)	
Lab SOP No.: 206	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Causes for an increase in total bilirubin may be divided into 3 categories: prehepatic—resulting from various haemolytic states; hepatic—resulting from hepatitis, cirrhosis, and other causes of hepatic necrosis; and posthepatic—resulting from an obstruction of the common bile or hepatic duct.
- B. The analysis is based on a reaction of bilirubin with a diazonium salt to produce a highly coloured dye. The intensity of the colour is proportional to the amount of total bilirubin in the sample.

II. PRINCIPLE

- A. 100 µl of patient sample is mixed with test reagent containing diazonium salt. The indirect bilirubin is liberated by the detergent. The total bilirubin is coupled with a diazonium compound to give the corresponding azobilirubin, which is a red azo dye. Water-soluble bilirubin glucuronides react directly with diazonium salt, whereas the albumin conjugated indirect bilirubin reacts only to diazonium salt in the presence of an accelerator.
- B. By measuring the amount of light reflected from the colour layer, the analyser can calculate the amount of total bilirubin present in the sample.

III. SPECIMEN

- A. Recommended specimen is 100 µl of serum or heparinised plasma. Collect specimens by standard venipuncture technique. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Protect specimens from light and analyse as soon as possible after collection. Direct exposure to sunlight is reported to cause as much as 50% loss of bilirubin in one hour, especially when the specimen is kept in a capillary tube. Exposure to normal room light can result in a significant loss of serum bilirubin after 2 to 3 hours.
- C. If analysis is not performed immediately, samples can be refrigerated for up to 24 hours at 2–5°C.

- D. Samples that come in contact with alcohol from sterile wipes may become haemolysed, which will increase the value of bilirubin.
- E. If concentration is greater than the analyser range, dilute with 5 volumes of normal saline and reanalyse. Multiply the result by 6 to obtain the original total bilirubin concentration. The results may show a positive bias of up to 30%.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Total bilirubin reagent: sulphanilic acid 14 mmol/L, hydrochloric acid 300 mmol/L, caffeine (accelerator) 200 mmol/L, sodium benzoate 420 mmol/L
- C. T-nitrite reagent for determination of total bilirubin 390 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Mix reagents according to manufacturer's recommendations.
- B. Label 2 tubes: In tube 1, or the sample blank pipette 1,000 µl of total bilirubin reagent and 100 µl of distilled; in tube 2 pipette 1,000 µl reagent solution, 100 µl of sample, and 1 drop of T-nitrite reagent. Mix well and incubate for at least 10 minutes at 20–25°C or for 5 minutes at 37°C and measure absorbance.
- C. When the screen displays MEASURE BLANK, press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RBB, place cuvette with the reagent blank solution into cuvette holder and aspirate the reagent blank solution by pushing lever [P].
- E. Press [WASH] to sip distilled water.
- F. When the screen displays MEASURE SB, place cuvette with the sample blank solution into cuvette holder and aspirate sample blank solution by pushing lever [P].
- G. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate sample solution by pushing lever [P].
- H. Press [WASH] to sip distilled water and measure the next sample.
- I. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in µmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 2–21 µmol/L
- B. Panic values: 50% difference from prior value
- C. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Specimens from haemodialysis patients should not be analysed for total bilirubin.
- B. Haemoglobin affects bilirubin results.

- C. Compounds that discolour serum, such as 4-aminosalicylic acid and phenazopyridine, may falsely increase bilirubin results.
- D. Results for predominantly unconjugated bilirubin (e.g., for neonates) may be up to 10% higher than the reference method.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Direct Bilirubin Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 207	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Causes for an increase in total bilirubin may be divided into 3 categories: prehepatic—resulting from various haemolytic states; hepatic—resulting from hepatitis, cirrhosis, and other causes of hepatic necrosis; and posthepatic—resulting from an obstruction of the common bile or hepatic duct.
- B. The analysis is based on a reaction of bilirubin with a diazonium salt to produce a highly coloured dye. The intensity of the colour is proportional to the amount of total bilirubin in the sample.

II. PRINCIPLE

- A. 100 µl of patient sample is mixed with test reagent containing diazonium salt. The indirect bilirubin is liberated by the detergent. The total bilirubin is coupled with a diazonium compound to give the corresponding azobilirubin, which is a red azo dye. Water-soluble bilirubin glucuronides react directly with diazonium salt, whereas the albumin conjugated indirect bilirubin react only to diazonium salt in the presence of an accelerator.
- B. By measuring the amount of light reflected from the colour layer, the analyser can calculate the amount of total bilirubin present in the sample.

III. SPECIMEN

- A. Recommended specimen is 100 µl of serum or heparinised plasma. Collect specimens by standard venipuncture technique. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Protect specimens from light and analyse as soon as possible after collection. Direct exposure to sunlight is reported to cause as much as 50% loss of bilirubin in one hour, especially when the specimen is kept in a capillary tube. Exposure to normal room light can result in a significant loss of serum bilirubin after 2 to 3 hours.
- C. If analysis is not performed immediately, samples can be refrigerated for up to 24 hours at 2–5°C.

- D. Samples that come in contact with alcohol from sterile wipes may become haemolysed, which will increase the value of bilirubin.
- E. If concentration is greater than the analyser range, dilute with 5 volumes of normal saline and reanalyse. Multiply the result by 6 to obtain the original total bilirubin concentration. The results may show a positive bias of up to 30%.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Direct bilirubin reagent: sulphanilic acid 14 mmol/L,
hydrochloric acid 300 mmol/L
- C. T-Nitrite reagent for determination of direct bilirubin 25 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Mix reagents according to manufacturer's recommendations.
- B. Label 2 tubes, in tube 1, or the sample blank, pipette 1,000 µl of total bilirubin reagent and 100 µl of distilled water. In tube 2 or sample pipette 1,000 µl reagent solution and 100 µl of sample, and 1 drop of T-nitrite reagent. Mix well and incubate for at least 10 minutes at 20–25°C or 37°C for 5 minutes before reading the absorbance
- C. When the screen displays MEASURE BLANK, press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RBB, place cuvette with the reagent blank solution into cuvette holder and aspirate the reagent blank solution by pushing lever [P].
- E. Press [WASH] to sip distilled water.
- F. When the screen displays MEASURE SB, place cuvette with the sample blank solution into cuvette holder and aspirate sample blank solution by pushing lever [P].
- G. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate sample solution by pushing lever [P].
- H. Press [WASH] to sip distilled water and measure the next sample.
- I. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in µmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: up to 4.3 µmol/L
- B. Panic values: 50% difference from prior value
- C. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Specimens from haemodialysis patients should not be analysed for direct bilirubin.
- B. Haemoglobin affects bilirubin results.
- C. Compounds that discolour serum, such as 4-aminosalicylic acid and phenazopyridine, may falsely increase bilirubin results.
- D. Results for predominantly unconjugated bilirubin (e.g., for neonates) may be up to 10% higher than the reference method.
- E. Exposure of samples to light may lower bilirubin levels.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Creatine Phosphokinase (CPK) Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 208	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Creatine kinase or creatine phosphokinase is an enzyme found mainly in skeletal and heart muscle, as well as in the brain. Measurements of total creatine kinase are used in investigating skeletal muscle disease and in diagnosing myocardial infarction and cerebrovascular accidents.
- B. The Photometer 5010 is a wet clinical chemical analyser where the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Analysis of creatine phosphokinase activity in 25 µl of serum or plasma is based on an enzymatic method that produces a coloured product.

II. PRINCIPLE

The 25 µl of patient sample (serum or plasma) is incubated with reagent containing the enzyme solution, followed by addition of the substrate. Creatine kinase converts creatine phosphate to creatine and ATP, which initiates a chain of reactions. Adenylate kinase, an enzyme present in red blood cells, is prevented from interfering by an inhibitor, diadenosine pentaphosphate. The change in reflection density is monitored at 37°C, and the rate of change is then used to measure enzyme activity in International Units per Litre (U/L).

III. SPECIMEN

- A. Recommended specimen is 25 µl of serum or heparinised plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated infectious waste.*
- B. Refrigerate specimens at 4°C for up to 24 hours if analysis is not done immediately.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—See Operator’s Manual for additional information.

B. Enzyme:

Imidazole buffer (pH 6.5)	0.1 mmol/L
Glucose	20 mmol/L
Magnesium acetate	10 mmol/L
EDTA	2 mmol/L
AMP	5 mmol/L
N-acetylcysteine	0.2 mmol/L
Diadenosine pentaphosphate	10 µmol/L
NADP	2 mmol/L
HK	>4 U/ml
SH-stabiliser	25 mmol/L
Sodium azide	0.095%

C. Substrate:

ADP	2 mmol/L
G6P-DH	>2.8 U/ml
Creatine phosphate	30 mol/L
Sodium azide	0.095%

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow through cuvette.

D. The length of transportation of the air and water will be displayed on the screen.

E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Mix 1,000 µl of reagent solution and 25 µl of sample and incubate for 3 minutes at 37°C. After 3 minutes of incubation, add 250 µl of the substrate solution and read the absorbance after 3 minutes.
- B. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- C. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- D. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- E. Print or write out the results. Press [WASH] to sip rinse solution or press [measure] to measure the next sample by pushing sip lever [P].

VII. CALCULATIONS

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values:
 - Males: 24–190 U/L
 - Females: 24–170 U/L
- B. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Grossly haemolysed specimens should not be used. The effect of trace haemolysis is minimized by AK inhibitors.
- B. There is 2.5% loss of activity of the enzyme within 7 days at 4°C or within 24 hours at 25°C.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Total Protein Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 209	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Total protein is essential for growth, the production of new tissue, and the repair of injured tissue. An increase in total protein levels may be the result of bone disease or severe dehydration. A decrease is seen with kidney damage, salt retention, and severe burn cases.
- B. The analysis is based on the biuret reaction, in which protein is treated with a cupric ion (Cu²⁺) to produce a violet-coloured complex. The intensity of the colour is proportional to the amount of total protein in the sample.

II. PRINCIPLE

20 µl of patient sample is mixed with 1,000 µl of solution containing cupric ions in alkaline medium. After incubation for 30 minutes, a reaction between protein and cupric tartrate takes place, forming a coloured complex. The amount of coloured compound formed is proportional to the amount of total protein in the specimen.

III. SPECIMEN

- A. Recommended specimen 20 µl of serum, heparinised or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Avoid haemolysed specimens. Refrigerate specimens for up to 6 days at 4–8°C if analysis is not performed immediately.
- C. Results from analysing plasma will be higher than serum because of fibrinogen remaining in the plasma.
- D. If concentration is greater than the analyser range, dilute with an equal volume of isotonic saline or distilled water and reanalyse. Multiply the result by 2 to obtain the original total protein concentration.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Sodium hydroxide 0.1 mol/L, potassium sodium tartarate 16 mmol/L, potassium iodide 15 mmol/L, cupric sulphate 6 mmol/L
- C. Sodium hydroxide 0.1mol/L, potassium sodium tartarate 16 mmol/L
- D. Protein 6 g/dL

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each day. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Mix reagents according to manufacturer’s recommendations. Mix 2 tubes: one blank with 1,000 µl of reagent solution 1; and tube 2 with 20 µl of serum or plasma and 1,000 µl of solution 1.
- B. Mix them well and incubate for 30 minutes at 20–25°C, then measure them immediately as indicated.

- C. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RB, place cuvette with the blank solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in g/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 6.3–8.2 g/dL (63–82 g/L)
- B. Panic values: <4 g/dL and >9 g/dL
- C. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Haemolysis causes an increase in measured values.
- B. Dextran causes an increase in measured values.
- C. For icteric, haemolytic, or lipemic sera, use assay with sample blank.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Triglycerides Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 210	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Triglycerides protein is essential for growth, the production of new tissue, and the repair of injured tissue. An increase in triglycerides levels may be the result of nephrosis, cholestasis, pancreatitis, cirrhosis, diabetes mellitus, and hepatitis. A decrease is seen with malnutrition.
- B. The analysis is based on the enzymatic hydrolysis of triglycerides with lipases in a quinoneimine formed from hydrogen peroxide used as a coloured indicator. The intensity of the colour is proportional to the amount of triglycerides in the sample.

II. PRINCIPLE

10 µl of patient sample is mixed and incubated with 1,000 µl of solution containing 4-aminoantipyrine and 4-chlorophenol in presence of hydrogen peroxide. After incubation for 10 minutes, a reaction between 4-aminoantipyrine and 4-chlorophenol and hydrogen peroxide takes place, forming quinoneimine, which is a coloured product. The amount of coloured compound formed is proportional to the amount of triglycerides in the specimen.

III. SPECIMEN

- A. Recommended specimen 10 µl of serum, heparinised or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Avoid haemolysed specimens. If analysis is not performed immediately, freeze specimens at -25°C for up to 4 months or refrigerate at $2-8^{\circ}\text{C}$ for up to 3 days.
- C. If concentration is greater than the analyser range, dilute 1 part of sample with 4 parts of isotonic saline or distilled water and reanalyse. Multiply the result by 5 to obtain the original triglycerides concentration.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—See Operator’s Manual for additional information.

B. Reagent	PIPES buffer (pH 7.5)	50 mmol/L
	4-chlorophenol	5 mmol/L
	4-aminoantipyrine	0.25 mmol/L
	Magnesium ion	4.5 mmol/L
	ATP	2 mmol/L
	Lipases	≥1.3 U/ml
	Peroxidase	≥0.5 U/ml
	Glycerol kinase	≥0.4 U/ml
	Glycerol-3-phosphate oxidase	≥1.5 U/ml
Standard	Triglyceride	2.28 mmol/L

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.

D. The length of transportation of the air and water will be displayed on the screen.

E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See SOP 217: *Chemistry Quality Control* for control procedures.

VII. PROCEDURE

- A. Mix 2 tubes: one blank with 1,000 µl of standard reagent, and tube 2 with 10 µl of serum or plasma and 1,000 µl of reagent solution.
- B. Mix each well and incubate for 5 minutes at 37°C, and measure the absorbance of the standard reagent and sample immediately as indicated.
- C. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RB, place cuvette with the blank solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in mmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 0.11–2.15 mmol/L.
- B. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Lipemic specimen usually generates turbidity of the sample reagent mixture, which leads to falsely elevated results.
- B. Ascorbate gives falsely low values.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Total Cholesterol Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 211	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Cholesterol is a steroid from which other steroids are derived, and in plasma exists esterified with fatty acids to form cholesterol esters. Total cholesterol may be increased in conditions such as hypothyroidism, obstructive jaundice, nephrosis, diabetes mellitus, pancreatitis, and pregnancy as well as by familial factors. It may be decreased in conditions such as hyperthyroidism, infections, malnutrition heart failure, malignancies, and during liver damage (e.g., caused by drugs, hepatitis, etc.).
- B. The analysis is based on the enzymatic hydrolysis of cholesterol esters and oxidation of resulting cholesterol to form cholestene-3-one and hydrogen peroxide. The peroxidase in presence of 4-aminophenazone and phenol forms the indicator whose absorbance is measured against blank reagent.

II. PRINCIPLE

10 µl of patient sample is mixed and incubated with 1,000 µl of reagent solution at 37°C for 5 minutes. The cholesterol esters in the specimen are hydrolysed in presence of cholesterol esterase to release cholesterol and fatty acids. The cholesterol released is oxidized by cholesterol oxidase to release cholestene-3-one and hydrogen peroxide, which in presence of 4-aminophenazone and phenol releases quinoneimine, which is a coloured product.

III. SPECIMEN

- A. Recommended specimen 10 µl of serum, heparinised or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. If the result is over 19.3 mmol/L, dilute the sample before precipitation 1:2 with normal saline and multiply the results by 3 to obtain the original cholesterol concentration.
- C. Cholesterol remains stable in serum for 6 days at 4–25°C and up to 4 months at –20°C.

- D. Lipemic specimens generate turbidity of the sample/reagent mixture, which leads to falsely elevated results.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—See Operator’s Manual for additional information.

B. Reagent (enzyme reagent)	Phosphate buffer (pH 6.5)	100 mmol/L
	4-aminophenazone	0.3 mmol/L
	Phenol	5 mmol/L
	Peroxidase	> 5 KU/L
	Cholesterolesterase	> 150 U/L
	Cholesteroxidase	> 100 U/L
	Sodium azide	0.05%
Standard	Cholesterol	5.17 mmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Label 2 tubes: In tube 1 or reagent blank pipette 1,000 µl of reagents solution, and 10 µl of distilled water and in tube 2 or sample tube pipette 1000µl of reagent solution and 10 µl of sample.
- B. Mix each well and incubate for 10 minutes at 20–25°C or 5 minutes at 37°C and measure their absorbance.
- C. When the screen displays MEASURE BLANK, press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RB, place cuvette with the blank solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in mmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 3.6–5.2 mmol/L.
- B. Each laboratory should confirm these values.

X. LIMITATIONS

- A. Bilirubin concentrations above 4 mg/dl interferes with tests
- B. If results are over 25.9mmol/l dilute the sample and repeat measurement
- C. High concentration of ascorbic acid (> 2.5 mg/dL) will give lower values.
- D. Haemoglobin levels higher than 200 mg/dL do not interfere with the test.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
HDL Cholesterol Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 212	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. High density lipoprotein (HDL) is lipoprotein which is involved in the transport of cholesterol from the cells to the liver. An increase in HDL levels may be the result of nephrosis, cholestasis, pancreatitis, cirrhosis, diabetes mellitus, and hepatitis. A decrease is seen with malnutrition.
- B. The analysis is based on the precipitation of chylomicrons, very low-density lipoprotein (VLDL), and low density lipoprotein (LDL) upon addition of phosphotungstic acid and magnesium chloride to the sample. The HDL is contained in the supernatant, which can be enzymatically assayed after centrifugation of the supernatant. The absorbance of the sample and the standard reagent is measured against blank reagent.

II. PRINCIPLE

When 200 µl of test sample is mixed with 500 µl of precipitant reagent containing phosphotungstic acid and magnesium chloride at room temperature for 10 minutes chylomicrons, VLDL, and LDL are precipitated. Centrifugation at 10,000 g for 2 minutes leaves only the HDL in the supernatant, where the concentration of HDL in the sample is determined enzymatically.

III. SPECIMEN

- A. Recommended specimen 200 µl of serum, heparinised or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other-based products. Discard contaminated materials with infectious waste.*
- B. If the supernatant is not clear, dilute the sample before precipitation 1:1 with normal saline and multiply the results by 2 to obtain the original HDL concentration.
- C. HDL cholesterol remains stable in serum for 6 days at 4–25°C and up to 4 months at –20°C.
- C. Serum must be separated from the blood clot as rapidly as possible.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—See Operator’s Manual for additional information.

B. Reagent (Precipitant)	Phosphotungstic acid	0.55 mmol/L
	Magnesium chloride	25 mmol/L
Standard	Cholesterol	1.29 mmol/L

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever “P”. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.

D. The length of transportation of the air and water will be displayed on the screen.

E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

A. Into a glass centrifuge tube mix 200 µl of test sample (serum or plasma) 500 µl of precipitant reagent. (Dilute precipitant reagent with distilled water 4 in 1.)

B. Mix them well and incubate for 10 minutes at room temperature.

C. Centrifuge at 4000 rpm for 10 minutes or 10,000 rpm for 2 minutes.

- D. After centrifugation remove the clear supernatant and into tube 1 (sample) add 100 µl of supernatant and 1,000 µl of total cholesterol reagent. In tube 2 (reagent blank) add 100 µl of distilled and 1,000 µl of cholesterol reagent.
- E. Mix them well and incubate for 10 minutes at 20–25°C or 5 minutes at 37°C and measure their absorbance.
- F. When the screen displays MEASURE BLANK, press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- G. When the screen displays MEASURE RB, place cuvette with the blank solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- H. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- I. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- J. Print or write out the results. Press [WASH] to sip rinse solution or press [measure] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in mmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 0–0.9 mmol/L.
- B. Each laboratory should confirm these values.

X. LIMITATIONS

- A. High triglyceride level interferes with HDL measure; dilute the sample before precipitation 1:1 with 0.9% saline and multiply the results by 2.
- B. High concentration of ascorbic acid (> 2.5 mg/dL) will give lower values.
- C. Haemoglobin levels higher than 100 mg/dL and bilirubin levels higher than 10 mg/dL interfere with the test.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital	
Laboratory Standard Operating Procedures	
LDL Cholesterol Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 213	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Low density lipoprotein (LDL) is lipoprotein which is involved in the transport of cholesterol to the cells. An increase in LDL levels may be the result of altered diet and vascular diseases. A decrease is seen in AIDS, haematological malignancies such as acute myeloid leukaemia (AML) or chronic myeloid leukaemia (CML), and disorders involving splenomegaly.
- B. The analysis is based on direct homogenous enzymatic assay for quantitative determination of LDL cholesterol combining two steps. The analysis involves removal of chylomicrons, very low density lipoprotein (VLDL), and high density lipoprotein (HDL), followed by enzymatic determination of LDL in the sample.

II. PRINCIPLE

10 µl of patient sample is mixed and incubated at 37°C for 5 minutes with 750 µl of enzyme solution containing cholesterol esterase and cholesterol oxidase, which allows the removal of chylomicrons, VLDL, and HDL from the specimen by formation of cholestenone and hydrogen peroxide. The LDL present in the sample is converted to cholestenone and hydrogen peroxide in the presence of cholesterol esterase, cholesterol oxidase, and specific surfactant. The chromogen in presence of hydrogen peroxide peroxidase is converted to a coloured dye (quinine) whose concentration is measured by colorimetric method.

III. SPECIMEN

- A. Recommended specimen 10 µl of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. If the serum concentration of LDL exceeds the measuring range, dilute the sample before precipitation 1:1 with normal saline, repeat the test, and multiply the results by 2 to obtain the original LDL concentration.
- C. LDL cholesterol remains stable in serum for 6 days at 4–25°C and up to 4 months at –20°C.
- D. Serum must be separated from the blood clot as rapidly as possible.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—See Operator’s Manual for additional information.

B. Reagent (enzymes)	Good’s buffer (pH 7.0)	50 mmol/L
	Cholesterol esterase	600 U/L
	Cholesterol esterase	500 U/L
	Catalase	600 kU/L
	TOOS	2.0 mmol/L
	Detergents	0.3% w/v
	Preservatives	<0.1% w/v

Substrate	Peroxidase	4000 U/L
	4-Aminoantipyrin	4 mmol/L
	Good’s buffer (pH 7.0)	50 mmol/L
	Sodium azide	0.05%
	Detergents	1% w/v
	Preservatives	<0.1% w/v

V. CALIBRATION

A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.

B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.

C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.

The length of transportation of the air and water will be displayed on the screen.

D. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Label 2 tubes and pipette 10 µl water and 750 µl of enzyme solution into tube 1 (blank), and into tube 2 pipette 10 µl of test sample and 750 µl of enzyme solution. Mix gently and incubate at 37°C for 5 minutes.
- B. Into each tube add 250 µl of substrate solution and mix them well and incubate for 5 minutes at 37°C, then measure absorbance of sample against reagent blank.
- C. When the screen displays MEASURE BLANK, press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- D. When the screen displays MEASURE RB, place cuvette with the blank solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- E. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- F. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- G. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in mmol/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

- A. Expected values: 0–0.9 mmol/L.
- B. Each laboratory should confirm these values.

NOTE: Direct measurement of LDL is required instead of the Friedwald, or “calculated”, method whenever triglyceride values exceed 400 mg/dL. The Friedwald or “calculated” method of LDL determination is determined by subtracting HDL and VLDL from the total cholesterol concentration. Total cholesterol = HDL + LDL + VLDL. VLDL is generally estimated by the triglyceride concentration divided by 5, but is inaccurate when triglyceride values exceed 400 mg/dL.

X. LIMITATIONS

- A. High triglyceride level interferes with LDL measure; dilute the sample before precipitation 1:1 with 0.9% saline and multiply the results by 2.

- B. High concentration of ascorbic acid (> 2.5 mg/dL) will give lower values.
- C. Haemoglobin levels higher than 100 mg/dL and bilirubin levels higher than 10 mg/dL interfere with the test.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Blood Urea Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 214	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Elevated levels of blood urea are most commonly associated with renal disease, but may also result from dehydration, a high-protein diet, excess destruction of body proteins, and gastrointestinal diseases, especially with intestinal obstruction.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. BUN/urea is determined in 10 µl of serum or plasma.

II. PRINCIPLE

- A. The analysis is based on hydrolysis of urea to ammonia and carbon dioxide. The ammonia reacts with an indicator to produce a highly coloured dye.
- B. The 10 µl of patient sample is reacted with reagent solution where urea is hydrolysed to ammonia and carbon dioxide. The ammonium ion produced reacts with salicylate and hypochlorite to give a green dye. By measuring the amount of UV light in a standard solution, the analyser can calculate the amount of urea nitrogen present in the sample.

III. SPECIMEN

- A. Recommended specimen is 10 µl of serum or EDTA plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*
- B. Refrigerate specimens up to 3 days at 4°C if analysis is not performed immediately.
- C. If concentration is greater than the analyser range, dilute with 9 volumes of isotonic saline or distilled water and reanalyse. Multiply the result by 10 to obtain the original urea nitrogen concentration.

IV. REAGENTS AND MATERIALS

A. Photometer 5010 Analyser—see Operator’s Manual for additional information.

B. Reagent 1:	Phosphate buffer (pH 7.0)	120 mmol/L
	Sodium salicylate	60 mmol/L
	Sodium nitroprusside	5.00 mmol/L
	EDTA	1 mmol/L
Reagent 2:	Phosphate buffer (pH < 13)	120 mmol/L
	Hypochlorite	~0.6 g/L
Enzyme:	Urease >	500 kU/L
Standard:	Urea	80 mg/dL or 13.3 mmol/L
	Equivalent to BUN	37.28 mg/dL or 6.2 mmol/L
	Sodium azide	0.095%

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 µl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 µl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: *Chemistry Quality Control* for control procedures.

VII. PROCEDURE

- A. Mix reagents according to manufacturer's recommendations.
- B. Label 2 tubes: in tube 1, or reagent control tube pipette 1,000 µl of reagent solution 1; and tube 2, or sample tube pipette 10 µl of sample and 1,000 µl of reagent solution 1. Mix them well and incubate for 3 minutes at 37°C.
- C. Add 1,000 µl of reagent solution 2 to each of the tubes. Mix well and incubate for 5 minutes at 37°C. Measure absorbance of sample against control within 60 minutes.
- D. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- E. When the screen displays MEASURE STANDARD, place cuvette with the control solution into cuvette holder and aspirate the blank solution by pushing lever [P].
- F. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].
- G. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- H. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

IX. CALCULATIONS

The results are reported in mmol/L. No further calculation is necessary unless the specimen has been diluted.

Conversion

$$\text{Urea} = 2.14 \times \text{BUN}$$

$$\text{BUN} = 0.466 \times \text{Urea}$$

X. RESULTS

- A. Expected values: (urea) 1.7–8.3 mmol/L
- B. Panic values: <1.07 mmol/L and >37.1 mmol/L
- C. Each laboratory should confirm these values.

XI. LIMITATIONS

- A. Do not use plasma collected with sodium fluoride.
- B. Specimens that contain haemoglobin increase urea nitrogen. Haemoglobin of 50 mg/dL (slightly haemolysed) increases BUN levels below 28 mg/dL by 1 mg/dL.
- C. Ammonium ions have shown an increase in urea nitrogen equivalent to their nitrogen content.

XII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Creatinine Analysis by Photometer 5010 (Boehringer Mannheim)	
Lab SOP No.: 215	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Creatinine measurement is used in the diagnosis and treatment of renal diseases and in monitoring patients on renal dialysis.
- B. The Photometer 5010 is a wet clinical chemical analyser in which the solution is measured either in disposable cuvettes (placing one after the other) or a flow-through cuvette that works in a built-in peristaltic pump. Analysis of creatinine activity in 100 µl of serum or plasma is based on an enzymatic method that produces a coloured product.

II. PRINCIPLE

- A. The 100 µl of patient sample mixed with picrate in alkaline medium produces a coloured complex with creatinine. The rate of formation of the complex is then measured.
- B. The creatinine present in the sample reacts with the picrate at 37°C, and the rate of complex formation is measured and is proportional to the creatinine concentration.

III. SPECIMEN

- A. Recommended specimen is 100 µl of serum or heparinised plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated infectious waste.*
- B. Refrigerate specimens at 4°C for 24 hours if analysis is not done immediately.

IV. REAGENTS AND MATERIALS

- A. Photometer 5010 Analyser—see Operator’s Manual for additional information.
- B. Picric acid 26 mmol/L
- C. NaOH 1.6 mmol/L
- D. Creatinine 2 mg/dL or 176.8 µmol/L

V. CALIBRATION

- A. Empty and wash flow-through cuvette by pressing [WASH] on the LCD display. An automatic return to the select menu will occur.
- B. Push sip by pressing lever [P] to drain the tube system. Do not sip any solution.
- C. Measure exactly 1,000 μl of distilled water into a sample cup and sip distilled water by pushing sip-press lever [P]. The sipping volume of 1,000 μl is automatically measured in the flow-through cuvette.
- D. The length of transportation of the air and water will be displayed on the screen.
- E. Press [↵] to finish calibration. The program will return to method select menu.

VI. QUALITY CONTROL

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyser. If the controls are out of range, do not report patient results until the problem is resolved and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See SOP 217: Chemistry Quality Control for control procedures.

VII. PROCEDURE

- A. Mix reagents according to manufacturer's recommendations.
- B. Label 2 tubes: in tube or standard tube pipette 1,000 μl of reagents solution and 100 μl of creatinine standard reagent; and in tube 2 or sample tube pipette 1,000 μl of reagents solution and 100 μl of sample.
- C. Mix them well and measure them immediately as indicated.
- D. Press **ZERO** and then push sip by pressing lever [P] to sip water and drain the system.
- E. When the screen displays MEASURE STANDARD, place cuvette with the standard solution into cuvette holder and aspirate the standard solution by pushing lever [P].
- F. When the screen displays MEASURE SAMPLE, place cuvette with the sample solution into cuvette holder and aspirate the sample solution by pushing lever [P].

- G. Press [RESULT] and await possible delay while measuring the sample solution. The result will be displayed on top line.
- H. Print or write out the results. Press [WASH] to sip rinse solution or press [MEASURE] to measure the next sample by pushing sip lever [P].

VIII. CALCULATIONS

The results are reported in $\mu\text{mol/L}$. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS

A. Expected values:

Males: 53–97 $\mu\text{mol/L}$
Females: 44–80 $\mu\text{mol/L}$

B. Panic values: < 27 $\mu\text{mol/L}$ and > 663 $\mu\text{mol/L}$

C. Each laboratory should confirm these values.

X. LIMITATIONS

A. EDTA and fluoride/oxalate anticoagulants will cause low creatinine results.

B. Lidocaine: Patients on long-term lidocaine therapy may show an increase of up to 1.0 mg/dL. Ninety percent of patients receiving intravenous lidocaine will show less than a 0.3 mg/dL increase.

C. Proline: Patients receiving hyperalimentation fluid that contains proline may show an increase of up to 2.0 mg/dL.

D. TRIS buffer (control fluid) causes an approximate 50% decrease in results.

E. Dipyrone (Metamizol) at 40 mg/dL shows a –0.6 mg/dL bias at a creatinine concentration of 1.0 mg/dL.

F. N-acetylcysteine: Patients receiving N-acetylcysteine (Fluimucil, Mucomyst) intravenously have been reported to show a large negative bias.

XI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures Chiron Diagnostic 644 Na⁺/K⁺/CL⁻ Analyser	
Lab SOP No.: 216	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Sodium is the major cation of the extracellular fluids. The kidneys regulate the sodium contents of the body. Low sodium levels may be caused by excessive urine loss, diarrhoea, Addison's disease, or renal tubular disease. High sodium levels may occur in severe dehydration, some types of brain injury, diabetic coma, and excessive intake of salt.
- B. Potassium is the major cation of intracellular fluids. Hypokalemia (low potassium levels) may be caused by prolonged diarrhoea, starvation, stress states, some kidney malfunctions, and malabsorption by the intestines. Hyperkalemia (high potassium levels) may occur with severe cell damage, hypoventilation, and acute kidney failure.
- C. Chloride is the major extracellular anion and is significantly involved in maintaining proper water distribution, osmotic pressure, and normal anion-cation balance in the extracellular fluid compartment. Chloride measurements are used primarily in the diagnosis of renal tubular disorders and metabolic acidosis. Abnormally high levels may also be caused by dehydration and decreased renal blood flow.

II. PRINCIPLE

- A. When an ion-selective membrane separates two solutions that differ in concentration of the ion, an electrical potential is developed across the membrane; the size of the potential is dependent upon differences in the ion concentration.
- B. The sodium ion-selective electrode (ISE) consists of a glass capillary that is selective to sodium ions. The sample passes through the capillary, with the outer surface in contact with the electrode filling solution. Electrical connection is via Ag/AgCl wire. The sodium potential from the sodium electrode is compared with a reference electrode.
- C. The potassium ISE consists of a valinomycin-based membrane. The valinomycin is in contact with both the sample on one side and the electrode filling solution on the other. The electrical connection is via Ag/AgCl wire.
- D. The chloride ion-selective electrode consists of an ion exchange membrane sensitive to Cl⁻ ions, immobilised in PVC. Electrical connection is via Ag/AgCl wire.

- E. The reference electrode consists of a shell filled with saturated KCl, separated from the sample by a cellulose membrane. The electrical connection is via Ag/AgCl wire coated with Nafion, an ion-permeable polymer.
- F. The Chiron Diagnostic 644 is a direct potentiometric ISE analyser used for *in vitro* quantitative measurements of sodium, potassium, and chloride, based on measuring the potential developed by the ISE with respect to the reference electrode. The sodium, potassium, and chloride electrodes are incorporated in a microprocessor-controlled sample fluid handling system. In an electrolyte solution, most simple salts dissociate their ions. An electrical exchange reaction occurs between the relevant electrode and the ions, producing a potential between the ISE and reference electrode.

III. SPECIMEN

- A. Serum, plasma, whole blood, or urine can be assayed.
 - 1. Whole blood sample must not be over one hour old and therefore should be sent to the laboratory immediately after being drawn.
 - 2. Plasma (anticoagulant of choice is lithium heparin) should be separated from the red cells as soon as possible. Keep sample capped to prevent evaporation until ready to assay.
 - 3. Dilute urine: one part urine sample to 10 parts Chiron Diagnostic 644 Analyser urine diluent.
- D. Minimum sample volume is 65 µl for blood, serum, plasma, and QC material, and 100 µl prediluted with Chiron Diagnostic 644 Analyser urine diluent for urine.

IV. REAGENTS AND MATERIALS

- A. Chiron Diagnostic 644 Analyser.
- B. Calibrating standard/flush solution used to calibrate the analyser and to rinse out the sampling tract: sodium 140 mmol/L, potassium 4.00 mmol/L, and chloride 100 mmol/L.
- C. Serum slope standard used to determine the sodium and potassium slope during the two-point calibration cycle: NaCl 110 mmol/L, KCl 8.00 mmol/L.
- D. Urine slope standard used to determine the slope and calibration for urine: Na = 100, K = 120, and Cl = 220.
- E. Chiron Diagnostic 644 Analyser urine diluent.
- F. Deproteinizing and cleaning solution: 0.075 mol/L HCl, non-ionic detergent, and pepsin.

- G. Electrode Conditioning Solution: sensitized for ISE systems.
- H. Na/K/Cl electrode-filling solution: sodium 140 mmol/L, potassium 4.00 mmol/L, and chloride 100 mmol/L.
- I. Reference filling solution: 4 mol/L KCl.

V. CALIBRATION

- A. Fully automated (can be manually controlled) to perform a one-point calibration every 10 to 60 minutes and a two-point calibration every fourth calibration for the monitoring of cal-drift.
 - 1. To initiate this calibration manually, press **NO** to message ANALYSE BLOOD and press **YES** for STANDARDIZATION to perform a one- or two-point calibration.
 - 2. The first step of the cycle is cleaning and calibrating the sample sensor, and checking the voltage of the sample to see if it is within acceptable range. If calibration is unsuccessful, out of range, unstable, or indicates a drift, the analyser will automatically recalibrate up to three cycles. After a third unsuccessful recalibration, the system will stop with the message: CAL FAILED-REPEAT? Recommend conditioning of electrodes.
 - 3. If the measuring concentration is within range, CALIBRATION OK will be displayed on the analyser.
 - 4. After a successful calibration, the slope will be checked (two-point calibration), and if slope is within range, SLOPE OK will be displayed.
 - 5. If calibration fails on any electrode, the proper error code message will be generated and troubleshooting should begin. Refer to the Chiron Diagnostic 644 Analyser Operator's Manual.

VI. QUALITY CONTROL (CERTAIN ISE AQUEOUS CONTROLS)

- A. The Chiron Diagnostic 644 assayed controls come in three levels—low, medium, and high—and are used for the Na⁺/K⁺/Cl⁻ assays performed to validate quantification of patient samples when necessary. The controls are supplied ready-to-use and require no reconstitution; treat them identically to patient samples. Date and initial the control ampoules when initially opened. Certain ISE controls are supplied at three levels to verify the instrument's performance at several points in the clinical range.
- B. Assay controls daily (at least once every 8 hours, or as frequently as necessary) to monitor the performance of the Chiron Diagnostic 644 Analyser.

C. Keep control limits within ± 2 SD of established ranges, which are initially provided by manufacturer's assays. Slight adjustments of the assayed means may be made on a monthly basis by the supervisor, according to the functioning of the equipment, provided that these ranges do not exceed manufacturer's assay limits. Store controls at 4–25°C; avoid direct sunlight.

D. Control procedure:

1. Restore all the liquid to the bottom part of the ampoule.
2. Snap open ampoule; care should be taken to avoid injury.
3. Aspirate contents into analyser either in ANALYSE BLOOD mode or QC NOW mode.
4. Operator can set QC prompts and limits (see Operator's Manual for details).

VII. PROCEDURE

A. To measure serum, plasma, and whole blood:

1. Press **YES** on the ANALYSE BLOOD display. Wait for the analyser to read OPEN PROBE, then place sample in probe.
2. When analyser is ready, PROBE IN SAMPLE will appear on the display; press **YES**. Ensure probe is immersed in the sample/control. Aspiration time is approximately 10 seconds, with display of SAMPLING WAIT appearing. If the sample sensor does not detect the sample/control within 15 seconds, a SAMPLE FAULT message will be displayed.
3. After sampling, wipe probe with a lintless tissue.
4. The system will instruct you to retract the probe. Ten seconds after the RETURN PROBE, a continuous beep will sound to remind you to return probe.
5. Results will be displayed within 35 seconds. A copy of the results will also be printed out.
6. To analyse urine, see Instruction Manual.

VIII. CALCULATIONS

The results are recorded in mmol/L. No further calculation is necessary.

IX. RESULTS

A. Expected values:

Sodium: 135–145 mmol/L

Potassium: 3.6–5.0 mmol/L

Chloride: 98–107 mmol/L

B. Panic values:

Sodium: <120 mmol/L and >160 mmol/L

Potassium: <2.5 mmol/L and >6.0 mmol/L

Chloride: <85 mmol/L and >115 mmol/L

X. MAINTENANCE

A. Daily maintenance to be performed:

1. Check levels of cal-pak (change weekly).
2. Ensure probe is straight and centred over weir.
3. Clean weir cover.
4. Clean external surfaces, sampling area, and calibration compartment with disinfectant.
5. Deproteinize and condition electrodes every 8 hours.

B. Quarterly maintenance to be performed:

1. Disinfect analyser.
2. Replace pump tube cassette; clean and lubricate roller assembly.
3. Replace weir cover if necessary.
4. Replace reference electrode cassette.
5. Refill $\text{Na}^+/\text{K}^+/\text{Cl}^-$ electrodes with fill solution as needed.
6. System check as required.

XI. LIMITATIONS

A. Sample must be free of haemolysis.

B. Linear range: CV 1.5% or less.

Whole blood/serum/plasma:

Sodium: 80–200 mmol/L
Potassium: 0.50–9.99 mmol/L
Chloride: 50–200 mmol/L

Urine:

Sodium: 10–350 mmol/L
Potassium: 5–250 mmol/L
Chloride: 10–350 mmol/L

C. Always replace standard and slope solution with fresh cal-pak of reagent. Never add fresh reagent to existing reagent in the old cal-pak.

D. Cleaning solution is irritating to the eyes, nose, and respiratory tract and is poisonous. Avoid contact with eyes or skin. If contact occurs, flush with a copious amount of water immediately.

E. System should always be run with front cover closed.

F. Water should never be used to clean or flush the sodium, potassium, or reference electrode.

XII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Chemistry Quality Control	
Lab SOP No.: 217	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. This SOP establishes the minimum guidelines to follow to ensure the quality and reliability of laboratory results. The principle of quality control is to ensure that the analytical values reported by the laboratory correspond to the clinically correct values
- B. The goal of the QC program is to assess the laboratory's performance in relation to theoretical results and results of other laboratories, to identify significant problems as they arise, to implement and document results of corrective actions, and to ensure problems are corrected in a routine and systematic manner.

II. DEFINITION OF TERMS

- A. Internal Quality Control (Intralaboratory Quality Control). An internal QC program is the system where controls with determined values (assayed or unassayed) are run, prior to patient samples, to determine whether the analytical test system is producing analytically reliable results for that particular test (analyte).
- B. External Quality Control (Interlaboratory Comparison Programs). In an external QC program, such as the United Kingdom National External Quality Assurance System (UK, NEQAS), unknowns are sent to participating laboratories for analysis. Survey results are compiled and reports are provided to the originating laboratories. The reports compare a laboratory's reported values with the mean responses and standard deviations obtained by peer laboratories. The number of peer laboratories and the standard deviation index (SDI) of all test systems are also included.
- C. Qualitative Control: controls which yield a positive or negative result based upon an expression of judgment by the observer, e.g., pregnancy test controls.
- D. Quantitative Control: controls which are numerical, chemical, or physical determinations yielding a "numerical" result.
 - 1. Assayed Control: commercial controls where the analytes have been assigned a mean value. The standard deviation has been derived by the vendor.
 - 2. Unassayed Control: commercial or prepared controls where the analytes are of unknown value. Each analyte must be thoroughly tested and, based upon the results, a mean and standard deviation may be derived.

- E. Accuracy Control: controls utilized in determining whether or not a test system is reporting accurate results as compared with the previously determined "known values" of the tested analyte.
- F. Precision Control: controls utilized throughout the operation of a test system to verify the precision of the test system for a given analyte.

III. CONTROLS

The following controls are performed on a routine basis. The list is not all encompassing. Individual SOPS contain QC specifics that apply to each procedure.

- A. PHOTOMETER 5010 (BOEINGER MANHEIM):
BUN γ -GT CK TP SGOT GLU TBIL AMYL CREAT SGPT
- B. Diagnostic 644 assayed controls come in three levels:
 - Level I: Low
 - Level 2: Medium
 - Level 3: High

IV. CONTROL PROCEDURES

- A. General QC guidelines: Total Quality Control must incorporate various aspects of laboratory procedures such as:
 - 1. Continuous training and education of laboratory personnel
 - 2. Preventive maintenance procedures
 - 3. Correct sample collection
 - 4. Proper pre- and post-analytical processing of samples and results.
 - 5. Proper identification of samples and results.
 - 6. Confirm that instructions in reagent package inserts for specific tests are being followed.
 - 7. Use good quality distilled or deionized water and only reagents and disposables provided or recommended for use by the manufacturer
- B. Document the daily performance of quality control sera.
- C. Running QC sera.
 - 1. Controls are run in the same way as regular samples.
 - 2. Two levels of Controls (high and low) should be run for each test each day the test is run, and always before any results are being reported.

3. Controls should also be run whenever a new bottle of reagent is loaded or recalibration of a test is performed.
4. Careful handling and preparation of the control sera is a must.
5. A report of the day's QC sera should be reviewed before any results are given out and entered in the register every day.

V. COMMON CONTROL RULES

Clinical laboratories use a variety of control rules based upon specifics of each individual system or analyte tested. See individual SOPs for control tolerance limits.

VI. CONTROL EVALUATION AND ACCEPTANCE CRITERIA

- A. Qualitative Controls—are acceptable when they perform according to their expected value, i.e., positive or negative.
- B. Semi quantitative Controls—are acceptable when they fall within their established control ranges. Results are usually reported as: a number in a range, positive or negative, small, moderate, or large, etc.
- C. Quantitative Controls—are acceptable when they meet the following guidelines:
 1. Controls consisting of 2 and 3 levels—All levels of the control are within + 2 SD of the meanOr
 2. Controls consisting of 3 levels—Two levels of the control are within + 2 SD of the mean and the other is within + 3 SD. When this situation occurs, review the previous QC run and ensure the control which is out of the + 2 SD (but within the + 3 SD) was not out of the + 2 SD previously.

VII. CONTROL REJECTION CRITERIA

- A. Qualitative Controls—are unacceptable if they do not perform according to the expected value.
- B. Semi quantitative Controls—are unacceptable if they do not fall within the established control range.
- C. Quantitative Controls—are unacceptable if:
 1. Controls consisting of 2 levels—if one level of the control is + 2 SD and the other level is >2 SD.

2. Controls consisting of 3 levels:
 - If one level is + 2 SD and the other two levels are >3 SD.
 - If two levels are + 2 SD and other level is >3 SD.
 - If two levels are + 2 SD and the other level is between + 2 SD and + 3 SD on two successive runs.

VIII. PROCEDURE TO TAKE WHEN CONTROLS ARE OUT

This is a generalized order that can be utilized to resolve control problems. Follow these steps sequentially; if the problem is not alleviated, go to the next step. If values are still out of control, inform your supervisor.

- A. Step 1. Rerun controls. If when repeated the result fall within the range, the patient results may be reported. If they are still out, go to Step 2.
- B. Step 2.
 1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
 2. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable. If this does not solve the problem, go to Step 3.
- C. Step 3. Prepare Fresh Control(s). If the repeated control(s) are still outside the established range, prepare new control(s) and try again. If the controls are still out, check the operator's manual. Go to Step 4.
- D. Step 4. Recalibrate instrument. If controls are still out, inform your supervisor.
- E. Step 5. Contact Medical Maintenance where applicable, or servicing engineer.

In cases where the QC value is falling outside the limit of the established range (per the manufacturer), the following corrective actions should be taken:

1. Repeat Same Control(s). If when repeated the result fall within the range, the patient results may be reported.
2. Prepare Fresh Control(s). If the repeated control(s) are still outside the established range, prepare new control(s) and try again.
3. Re- Calibrate (where applicable). If the results of fresh control(s) are out of range, re-calibrate the test(s) in question and try the control(s) again
4. Prepare Fresh Reagents. If the controls are still out of range, prepare new reagents, re-calibrate and try the control(s) again. If the results are acceptable, repeat the test with patient specimens.

Note: If none of the above works, call for Company Service.

IX. ADMINISTRATIVE NOTES

- A. All technical SOPs will define the quality control material being utilized in addition to what is determined to be acceptable performance.
- B. This SOP establishes the minimum guidelines to follow. Specific guidelines are addressed in the appropriate technical SOPs.
- C. Factors to consider for out of control values: contaminated reagents, calibration, and pipettes or technical technique.
- D. Any deviation from these guidelines must be approved by the head of the laboratory or his/her representative.

X. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

**Coast Provincial General Hospital
Antiretroviral Programme**

**Haematology
Standard Operating Procedures**

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Complete Blood Cell Count (CBC) by Coulter Analyser	
Lab SOP No.: 301	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

- A. The Coulter is a quantitative, automated haematology analyser that calculates WBC count, red blood cell count, haemoglobin concentration, haematocrit, platelet count, mean corpuscular volume, and mean corpuscular haemoglobin concentration. This provides information concerning the oxygen-carrying capability of haemoglobin, vascular integrity, and the presence of infection in the body.
- B. A suspension of blood cells is passed through a small aperture simultaneously with an electric current. The individual blood cell passing through the aperture introduces an impedance change in the aperture determined by the size of the cell. The system counts the individual cells and provides the cell size distribution. The lytic reagents rapidly and simultaneously destroy the erythrocytes and convert haemoglobin to a stable cyanide-containing pigment whose absorbance is directly proportional to the haemoglobin concentration of the sample.

II. SPECIMEN

A salt (K₂, K₃, or Na₂) EDTA anticoagulated blood sample not more than 24 hours old and at least 2 ml.

III. REAGENTS AND EQUIPMENT

- A. Coulter A^CT Pack or Trainer, which contains:
- Reagent 1 diluent
 - Reagent 2 lytic reagent
 - Reagent 3 shutdown diluent
- B. Coulter computer software card
- C. Printer
- D. Coulter Analyser
1. Complete the preliminary procedures, reproducibility check, and carry-over to ensure that the instrument measures blood parameters consistently.
 - a. Set analysing mode to whole blood.

- b. Cycle one fresh, normal, properly mixed whole blood sample to prime the instrument.
- c. Analyse one fresh, normal, properly mixed whole blood specimen 10 times; record results for WBC, RBC, Hb, MCV, and platelets; and calculate the mean, standard deviation, and CV% for the 10 cycles.
- d. The CV% of WBC and RBC should be $\leq 3.0\%$, HB and MVC should be $\leq 2.0\%$, and Plt should be $\leq 7.0\%$.

$$\text{CV\%} = (\text{standard deviation} \div \text{mean}) \times 100$$

- e. Perform the carry-over check by pressing the aspirate switch 3 times, and record the results during each cycle and compute the carry-over as follows:

$$\text{Carry-over} = \frac{\text{first cycle} - \text{third cycle}}{\text{Result \# 10 reproducibility check}} \times 100$$

For WBC, RBC, Hb, and Plt, the carry-over percentage should be $\leq 2.0\%$.

2. Shut down the Coulter Analyser in a shutdown diluent. Perform startup.

IV. QUALITY CONTROL

- A. Use S-CAL calibration kit provided by Coulter. Perform calibration checks when necessary or every 6 months. You will need the following:
 1. S-CAL calibration sheet provided with correct assigned values for the calibrator.
 2. S-CAL calibration sample provided in a closed vial (Coulter 4C PLUS cell control).
 3. Check that you have enough supply of reagent to complete calibration.
- B. Conduct the calibration check:
 1. Prepare the S-CAL calibrator according to the instructions in the S-CAL calibrator package insert.
 2. Mix the Coulter 4C cell control vial gently and thoroughly before opening the vial.
 3. Hold the tube to the probe and allow the probe to aspirate the sample and to move up into the instrument. Remove the tube when you hear the audible alarm.
 4. Use of personal protective equipment (PPE) and universal precautions is required when handling biological specimens.

5. Complete the reproducibility check and carry-over check before calibration.
6. Allow the Coulter to analyse and give the results on the screen.
7. Record results for WBC, RBC, Hb, MCV, and Plt on the S-CAL calibration worksheet.
8. Calculate the mean for each parameter using the current and previous Coulter 4C cell control sample results.
9. Copy the S-CAL calibration assigned value from the package insert on the worksheet.
10. Calculate the absolute difference between the assigned value and the mean value calculated above and write this number in the provided row (row C) of the calibration worksheet.
11. Determine the calibration status for each parameter and compare the absolute difference to the Coulter series calibration criteria table in the S-CAL calibrator kit.
12. If the absolute difference is less than the value in column 1, no calibration is required and the calibration for that parameter has therefore been verified.
13. If the absolute difference is between the values found in column 1 and column 2, calibration is then required and new calibration factors need to be calculated as follows:
 - a. Obtain the current calibration factors that are in memory in the instrument for the parameter that requires calibration.
 - b. Write these factors down on the S-CAL calibration worksheet under the current calibration factor.
 - c. Calculate the new calibration factor by dividing the assigned value by the mean value; then multiply this number by the current calibration factor.
$$\text{New calibration factor} = \frac{\text{Assigned value}}{\text{Mean value}} \times \text{Current calibration factor}$$

Enter the new values on the calibration factors screen in the instrument.
 - d. Verify that the calibration is acceptable by analysing 4C PLUS cell control or whole blood with known values or other quality control materials. The control results should fall within the expected ranges. If the control results are not within the expected range, run one more sample. If the second sample results are not within the expected range, follow the procedure in the control package insert and contact your Coulter representative.

V. PROCEDURE

A. Sample preparation:

1. Switch on the Coulter Analyser. The switch is located on the right-hand side. The instrument will aspirate the diluent twice and indicate blank values for WBC, RBC, Hb, and Plt.
2. Enter the patient ID or sample ID and check its correctness on the touch screen display.
3. Mix the sample gently but thoroughly before cycling it by inverting the sample at least 8 times.
4. Hold the tube up to the probe with the probe well into the sample.
5. Allow the probe to aspirate the sample and to move up into the instrument.
6. Remove the tube when you hear the audible alarm.

B. Analysis:

1. Allow the Coulter to analyse and give the results on the screen of WBC, RBC, Hb, and Plt.
2. Note the values indicated. Abnormal low values may indicate incomplete aspiration. If this happens, cycle the sample again.
3. Ignore high and low flag is next to the 4C PLUS cell control results.
4. If any result is over the linearity range of + or over the operating range of +++++, run a blank sample.
5. Wait for cycle to finish before running another sample.
6. Print out the results on the printer of the system.

VI. RESULTS

A. Normal values:

1. WBC:..... $4.3-11 \times 10^9/L$
2. RBC:..... $4.5-6.3 \times 10^{12}/L$
3. HGB:.....12-18 g/dL
4. HCT:.....35.0-60.0
5. MCV:.....80.0-99.9 fl
6. MCHC:.....27.0-31.0%
7. Plt:..... $140-440 \times 10^9/L$

- B. HCTs with values less than 30% should be rechecked by microhaematocrit method.
- C. A differential should be performed on WBC counts less than $3 \times 10^9/L$ and greater than $15 \times 10^9/L$.
- D. Granulocytes with absolute count less than $4 \times 10^9/L$ and greater than $10 \times 10^9/L$ should be confirmed by differential.

VII. LIMITATIONS

- A. Haematology parameters—readings are valid only within the following parameters:
 - 1. Haematocrit: 25–55%
 - 2. White cell count: $2.0\text{--}30.0 \times 10^9/L$
 - 3. Platelet count: $80\text{--}600 \times 10^9/L$
- B. Venous sample—EDTA-anticoagulated blood not more than 4 hours old.
- C. Specifications:
 - 1. Operational temperature: 16–35°C
 - 2. Relative humidity: 30–85% noncondensing
 - 3. Power: 100–240 volts, 50/60 Hz, 6 amps

VIII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Manual White Cell Differential Count and Platelet Estimate	
Lab SOP No.: 302	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

The differential white cell count is performed to determine the relative number of each type of white cell present in the blood. This provides valuable information concerning infections and other disease processes. At the same time, a study of red cell, white cell, and platelet morphology is performed. Performing the differential smear after counting the cells allows the smear to be used as a double-check of the white cell count and platelet count.

II. SPECIMEN

Whole blood from a capillary puncture or EDTA tube at least 3/4 full.

III. REAGENTS AND EQUIPMENT

- A. Microscope
- B. Immersion oil
- C. Stain: Leishman or Giemsa stain
- D. Differential cell counter

IV. QUALITY CONTROL

- A. Slide.
 1. The RBCs should appear buff pink to orange.
 2. WBCs should have a blue nucleus with a lighter-staining cytoplasm.
- B. Technicians should be evaluated regularly on their ability to perform a differential. Results and appropriate actions taken should be documented.

V. PROCEDURE

- A. Differential count:
 1. Stain the slide with appropriate stain (See SOP 303: *Preparation and Staining of Blood Films*) and place the slide on the microscope stage with the smear side up and focus using the low power objective and low light.

2. Scan the blood smear, noting any unusual or irregular cells or rouleaux formation.
3. Locate the portion of the smear where there is no overlapping of cells (thin area).
4. Place a drop of immersion oil on the slide.
5. Carefully change to the oil immersion objective (100×), focus and increase light intensity as needed.
6. Begin in the thin area of the smear.
7. Scan the slide in a figure “S”.
8. Counts:
 - a. White cell counts:
 - (1) For WBC counts less than 20×10^9 WBC/L, count and classify 100 cells.
 - (2) For WBC counts of 20 to 50×10^9 WBC/L, count and classify 300 cells.
 - (3) If there is an abnormal percentage of cells in the differential count, classify 200 cells.
 - (a) More lymphocytes than neutrophils (except in children).
 - (b) Over 11% monocytes.
 - (c) Over 10% eosinophils.
 - (d) Over 2% basophils
 - b. Nucleated red blood cells (NRBCs):
 - (1) Count number of NRBCs per 100 WBCs on a separate counter; report.
 - (2) Recalculate the WBC count if the NRBC count is greater than 5 NRBCs per 100 WBCs.
9. Report any abnormalities of white cells.

B. Platelet estimate:

1. Scan the thin area, using the oil immersion lens.
2. Observe 10 fields, counting the platelets in each field, observing granulation and morphology.

3. Determine the average number of platelets observed per oil immersion field (OIF).
4. Report the platelet estimate and any abnormal morphology.

C. WBC estimate:

1. Scan the thin area, using the 50x oil immersion lens.
2. Observe 10 fields, counting all WBCs in each field.
3. Average the number of WBCs seen per oil immersion field (OIF).
4. Document performance of WBC estimate. Report any discrepancies to supervisor.

D. RBC morphology:

1. Scan the thin area, using the oil immersion lens.
2. Observe 10 fields.
3. Report RBC size and shape. Use the nucleus of a typical small lymphocyte as a comparison for normal size
4. Report any alterations in colour, the amount of haemoglobin, or inclusions.

E. Save all differential slides for 7 days.

VI. RESULTS

A. Leukocyte differential normal values:

- | | |
|---------------------------|--------|
| 1. Segmented neutrophils: | 54–62% |
| 2. Lymphocytes: | 20–40% |
| 3. Band neutrophils: | 1–10% |
| 4. Monocytes: | 4–10% |
| 5. Eosinophils: | 1–3% |
| 6. Basophils: | 0–1% |

B. NRBCs reported as number of NRBCs per 100 WBCs counted.

C. Platelets estimate:

- | | |
|--------------------|--------------|
| 1. Adequate/normal | 8–20 plt/OIF |
| 2. Decreased | < 8 plt/OIF |
| 3. Increased | > 20 plt/OIF |

D. WBC estimate: $\pm 1.5 \times 10^9$ WBC/L of WBC count

E. RBC morphology:

1. Normal findings:
 - a. Normocytic: normal cell size and shape
 - b. Normochromic: normal hemoglobin content and colouration
2. Abnormal findings:
 - a. Use specific terms
 - b. Grade degree of abnormalities
 - (1) Slight: 1–5 cells/10 fields
 - (2) Moderate: 6–15 cells/10 fields
 - (3) Marked: > 15 cells/10 fields

VII. PROCEDURAL NOTES

A. RBC morphology

1. Anisocytosis: variation in size of the cells
 - a. Macrocyte: larger than normal
 - b. Microcyte: smaller than normal
2. Poikilocytosis: variation in shape of the cell
 - a. Drepanocyte (sickle-like cell)
 - b. Ovalocyte (elliptocyte)
 - c. Spherocyte
 - d. Dacrocyte (teardrop cell)
 - e. Echinocyte (burr cell)
 - f. Stomatocyte (cup cell)
 - g. Codocyte (target cell)
 - h. Schizocyte (red blood cell fragments)
 - i. Acanthocyte (irregularly spaced projections)
 - j. Crenated cells (mechanically produced, not reported)
3. Alteration in colour
 - a. Hypochromasia—increase central pallor
 - b. Polychromasia—diffuse basophilia
4. Inclusions
 - a. Basophilic stippling
 - b. Howell-Jolly bodies
 - c. Cabot's rings
 - d. Malaria

VIII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Erythrocyte Sedimentation Rate (ESR) by Westergren Method	
Lab SOP No.: 303	Version No.: 1.0
Date prepared:	Date adopted:

I. PRINCIPLE

Anticoagulated whole blood placed in a tube will separate into an upper plasma layer and a lower cell layer as a result of gravity. The ESR is the distance at which the cells settle out in a given time period and is expressed in mm/hr.

II. REAGENTS

- A. Westergren tubes
- B. Westergren vertical racks

III. PROCEDURE (Westergren procedure)

- A. Dilute blood collected in EDTA tubes with 3.8% sodium citrate in a dilution of 1:5.
- B. Place Westergren tube in vertical Westergren rack.
- C. At the end of 60 minutes, record the distance.

IV. REFERENCE RANGES

- A. Males: 0 to 7 mm/hr
- B. Females: 0 to 9 mm/hr

V. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
 Full name: _____
 Designation: _____

Section Supervisor

Signed: _____ Date: _____
 Full name: _____
 Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Handling Specimen for CD4+ T Cell Enumeration	
Lab SOP No.: 304	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Obtaining accurate and reliable measures of CD4⁺ T lymphocytes (CD4⁺ T cells) is essential for assessing the immune system and managing the health care of persons infected with human immunodeficiency virus (HIV).
- B. Absolute CD4⁺ T lymphocytes (CD4⁺ T cells) can be measured from whole blood collected on K₃EDTA anticoagulant.

II. SPECIMEN

- A. Collect blood specimens by venipuncture into evacuated tubes containing K₃EDTA anticoagulant, completely expending the vacuum in the tubes.

Use paediatric tubes to obtain specimens from children. Ensure that the tube is full.

Mix the blood well with the anticoagulant to prevent clotting.
- B. Label all specimens with the date, time of collection, and a CCC patient number. Ensure that patient information and test results are kept confidential.
- C. Place the samples on a gentle blood rocker to ensure that the samples are uniformly distributed while awaiting transportation to the laboratory.

III. SPECIMEN TRANSPORT

- A. Maintain and transport specimens at room temperature (64–72°F [18–22°C]). Specimens should not be exposed to extreme temperatures that could allow them to freeze or become too hot. Temperatures >99°F (37°C) might cause cellular destruction and affect flow cytometry measurements.

Transport specimens to the immunophenotyping laboratory as soon as possible.
- B. In hot weather, pack the specimen in an insulated container. If necessary, place this container inside another containing an ice pack and absorbent material. This method helps retain the specimen at ambient temperature.
- C. For transport to locations outside the hospital, pack the specimens in a tube containing the leak proof container (e.g., a sealed plastic bag) and pack this container inside a cardboard canister containing sufficient material to absorb all

the contents should the tube break or leak. Cap the canister tightly. Fasten the request slip securely to the outside of this canister with a rubber band.

- D. Arrange appropriate times of collection and transport from the hospital collecting the specimen. This should be by 2:00 p.m.

IV. SPECIMEN INTEGRITY

Before and on delivery of the specimen, inspect the tube and its contents to verify that the specimen is suitable for CD4+ T cell determination.

- A. Check the temperature of the specimen. If the specimen is not hot or cold to the touch, and if it is not obviously haemolysed, or frozen, allow it to be processed; however, the temperature condition should be noted on the worksheet and report form.
- B. The specimen should not be rapidly warmed or chilled to bring it to room temperature because this may adversely affect the immunophenotyping results. Abnormalities in light-scattering patterns may reveal a compromised specimen.
- C. If blood is haemolysed or frozen, the specimen should NOT be transported to the testing laboratory. Another specimen should be taken.

If the specimen has stayed for >72 hours after collection, it should NOT be transported to the testing laboratory. Another specimen should be taken.

- D. Tests should be performed within 48 hours (preferred), but no later than 72 hours after drawing the blood specimen.
- E. Samples should be placed on a gentle blood rocker for 5 minutes to ensure that the samples are uniformly distributed before processing.

V. UNIVERSAL PRECAUTIONS

Use universal precautions with all specimens. Adhere to the following safety practices:

- A. Wear laboratory coats and gloves when processing and analysing specimens.
- B. Never pipette by mouth. Use safety-pipetting devices.
- C. Never recap needles. Dispose of needles and syringes in puncture-proof containers designed for this purpose.
- D. After working with specimens, remove gloves and wash hands with soap and water.

VI. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
CD4+/CD8+ T-Lymphocyte Cell Determination by Cytoflow	
Lab SOP No.: 305	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. Obtaining accurate and reliable measures of CD4⁺ T lymphocytes (CD4⁺ T cells) is essential for assessing the immune system and managing the health care of persons infected with human immunodeficiency virus (HIV).
- B. Absolute CD4⁺ T lymphocytes (CD4⁺ T cells) can be measured from whole blood collected on K₃EDTA anticoagulant.

II. PRINCIPLE

CyFlow two-colour reagents use a time-saving No Lyse–No Wash method for direct immunofluorescence staining of human peripheral blood specimens. When whole blood specimen is added to test tubes (Partec) containing CyFlow reagents, the fluorochrome-labelled antibodies bind specifically to antigens on the surface of lymphocytes. The CyFlow cytometer detects forward scattered (FSC) and side scattered (SSC) light from the cells to give the appropriate volumetric cell-counting event. A sample passes the flow cuvette where the detection takes place. The upper sensor electrode starts the counting process when the meniscus of the sample is disconnected, whereas the lower sensor electrode stops the measurement in the same way. The measuring volume of 200 µl is defined by the distance of the electrodes and the inner diameter of the sample tube. The cell concentration is automatically calculated by the FlowMax software as a function of counted events in 200 µl volume.

III. SPECIMEN

- A. Collect blood specimens by venipuncture into vacutainer tubes containing K₃EDTA anticoagulant, completely expending the vacuum in the tubes. Label all specimens with the date, time of collection, and a CCC patient number. Ensure that patient information and test results are accorded confidentiality. Use paediatric tubes to obtain specimens from children, and ensure that the tube is full. Mix the blood well with the anticoagulant to prevent clotting.
- B. Place the samples on a gentle blood rocker to ensure that the samples are uniformly distributed when awaiting transportation to the laboratory.

IV. REAGENTS AND MATERIALS

A. Equipment

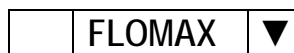
1. Flow cytometer: Partec CyFlow
2. Computer Monitor
3. Printer HP 5550
4. FlowMax software
5. Pipette calibrated to deliver 50 µl of sample and 10 µl of reagents.

B. Consumables

1. Sheath fluid
2. Dilution buffer
3. CD4 monoclonal antibody: CD4 No Wash–No Lyse Kit
4. CD8 monoclonal antibody: CD8 No Wash–No Lyse Kit
5. Partec tubes
6. Distilled/deionised water
7. Test tube racks
8. Latex gloves
9. Pipette tips

V. CALIBRATION

- A. Under setup, go to [Partec FloMax]. Click **OK** to initialize instrument. A new file will open on the screen. From the file menu, close this file.
- B. When the screen goes blank, go to file menu and open the window screen again.
- C. Select FlowMax folder in the look-in window:



- D. Select **calibration template for beads.FCS** and open file. The screen will open with the previous calibration graphs.
- E. Select instrument settings from the panel at the bottom of the screen. The graph peaks will clear, and a window for settings will open; select **LOAD** from the left bottom screen. The window will open with the FlowMax folder files.
- F. Select **3µm beads calibration file** and open the file. The screen will open, indicating graphs for calibration, but without peaks.
- G. Run detergent or cleaning solution (green) for some time and then run calibration beads. Ensure proper mixing of beads before running.
- H. Get the peaks in the RNI region identified in the graphs. If not, use the micrometer screw to bring the peaks into the region. Do not make any other adjustment in the gain levels.

	Parameter	GAIN	Log	Lower level	Upper level
Speed 2.2	SSC	180	Log 3	10	999.9
	FLI	310	Log 4	170	999.9

- I. Both peaks should be at approximately 100 in the FLI graph and the SSC graph. If peaks are not narrow, check for an air bubble in flow cell.
- J. After running beads, run cleaning solution again to clear the beads.
- K. Close the file from the File Menu. When the window for change appears, click **NO** changes to be made.
- L. Open blood template from file. The screen will open with the template graphs. Go to instrument setting from panel below screen and go to **LOAD** → **blood analysis.ist file-m**. Open from FlowMax folder. The equipment is ready to run samples.

VI. PROCEDURE

A. Sample preparation:

1. Label two Partec tubes for each specimen/test with the same ID number as the sample tube. Then mark the two tubes A and B.
2. To tube A add 50 µl of whole blood and 10 µl CD4 PE reagent, using the appropriate pipette. To B add 50 µl of whole blood and 10 µl CD8 PE reagent, using the appropriate pipette.
3. Mix the two tubes gently and incubate them for 15 minutes in the dark.
4. Into each tube add 0.8 ml of dilution buffer and shake or vortex gently.
5. Carry out a count analysis on the CyFlow system.

***NOTE:** Samples can be stained 24 hours after blood collection and analysed 24 hours after staining with no loss in precision. Unstained samples must be kept at room temperature.*

B. Starting up CyFlow:

1. Fill sheath bottle with clean, filtered, and degassed sheath fluid and close it lightly with the screw top. Empty the waste bottle.
2. Switch **ON** the CyFlow by switching on the power at the back.

3. Switch on the peripheral devices.
 - a. Switch on the HP printer.
 - b. Switch on computer by the computer power switch. After a short while the computer display shows Windows® desktop.
4. After the system starts up, double click **Partec FlowMax** icon and click **OK**. The system will initialise the FlowMax software.
5. Go to file and close the file again. Select and open the blood template FCS file. Go to instrument settings and load **Blood analysis.ist** file.
6. After a few seconds, display shows FlowMax welcome window. Click **OK** to display empty histogram. The equipment is ready to run samples from the acquisition panel.

C. Running the samples:

Make sure that the flow cytometer is ready for analysis and operating software is ready for the measurement.

1. From the acquisition panel, select run tube panel and open patient sample folder.
2. Type patient name and click **OK**. When the tube panel screen opens, click **OK**.
3. Run CD4 sample followed by CD8 sample.
4. Fill up the sample tube with 1–2 ml of the ready prepared sample suspension.
5. Insert sample tube onto the sample port until you hear a click. Wait for measurement or acquisition to start. The FlowMax software will indicate **pre run, stabilise, run, and count** status. Wait until cells are counted and acquisition is finished.
 - a. In the prerun phase, cells are quickly transported to the position for analysis, the flow cuvette.
 - b. During the stabilise phase, the system gives time for the flow to reach the slow speed required for analysis.
 - c. In the run phase, the cells are analysed and classified into histogram on the display in real time.
 - d. In the count phase, cells are counted for a given volume.
6. After the count phase, the acquisition finishes automatically. After acquisition remove the sample tube from the sample port.

7. When the report generates, fill in patient details and go to file and save as patient CD4–CD8 report. Type patient’s name in the file and save.

D. Printing Patient Reports:

1. From main screen go to **START** and select **My Documents** folder.
2. Double click to open the patient CD4–CD8 report folder.
3. Select patient file to be printed and double click the file to open. The patient CD4–CD8 report will open.
4. Type patient’s name in the file and click save, and then click on printer icon to print out the report. Print out the report and close the window by clicking **X**.
5. In the file menu select **exit** and click **NO** changes to be saved.
6. Exit the report file and go back to the acquisition menu to run the next patient sample.
7. Wait for biosafety cycle to start to clean the instrument. Save results and put on the next sample by inserting the sample tube onto the sample port.

VII. SHUTDOWN

Make sure data from the previous acquisition have been saved.

- A. Fill a new sample tube with cleaning solution and insert tube onto the sample port and wait until you hear a “click”. The flow cuvette will be floated by the tube fluid.
- B. Perform a biosafety clean by clicking the green icon or the CLEAN button. Repeat “A” and “B” with distilled water.
- C. Let the final sample tube half with some 1 ml clean distilled water connected to the sample port to avoid drying and crystallising of any remaining materials.
- D. Exit the program by clicking the close button **X** in the upper right corner of the window or select FILE EXIT from the file menu.
- E. Turn OFF the computer → turn OFF. The computer will shut down. Switch off analyser, CPU, monitor, laser unit, and printer. Cover the equipment. Check waste and sheath levels.

VIII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Cell Separation: PBMC	
Lab SOP No.: 306	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

This standard operating procedure defines the methodologies to be used to separate plasma and PBMC for patients undergoing both viral load testing and resistance testing.

II. REAGENTS AND EQUIPMENT REQUIRED

- A. Falcon tubes (15 and 50 ml)
- B. RPMI 1640 with L-glutamine and HEPES (Gibco BRL)
- C. Fetal calf serum, heat inactivated at 56°C for 30 minutes
- D. Antibiotics/antifungals
- E. Cell-culture grade dimethyl sulfoxide (DMSO) sigma
- F. Histopaque
- G. Cryovials (Nunc. 363401)
- H. Cryopens
- I. Trypan Blue
- J. Centrifuge tubes
- K. Counting chambers
- L. Controlled rate freezer (cryosaver)
- M. 70% ethanol
- N. 1% Jik (hypochloride)
- O. NaHCO₃

III. PREPARATION OF RPMI 1640 SOLUTION

- A. Weigh 16.2 g of RPMI 1640 powder.
- B. Weigh 2.0 g of NaHCO₃ and dissolve in 950 ml of distilled water.
- C. Adjust pH to be between 7.2 and 7.4, using 5 M of NaOH.
- D. Top up to 1 L with distilled water.
- E. Sterilise filter.
- F. Always keep at 4°C when not in use.

IV. PREPARATION OF RIO (10% FCS)

Add 100 ml of FCS to 900 ml of RPMI 1640 solution into a sterile bottle; mix well and keep at 4°C.

V. PREPARATION OF FREEZING MEDIUM:

Add the following in a sterile 50 ml falcon tube:

RPMI 1640 = 20 ml = 40%

FCS = 25 ml = 50%

MSO = 5 ml = 10%

50 ml = 100%

or

FCS = 45 ml = 90%

DMSO = 5 ml = 10%

50 ml = 100%

VI. METHODS:

- A. The hood should be cleaned with 70% alcohol and turned on 10 minutes before starting.
- B. Blood is taken from patients in vacutainer tubes with heparin (green top).
- C. Add 10 ml of histopaque solution into a 50 ml labelled sterile tube.
- D. Using a 10 ml pipette, overlay 25 ml of whole blood on the histopaque solution. The over-layering can be done by holding the tube containing the histopaque solution at an angle and gently running the blood down the side of the tube. As the tube fills up, the tube is brought to the upright position.
- E. Centrifuge the tube at 3,000 rpm for 20 minutes at room temperature with brakes off, making sure the tubes are properly balanced.
- F. Inspect the tube to make sure there is a distinct mononuclear fraction.
- G. Gently pipette 1.5 ml of plasma into each of the 2 serum vials.
- H. Store plasma samples at -70°C in the appropriate box and record the location in the laboratory book.
- I. With a sterile transfer pipette, gently reach the monolayer fraction and pipette the entire "band" of lymphocytes into a 15 ml sterile tube.
- J. Add 10 ml of R10 wash medium to the lymphocytes. Invert the tube several times and centrifuge at 1,500 rpm for 10 minutes at room temperature with brakes on. Flick the cell pellet and resuspend in 10 ml of R10.

- K. Spin at 1,500 rpm for 10 minutes at room temperature. Decant the supernatant and resuspend the pellet in 10 ml of R10.

- L. Perform cell count using Trypan Blue method and prepare the rest of the cells for freezing.

VII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Preparation and staining of Blood Films	
Lab SOP No.: 307	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. A thin blood film is a small drop of blood that is spread thinly on a glass slide so that the red cells do not overlap. In this way, individual blood cells can be examined.
- B. The differential white cell count is performed to determine the relative number of each type of white cell present in the blood. This provides valuable information concerning infections and other disease processes. At the same time, a study of red cell, white cell and platelet morphology is performed. Performing the differential smear after counting the cells allows the smear to be used as a double-check of the white cell count and platelet.

II. SPECIMEN

Whole blood from a capillary puncture or EDTA tube at least 3/4 full. The capillary venous blood or salt EDTA anticoagulated, blood should not be more than 4 hours old

III. MATERIALS

- A. Surgical gauze
- B. Absolute ethanol
- C. Anticoagulated blood
- D. Pasteur pipette/ applicator sticks
- E. Cover slide
- F. Leishmans stain
- G. Distilled water
- H. Staining rack
- I. Forceps

IV. PROCEDURES

- A. Precautions and specifications
 - 1. Ensure that you wear a lab coat and gloves
 - 2. Ensure that other universal precautions are taken care of when handling biological specimens.

3. Ensure that the operational temperature is between 16 and 35°C and the relative humidity is between 30 and 85% non-condensing
4. Spillage of potentially infectious materials or positive control should be removed immediately with absorbent tissue paper, and the contaminated area be swabbed with appropriate strength disinfectant e.g. 1.0 % sodium hypochlorite. Sodium hypochlorite should not be used to clean acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially bio-hazardous waste.

B. Sample preparation

1. Report on the Manual FBC worksheet the subject number of the samples to be processed during this run.
2. Clean a glass slide with gauze soaked in absolute ethanol. Allow the slide to dry.
3. Mix the blood well.
4. From anticoagulated venous blood using a clean Pasteur pipette with a rubber teat suck and drop a small drop of blood on to the slide, about 1cm from one end, Place the slide horizontally on a flat surface. If using a sample of capillary venous blood, carefully touch the drop of blood on to the slide, about 1cm from one end, making sure that the slide does not touch the patient's skin.
5. Hold a spreader (or cover slip) in the centre of the slide at an angle of 50⁰C.
6. Move the spreader back until it is in contact with the blood, and allow the blood to spread along the base of the spreader.
7. Now reduce the angle of the spreader to about 30⁰C and move the spreader steadily across the slide. A good thin film should end in a conical "tail" and should cover two-thirds of the slide.
8. If the drop of blood is too large, transfer a small portion of blood by touching it with the edge of the spreader and start the film in a fresh place ahead of the large drop. If the blood is anaemic, hold the spreader at a greater angle and move it faster. This makes a slightly thicker film.
9. Dry the film quickly by waving it in the air. Slow drying results in shrinkage of red cells. Do not dry the film by heating over a flame or placing on a hot object.
10. Label the slide with the patient's study number by writing with lead pencil across the thickest part of the film or on the frosted end of the slide.

C. Staining procedure

1. Fix the film by dipping for 2 seconds in absolute methanol. Allow drying.
2. Stain using leishmann stain by flooding the slide for 1 to 3 minutes.

3. Dilute the stained slide with buffer (ph 6.8) for 4 to 7 minutes.
4. Using a pair of forceps, hold one end of the slide and rinse with water to remove excess stain.
5. Keep the slides on the rack in a standing position and allow drying for 5 minutes before doing differential counting.

D. Analysis

1. Do the differential blood count(See SOP 302 Manual White Blood Cell Differential Count And Plate Estimate)
2. Report the results on the Differential blood count and HCT worksheet

V. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

Section Supervisor

Signed: _____ Date: _____
Full name: _____
Designation: _____

**Coast Provincial General Hospital
Antiretroviral Programme**

**Molecular Methods
Standard Operating Procedures**

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Specimen Collection, Storage, and Delivery for Viral Load Testing	
Lab SOP No.: 401	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. This standard operating procedure defines the procedures to be followed when taking, handling, and transporting specimens for viral load testing to a reference or designated laboratory. The plasma viral load is the chief predictor of the risk of heterosexual transmission of HIV-1 and for progression of HIV disease.
- B. The plasma viral load can be measured by the RT-PCR method, bDNA method, or NASBA method. All use different methods but give comparable results. The AMPLICOR HIV-1 MONITOR uses plasma specimens only.
- C. Collect blood in sterile tubes using EDTA (lavender top) or ACD (yellow top) as the anticoagulant. Do not collect specimens in heparin-containing containers (the purple top). Handle all specimens as if they are capable of transmitting infectious agents.

II. GENERAL INSTRUCTIONS

- A. Use sterile technique; close all containers tightly.
- B. Label all containers with patient's name, hospital number, date and time of collection, and physician's name.
- C. Specimens should be delivered to the receiving laboratory within 24 hours of collection.
- D. Always call the receiving laboratory to let them know that the specimen is coming.

III. SPECIMEN COLLECTION

- A. Prior to drawing the specimen, confirm that the information on the request form is correct.
- B. Properly identify the patient. If patient is an outpatient, ask him/her to state his/her full name and date of birth. If patient is an infant or confused adult, ask the person accompanying the patient for identification.
- C. If there is a discrepancy or difference in any of the above information, **DO NOT PROCEED**. Take the corrective action necessary to ensure that the patient name and number on the request form are identical.

- D. If there is no discrepancy or difference after the identification process is completed, draw the specimen.
- E. Determine appropriate tube type and correct blood volume for the test. This can either be a lavender or yellow top tube: *Use a lavender tube and draw 4 ml of blood only for viral load testing.*
- F. After drawing blood but before leaving the patient, label the tube(s) with the patient's number, physician's name, CCC number, patient's diagnosis, test needed, and the date and time the specimen was taken.

IV. TUBES STORAGE INSTRUCTIONS

- A. Do not store whole blood for more than 6 hours after collection. Even in temperatures between 2 and 25°C, whole blood should not be left for more than 6 hours.
- B. Separate plasma from whole blood within 6 hours as follows:
 - 1. Centrifuge whole blood at 800–1600 × g for 20 minutes at room temperature.
 - 2. Aliquot 225–250 µl of plasma in a sterile 2 ml polypropylene screw-cap tube.
 - 3. Transport plasma at room temperature within 24 hours. If this is not possible, store at 2–8°C and transport within 5 days. If plasma has been frozen at –80°C, make sure it is transported at the same temperature.
 - 4. Store plasma frozen at –80°C until time for transport.

V. DELIVERY INSTRUCTIONS

- A. Package the specimen carefully to protect it from breakage and insulate it from extreme temperature.
- B. Label for “viral load.”
- C. Samples should be transported to the receiving laboratory by courier.
 - 1. Within the CPGH, send sample to the OPD Lab for courier pickup.
 - 2. CPGH “courier of choice is” (telephone number).
 - 3. During workdays (Mon.–Fri./8:00 a.m.–5:00 p.m.), call the receiving KEMRI lab at telephone number..... to dispatch courier.
 - 4. No transport should be done during weekends and holidays unless prior arrangement has been made with the receiving laboratory.

VI. TRANSPORTATION

Maintain and transport specimens at appropriate temperatures (frozen). During packaging, place the tube containing the specimen in a leak proof container (e.g., a sealed plastic bag) and pack this container inside a cardboard canister containing sufficient material to absorb all the contents should the tube break or leak. Cap the canister tightly. Fasten the request slip securely to the outside of this canister with a rubber band.

VII. UNIVERSAL PRECAUTIONS

Use universal precautions with all specimens. Adhere to the following safety practices:

- A. Wear laboratory coats and gloves when processing and analysing specimens.
- B. Never pipette by mouth. Use safety-pipetting devices.
- C. Never recap needles. Dispose of needles and syringes in puncture-proof containers designed for this purpose.
- D. Handle and manipulate specimens (e.g., aliquot, add reagents, vortex, and aspirate) in a Class I or II biological safety cabinet. Centrifuge specimens in safety carriers.
- F. After working with specimens, remove gloves and wash hands with soap and water.

VIII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Sample Preparation for Storage for Viral Load Analysis by Amplicor HIV-1 Monitor	
Lab SOP No.: 402	Version No.: 1.0
Date prepared:	Date adopted:

I. INTRODUCTION

- A. The plasma viral load is the chief predictor of the risk of heterosexual transmission of HIV-1 and for progression of HIV disease.
- B. The plasma viral load can be measured by RT-PCR method, bDNA method, or by NASBA method. All use different methods but give comparable results.

II. SPECIMEN COLLECTION

- A. Collect 10 ml of venous blood using an EDTA lavender vacutainer top or an ACD yellow vacutainer top. *Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.*

Please note:

- 1. AMPLICOR HIV-1 Monitor Test version 1.5 uses plasma samples, anti-coagulated with either EDTA or ACD. **Heparin is unsuitable!**
- 2. The volume of blood indicated on the vacutainer tube should not be exceeded.
- B. Gently invert twice each vacutainer tube after collection of blood to ensure mixing of the anticoagulant with blood.
- C. Blood samples can be kept at 20–24°C before processing for not more than 6 hours.

III. SPECIMEN PROCESSING:

- A. Wear dust free (powder less) gloves, and using sterile pipette tips transfer 10 ml of whole blood into 15 ml centrifuge tube.
- B. Centrifuge whole blood at 800–1,600 × G for 20 minutes to separate plasma from whole blood.
- C. Using sterile pipette tips, transfer plasma (the upper layer) in aliquots of 225–250 µl into 1.8–2.0 ml sterile polypropylene screw-cap tubes. Make sure that, for each sample, 4 vials of plasma are prepared.

- D. Label the 1.8–2.0 ml sterile polypropylene screw-cap tubes with date, name of site, patient identification number, and type of specimen (P for plasma).
- E. Place the plasma aliquots of 225–250 µl into cryogenic storage boxes and store them at –80°C.

IV. SPECIMEN STORAGE

- A. Aliquot 225–250 µl of plasma in a sterile 2 ml polypropylene tube screw-cap tubes.
- B. Store the specimen at:
 - 1. 2–8°C for up to 5 days *or*
 - 2. Transport the specimen at 2–8°C to the viral load laboratory *or*
 - 3. Freeze the specimen at –80°C until time for transportation. At –80°C the specimens are better placed in cryogenic storage boxes.

V. TRANSPORTATION

- A. Maintain and transport specimens at appropriate temperatures (frozen).
- B. Package the specimen for transport by placing the tube containing the specimen in a leak proof container (e.g., a sealed plastic bag), and pack this container inside a cardboard canister containing sufficient material to absorb all the contents should the tube break or leak. Cap the canister tightly. Where liquid nitrogen exists, such specimen should be transported using the liquid nitrogen tank or canister.
- C. Fasten the request slip securely to the outside of this canister with a rubber band.

VI. UNIVERSAL PRECAUTIONS

Use universal precautions with all specimens. Adhere to the following safety practices:

- A. Wear laboratory coats and gloves when processing and analyzing specimens.
- B. Never pipette by mouth. Use safety-pipetting devices.
- C. Never recap needles. Dispose of needles and syringes in puncture-proof containers designed for this purpose.
- D. Handle and manipulate specimens (e.g., aliquot, add reagents, vortex, and aspirate) in a class I or II biological safety cabinet. Centrifuge specimens in safety carriers.
- E. After working with specimens, remove gloves and wash hands with soap and water.

VII. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Coast Provincial General Hospital Laboratory Standard Operating Procedures	
Quantitative RNA PCR in Plasma Using Roche Amplicor HIV-1 Monitor Version 1.5	
Lab SOP No.: 403	Version No.: 1.0
Date prepared:	Date adopted:

I. PURPOSE

The purpose of this standard operating procedure is to define procedures to be used in assessing patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels during the treatment.

II. BACKGROUND

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). Within 3 to 6 weeks after exposure to HIV, infected individuals usually develop a brief show of symptoms and show high levels of viremia in the peripheral blood. This is followed by an HIV-specific immune response and a decline in plasma viremia, usually within 4 to 6 weeks of the onset of symptoms. After sero-conversion, infected individuals enter a clinically stable, asymptomatic phase with low levels of plasma viremia. Quantitative measurements of HIV viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV disease and that reductions in plasma viremia may be associated with decreased risk of disease progression. HIV RNA in plasma can be quantified by polymerase chain reaction (PCR).

III. PRINCIPLE OF THE AMPLICOR HIV-1 MONITOR VERSION 1.5

- A. The Amplicor HIV-1 Monitor version (vs.) 1.5 test kit is based on sample preparation and reverse transcription of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HIV-1 specific complementary primers, and hybridisation of the amplified DNA to oligo-nucleotide probes specific to the target(s); and detection of the probe-bound amplified DNA by colorimetric determination.
- B. The standard process involves direct isolation of HIV-1 RNA from plasma by lysis of the viral particles, followed by precipitation of the RNA with alcohol.
- C. A known number of quantified standard RNA molecules are introduced into each specimen with the lysis reagent. The HIV-1 quantification standard (QS) is carried throughout the procedure and is used for quantification of HIV-1 RNA in the specimen.

- D. The reverse transcription and PCR amplification reactions occur in the same reaction mixture. This is followed by the chemical denaturation of the HIV-1 amplicon to form single-stranded DNA by the addition of the denaturation solution.
- E. Following the hybridisation reaction, detection is initiated by washing and addition of Avidin-horseradish peroxidase conjugate that binds to the biotin-labelled amplicon. This is then washed to remove unbound conjugate, and a substrate solution containing hydrogen peroxide is added. This produces colour change; the reaction is stopped by the addition of a weak acid. The optical density (OD) is measured at 450 nm single wavelength. The OD in each well is proportional to the amount of HIV-1 amplicon in each well.

IV. SPECIMEN COLLECTION, HANDLING, AND STORAGE

For sample collection refer to SOP 106: Blood Collection by Venipuncture in Adults and Children.

NOTE:

- A. The AMPLICOR HIV-1 Monitor test vs. 1.5 is for use with plasma samples, anti-coagulated with either EDTA or ACD. **Heparin is unsuitable!**
- B. Samples may be kept for a maximum of 4 hours at room temperature (20–24°C) before plasma is separated from whole blood by centrifugation (800–1,600 × G for 20 minutes).
- C. Whole blood should be transported at 20–24°C. Plasma may be transported at 2–8°C or frozen.
- D. Plasma specimens may be stored at 2–8°C up to 5 days or frozen at –70°C. Store aliquots of 500–1,000 µl in sterile 1.8–2.0 ml polypropylene screw-cap tubes. Plasma specimens may be frozen and thawed up to 3 times.

V. PROCEDURE

A. Procedural precautions:

1. Good laboratory technique is essential to the proper performance of any test to ensure accurate and reliable results.
2. The work-flow of the PCR procedure must begin in the Pre-Amplification area and move to the Post-Amplification area.
3. Different work areas (i.e., hoods) should be dedicated to reagent preparation and specimen preparation.

4. Supplies and equipment must be dedicated to each Pre-Amplification activity and not used for other activities or moved from one area to another.
5. Gloves must be worn in each area and discarded when leaving that area.
6. Equipment and supplies used for reagent preparation **must not** be used for specimen preparations or for pipetting or processing of amplified DNA.
7. Post-Amplification equipment must remain in the Post-Amplification area at all times.
8. To avoid splashing and potential cross-contamination, always use screw-cap tubes (not snap-tops) for specimen and control preparations.

NOTE: Handle all specimens as if they are capable of transmitting infectious agents.

B. Preparations:

All plasma specimens and reagents must be at ambient temperature (20–24°C) before use. Make sure you have sufficient quantities of reagents and specimen before you start the procedure.

1. Extraction: Pre-Amplification Area (Hood in Clean Room)
 - a. Consumable reagents and supplies
 - Roche Amplicor HIV-1 Monitor vs. 1.5 Kit
 - Quantitation Standard (QS)
 - Lysis Reagent
 - Negative Human Plasma (NHP)
 - HIV-1 Negative, Low Positive, and High Positive Controls
 - Specimen diluent **HIV-1 DIL**
 - Clean lab coat
 - Powder-free gloves
 - Wipes
 - 70% Ethanol in a spray bottle
 - 10% Bleach in a spray bottle
 - 70% Ethanol (11 ml 200 proof Ethanol + 4 ml DEPC water) sufficient for 12 tests
 - 50 ml sterile polypropylene tubes
 - Sterile individually wrapped serology pipettes (5 ml, 10 ml, 25 ml)
 - 1.8–2.0 ml sterile screw-cap tubes
 - Tube racks
 - RNase free sterile, aerosol filtered pipette tips for pipettes
 - 100% isopropanol
 - Sterile fine-tip transfer pipettes
 - 2 Beakers of 10% bleach in a 600 ml beaker (25 ml bleach + 225 ml water)

- Bench top autoclave bag holder
- Bench top autoclave bags 8" × 12"

b. Equipment

- Vortex
- Water bath set at 37°C
- Micro centrifuge
- Pipettes (100 µl, 200 µl, 1,000 µl)

c. Procedure:

- (1) Put on clean lab coat and gloves and wipe the hood out with 10% bleach and then 70% ethanol.
- (2) Confirm that reagents and plasma specimens have attained room temperature (RT).
- (3) Prepare sufficient quantity of 70% ethanol in 50 ml sterile polypropylene tubes and vortex.
- (4) Vortex lysis reagent and place in a 37°C water bath for approximately 30 minutes to dissolve all crystals.
- (5) Label one 1.8–2.0 ml V-bottom screw-cap tube for each sample and three additional tubes for HIV-1 Negative, High Positive, and Low Positive controls.
- (6) Prepare Standard Working Lysis Reagent. (This is enough for 12 specimens/controls.)
 - (a) Vortex Lysis Reagent and QS for 15 seconds.
 - (b) Add 100 µl of QS to one bottle of Lysis reagent using a pipette µl 100 and mix well.

NOTE: *The Standard Working Lysis reagent is stable for 4 hours.*

- (7) Add 600 µl standard lysis reagent to each labelled tube using 1,000 µl pipette and cap the tubes.
- (8) Prepare the controls:
 - (a) Vortex NHP + the negative, low, and high-positive controls for 5 seconds.
 - (b) Add 200 µl NHP in each of the three control tubes using a 200 µl pipette.

- (c) Add 50 µl of the Negative, Low, and High Positive controls in the tubes labelled accordingly using a 100 µl pipette. *Do not have more than one tube open at a time.*
- (9) Vortex for 5 seconds.
 - (a) Vortex plasma specimens for 5 seconds.
 - (b) Add 200 µl of each specimen to the appropriately labelled tube using 200 µl pipette with sterile, filtered tips. *Do not have more than one tube open at a time.*
 - (c) Cap the tubes and vortex for 5 seconds.
- (10) Incubate the specimens and control tubes for 10 minutes at room temperature. Add 800 µl isopropanol to each tube, re-cap, and vortex for 5 seconds using a 1,000 µl pipette. *Do not have more than one tube open at a time.*
- (11) Put an orientation mark on each tube.
- (12) Place the tubes in the micro centrifuge with the marked side of the tube facing outward. This is the side of the tube where the pellet will form.
- (13) Centrifuge at 14,000 rpm for 15 minutes at RT.
- (14) Take the tubes out of the centrifuge, carefully avoiding any mixing or shaking. Place the tubes in a rack with the marked side of the tube facing you.
- (15) Using a new sterile fine-tip transfer pipette for each tube, go down the side of the tube opposite the mark and take the liquid off. Place the liquid in the beaker with 10% bleach solution and put the transfer pipette in the small autoclave bag inside the hood to be disposed of later.
- (16) Add 1,000 µl of 70% ethanol (RT) to each tube using 1,000 µ pipette. *Do not open more than one tube at the time.*
- (17) Re-cap and vortex for 5 seconds.
- (18) Place in the micro-centrifuge with the orientation mark facing outward and spin for 5 minutes at 14,000 rpm.
- (19) Aspirate as much of the ethanol as possible. Take each tube and tilt it 45 degrees away from the marked side. If there is any remaining ethanol left in the tube, pipette it out with a transfer pipette.

- (20) Using 1,000 µl pipette, add 400 µl of **HIV-1 DIL** specimen diluent to each tube. Re-cap the tube.
- (21) Vortex each tube for 10 seconds and then either place the samples in freezer boxes and store at -70°C or proceed to the Amplification step within 2 hours.
- (22) Wipe out the hood with 10% bleach followed by 70% ethanol.
- (23) Close the glass and turn the UV light on for approximately 15 minutes.

2. Amplification

a. Consumable reagents and supplies

- Roche Amplicor HIV-1 Monitor vs. 1.5 kit
 - Magnesium (**HIM-1 Mn²⁺**)
 - Master Mix (**HIM-1 MMX**)
 - Denaturation solution
- Clean lab coat
- Powder free gloves
- Wipes
- 70% Ethanol in a spray bottle
- 10% Bleach in a spray bottle
- Micro Amp tubes
- Micro Amp caps (12 caps/strip)
- Micro Amp base and tray retainer for Micro Amp tubes
- Re-sealable plastic bags
- RNase free, aerosol filtered, sterile pipette tips
- Reagent reservoirs (used for Denaturation Solution)
- Bench top Autoclave bags 8" × 12"
- Bench top Autoclave holder

b. Equipment

- PCR enclosure ("Clean Spot")
- Vortex
- Pipette 100 µl
- Thermocycler
- Multichannel Pipettor 200 µl
- Strata linker-UV cross linker 230V

c. Procedure: Preparing the Master Mix

The Master Mix can be made before the Extraction Procedure, during the 15 minute isopropanol spin, or after the Extraction Procedure. If the Master Mix is made at the beginning of the day, place the Micro Amp tray in a re-sealable plastic bag and place in the refrigerator. The Master Mix is stable for 4 hours at 2–8°C.

NOTE:

- *The Master Mix must be made up according to the following procedure in the Clean Room inside a PCR Enclosure “Clean Spot” which is used for this purpose only.*
 - *Remember to change lab coat and gloves before entering the Clean Room.*
- (1) Using 10% bleach followed by 70% ethanol, wipe out the PCR Enclosure.
 - (2) Using DNA/RNA decontamination solution (DNA away solution), wipe out the pipettes and the Micro Amp tray.
 - (3) Place reaction tubes in the Micro Amp tray and lock tubes in place with retainer. Arrange the tubes in rows of 12 tubes each. Close up the bag of Micro Amp tubes and leave inside the PCR Enclosure.
 - (4) Decontaminate the tray and the pipettes in the UV-cross linker.
 - (5) Vortex the Master Mix and the manganese solution for 5 seconds.
 - (6) Add 100 µl of manganese solution to one vial of Master Mix using 100 µl pipette.
 - (7) Mix by inverting 10–15 times.
 - (8) Pipette 50 µl of Master Mix into each reaction tube using 100 µl pipette.
 - (9) Cap each tube.
 - (10) Take tray and tubes out of the PCR Enclosure and put in a re-sealable plastic bag.
 - (11) Wipe out the PCR Enclosure with 10% bleach followed by 70% ethanol.
 - (12) Turn on the UV light for approximately 15 minutes.

- (13) In a clean hood, open each sample tube separately and pipette carefully 50 µl of each extracted sample into the appropriately labelled Micro Amp tube containing 50 µl of Master Mix using 100 µl pipette with sterile, aerosol filtered tips. Mix up and down with the pipette 3 or 4 times. **Avoid disturbing the pellet while pipetting!**

NOTE:

- *If processed specimens and controls have been stored frozen, thaw at room temperature and vortex for 5 seconds prior to use. **Avoid pipetting pellet, which can inhibit the reaction.***
- *The remainder of the processed specimens may be frozen and stored for up to one week*

- (14) Cap the tubes and place samples in the Thermocycler and run the 1.5 plus 25 cycles-program:

NOTE: *The Thermocycler needs to be turned on a minimum of 30 minutes before use.*

CYCLE PROGRAM

- HOLD Program: 2 minutes at 50°C
- HOLD Program: 30 minutes at 60°C
- CYCLE Program (8 cycles): 10 sec at 95°C, 10 sec at 52°C, and 10 sec at 72°C
- CYCLE Program (25 cycles): 10 sec at 90°C, 10 sec at 55°C, and 10 sec at 72°C
- HOLD Program: 15 minutes at 72°C

- (15) Do not forget to clean out the hood using 10% bleach followed by 70% ethanol and turn on the UV light for approximately 15 minutes.

- (16) Take the samples out during the 15-minute HOLD cycle.

NOTE: *Do not leave the samples past the 15 minutes.*

- (17) Bring the samples to the Post-Amplification Area and remove the caps carefully to avoid aerosols of the amplified product.

- (18) With a Multichannel Pipettor and sterile filtered RNase free tips add 100 µl of Denaturation Solution to each tube. Mix up and down 8 to 10 times.

- (19) Place in a refrigerator or continue to the Detection procedure.
Amplified specimens are stable when stored in the refrigerator for up to 72 hours.

VI. DETECTION

A. Consumable Reagents and Supplies

- Roche Amplicor HIV-1 Monitor vs. 1.5 kit
 - Wash Buffer Mix (100 ml of buffer to 900 ml of DI water)
 - HIV-1 Monitor Micro Well Plate (MWP)
 - Reagent 2 (Hybridization Buffer)
 - Reagent 3 (Conjugate)
 - Reagent 4A & 4B – Pipette 12 ml of 4A + 3 ml of 4B into a 15 ml polypropylene tube.
 - Reagent 5 (Stop Solution)
- RNase free, sterile, aerosol-filtered tips
- Reagent Reservoirs for reagents 2, 3, 4, and 5
- Sterile serology pipettes (5 ml, 10 ml, and 25 ml)
- 15 ml sterile polypropylene tubes

B. Equipment

- Pipette 100 μ l
- Multichannel Pipettor and tips
- Incubator
- Plate washer
- Plate reader

C. Procedure

1. Bring all reagents to room temperature.
2. Prepare working wash solution by adding 1 volume of 10X wash buffer to 9 volumes of DI water. Mix well. Stored at room temperature (RT), the wash solution is stable for 2 weeks.
3. Remove the monitor MWP (Micro Well Plate) from the foil pouch and add 100 μ l of hybridisation buffer to each well of the MWP to be tested using the 100 μ l pipette.
4. Pick up 25 μ l of denatured amplicon and add to the HIV-1 wells in row A.
5. Mix up and down 10 times with a multichannel pipettor with aerosol filtered tips.
6. Pick up 25 μ l and transfer to row B.

7. Mix 10 times; pick up 25 μ l and transfer to row C.
8. Continue to make serial fivefold dilutions until row F.
9. Mix, remove 25 μ l, and discard with pipette tips.
10. Pick up 25 μ l of denatured amplicon and add to row G.
11. Mix 10 times; pick up 25 μ l, and move to row H.
12. Mix, pick up 25 μ l, and discard with pipette tips.
13. Cover the MWP with the MWP lid and incubate at 37°C for 1 hour.
14. Just before taking plate out of incubator, run an empty plate through the washer for a few strips.
15. Take the MWP out of the incubator and wash 5 times using Plate Washer.
16. Blot the MWP by wrapping with a tissue and banging plate on table to get rid of excess liquid.
17. Using a multichannel pipettor, add 100 μ l of conjugate (Reagent 3) to each well.
18. Cover with lid and incubate for 15 minutes at 37°C.
19. Wash and blot the MWP.
20. During the wash cycle, make up reagent 4 (substrate).
21. Using a Multichannel Pipettor, add 100 μ l of substrate into each well.
22. Incubate at room temperature in the dark for 10 minutes.
23. Using the Multichannel Pipettor, add 100 μ l of Stop Solution (Reagent 5) to each well.

VII. RESULTS

- A. Read at 450 nm single wavelength on the Plate Reader within 10 minutes of adding Stop Solution.
- B. Calculate the results by using the Roche RNA PCR EXCEL Calculation Sheet.

VIII. QUALITY CONTROL

- A. Always include one each of the AMPLICOR HIV-1 MONITOR Negative, High, and Low Positive controls in each test run.
- B. Negative Control should yield all HIV-1 O.D values < 0.2. **If this is not the case, the whole run is invalid.**
- C. The assigned range for the positive controls is specific for each lot of control and based on the specimen preparation method. The range is provided on the data card supplied with each kit, and both controls should fall inside the indicated range. **If this is not the case, the whole run is invalid.**
- D. If any of the controls are consistently out of range, contact the Roche representative.

IX. APPROVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Laboratory Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

Section Supervisor

Signed: _____ Date: _____

Full name: _____

Designation: _____

