

STANDARD OPERATING OPERATIONAL FRAMEWORK

Specimen Processing Flowchart, Diagnostic Test Menu, SOP Master Index & Analytical Test Methods

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Institutional Statement:

This technical manual outlines the standard operating procedures, diagnostic capabilities, quality control protocols, and analytical methodologies enforced at Evergreen Medical Center (EMC). Our laboratory operations integrate biometric candidate verification, cold-chain integrity, continuous internal quality control (IQC), and automated diagnostic platforms. This document comprises four primary technical sections: (1) Specimen Processing Flowchart & Chain of Custody, (2) Complete List of Diagnostic Tests Performed, (3) Master Index of Standard Operating Procedures (SOPs), and (4) Detailed Diagnostic Test Methodologies.

SECTION 1: SPECIMEN PROCESSING FLOWCHART & CHAIN OF CUSTODY

The specimen processing protocol strictly adheres to ISO 15189 pre-analytical quality criteria and institutional safety guidelines. To eliminate risk of sample mix-ups, candidate identity is verified across reception, phlebotomy, and diagnostic testing stations.

STAGE 1: CANDIDATE REGISTRATION & BIOMETRIC VERIFICATION

- Verification of candidate identity using original Passport / National ID.
- Live webcam facial image capture and thumbprint biometric verification against internal registration system.
- System generates a unique 1D/2D Barcode Sample Tracking Number linked directly to the candidate profile.



STAGE 2: PHLEBOTOMY & SPECIMEN COLLECTION

- Phlebotomist verifies candidate identity using barcode scanner prior to sample collection.
- Phlebotomy executed in dedicated sanitary bays using single-use vacuum tube collection system (BD Vacutainer®).
- Primary sample tubes (EDTA, Clot Activator/Gel, Sodium Fluoride) and sterile urine/stool containers labeled with barcodes directly at the collection bay in front of the candidate.



STAGE 3: SPECIMEN ACCEPTANCE, LOG-IN & CENTRIFUGATION

- Central Specimen Receiving Area checks sample volume, container integrity, color coding, and absence of hemolysis/clots.
- Rejection criteria applied immediately for invalid samples (re-draw initiated with logged incident report).
- Accepted samples logged into Laboratory Information Management System (LIMS). Serum tubes centrifuged at 3,000 RPM for 10 minutes under temperature-controlled conditions.



STAGE 4: PARALLEL LABORATORY PROCESSING UNITS

A. HEMATOLOGY & SEROLOGY

- Whole blood EDTA -> Fully Automated 5-Part Hematology Analyzer.
- Serum -> Fully Automated Chemiluminescent Immunoassay (CLIA) for HIV, HBsAg, Anti-HCV.
- Carbon Flocculation & TPHA for Syphilis Serology.

B. CLINICAL BIOCHEMISTRY

- Serum -> Fully Automated Clinical Chemistry Analyzer.
- Photometric assay for Glucose (RBS), Serum Creatinine, Liver Enzymes (ALT/AST), Bilirubin.
- Automated calibration & daily IQC level 1 & 2 verification.

C. MICROSCOPY & URINE TOX

- Giemsa thick/thin blood film microscopy for Malaria & Microfilaria.
- Routine urinalysis & microscopy.
- 3-Panel Urine Toxicology Immunoassay (Opiates, Cannabis, Amphetamines).



STAGE 5: ANALYTICAL QUALITY CONTROL & PATHOLOGIST REVIEW

- Analysis executed on fully automated diagnostic systems with automated result interface to LIMS.
- Consultant Pathologist & Senior Microbiologist review all abnormal findings, blood smears, and serology cut-off indices.
- Repeat testing initiated for borderline or reactive serology samples.



STAGE 6: ELECTRONIC RESULT ENTRY & REPORT ISSUANCE

- Validated medical fitness reports approved and uploaded directly to secure electronic database within 24–72 hours.
- Digital signature and institutional seal applied by Consultant Clinical Pathologist.
- Specimens archived at 2°C–8°C for a 7-day post-testing retention period prior to biohazard disposal.

SECTION 2: COMPREHENSIVE LIST OF DIAGNOSTIC TESTS PERFORMED

Evergreen Medical Center performs a full spectrum of diagnostic screening examinations across serology, hematology, clinical chemistry, microbiology, toxicology, and digital radiology.

Diagnostic Category	Specific Test / Investigation Name	Sample Type	Department / Scope	Clinical Purpose / Medical Criteria
Infectious Disease Serology	HIV I & II Antibody / Antigen Screening	Serum	Immunology / Serology	Screening for HIV I & II infection.
	Hepatitis B Surface Antigen (HBsAg)	Serum	Immunology / Serology	Screening for active Hepatitis B infection.
	Anti-Hepatitis C Virus Antibody (Anti-HCV)	Serum	Immunology / Serology	Screening for Hepatitis C infection.
	Syphilis Serology (VDRL / RPR & TPHA)	Serum	Serology	Detection of Treponema pallidum infection.
Blood Parasitology	Malaria Blood Smear (Thick & Thin Film)	Whole Blood (EDTA)	Parasitology	Microscopic identification of Plasmodium species.
	Microfilaria Microscopy	Whole Blood (EDTA)	Parasitology	Microscopic detection of Wuchereria bancrofti.
Hematology	Complete Blood Count (CBC) & Hemoglobin	Whole Blood (EDTA)	Hematology	Evaluation of blood cells, severe anemia (Hb < 7.0 g/dL), infections.

Diagnostic Category	Specific Test / Investigation Name	Sample Type	Department / Scope	Clinical Purpose / Medical Criteria
Clinical Biochemistry	Random Blood Sugar (RBS)	Serum / Plasma	Clinical Biochemistry	Assessment of blood glucose level.
	Serum Creatinine & eGFR	Serum	Clinical Biochemistry	Renal function assessment (eGFR < 60 mL/min).
	Liver Function Tests (ALT, AST, Bilirubin)	Serum	Clinical Biochemistry	Hepatic function and liver enzyme evaluation.
Urinalysis & Toxicology	Routine Urinalysis (Protein, Glucose, Microscopy)	Mid-stream Urine	General Pathology	Screening for renal pathology, proteinuria, hematuria.
	Urine Toxicology (Opiates, Cannabis/THC, Amphetamines)	Fresh Urine	Toxicology	Screening for substances of abuse.
Stool Parasitology	Stool Routine & Microscopy (Ova, Cysts, Parasites)	Fresh Stool Container	Parasitology	Detection of intestinal parasites, helminthes, protozoal cysts.
Radiology	Digital Chest Radiography (PA View)	Digital Image (DR)	Radiology & Imaging	Chest examination to rule out pulmonary lesions, active TB, or fibrosis.

SECTION 3: MASTER INDEX OF TEST STANDARD OPERATING PROCEDURES (SOPS)

Evergreen Medical Center operates under a standardized Quality Management System (QMS). Below is the certified master index of operational Standard Operating Procedures controlling laboratory operations, biosafety, and data security.

SOP Ref Number	Standard Operating Procedure Title	Category / Scope	Key Operational Standard
EMC-SOP-ADM-01	Candidate Identification & Biometric Verification Protocol	Administration & IT	Dual identity check via fingerprint & webcam photo capture prior to collection.
EMC-SOP-LAB-01	Phlebotomy, Specimen Collection & Barcode Labeling	Pre-Analytical	Single-use vacuum collection systems, bedside primary barcode labeling.
EMC-SOP-LAB-02	Specimen Acceptance, Rejection Criteria & Sample Transport	Pre-Analytical	Visual inspection for hemolysis, volume sufficiency, and LIMS entry logging.
EMC-SOP-LAB-03	Internal Quality Control (IQC) & Instrument Calibration	Quality Assurance	Daily 2-level QC runs prior to testing; calibration logs maintained systematically.
EMC-SOP-LAB-04	Fully Automated Immunoassay Screening (HIV, HBsAg, HCV)	Serology / Virology	Automated CLIA analyzer protocol; repeat testing for reactive samples.
EMC-SOP-LAB-05	Syphilis Serological Testing (VDRL Screening & TPHA Confirmation)	Serology	Qualitative carbon agglutination followed by quantitative TPHA for reactive samples.
EMC-SOP-LAB-06	Automated Hematology Analysis & Blood Smear Preparation	Hematology	5-Part differential evaluation; manual microscopic review for abnormal morphology.

SOP Ref Number	Standard Operating Procedure Title	Category / Scope	Key Operational Standard
EMC-SOP-LAB-07	Biochemical Testing Protocol (Glucose, Creatinine, LFTs)	Clinical Biochemistry	Automated photometric chemistry analyzer with multipoint calibration verification.
EMC-SOP-LAB-08	Giemsa Stain Microscopy for Malaria & Microfilaria	Parasitology	Thick & thin blood smear examination under 100x oil immersion by senior staff.
EMC-SOP-LAB-09	Routine Urinalysis & 3-Panel Urine Toxicology Screening	Urinalysis / Tox	Reflectance strip photometry & lateral flow immunoassay for Cannabis, Opiates, AMP.
EMC-SOP-RAD-01	Digital Chest Radiography (DR) & PACS Image Archiving	Radiology	High-resolution DR ($\geq 200\text{mA}$); radiologist interpretation; PACS cloud digital backup.
EMC-SOP-INF-01	Infection Control, PPE Standards & Needle-Stick Injury Protocol	Biosafety	Universal safety precautions, mandatory personal protective equipment, PEP protocol.
EMC-SOP-WST-01	Biohazardous Medical Waste Segregation & Disposal	Environmental Safety	Color-coded waste segregation, puncture-proof sharp containers, licensed disposal.
EMC-SOP-DAT-01	Data Security, Confidentiality & Electronic Portal Protocol	Health Informatics	256-bit SSL encrypted data transfer, restricted server access, candidate privacy.

SECTION 4: DIAGNOSTIC TEST METHODS & ANALYTICAL PROTOCOLS

This section outlines the detailed analytical principles, equipment specifications, quality control standards, and interpretation criteria for diagnostic procedures performed at Evergreen Medical Center.

4.1 Infectious Disease Serology (HIV, Hepatitis B, Hepatitis C)

Principle: Chemiluminescent Immunoassay (CLIA) and Chemiluminescent Microparticle Immunoassay (CMIA) utilizing fully automated closed-system analyzers.

Equipment: Fully Automated Immunoassay Analyzer.

Reagent & Solution Quality: CE-IVD marked, high-sensitivity diagnostic reagents with complete lot tracking.

Methodology Protocol:

- Serum sample (100 μL) is incubated with microparticles coated with specific recombinant antigens or monoclonal antibodies.
- After magnetic separation and wash cycles to remove unbound components, isoluminol or acridinium-labeled conjugates are introduced.
- Starter reagents trigger a chemiluminescent reaction measured in Relative Light Units (RLU). RLU values are proportional to the concentration of target analyte.

Quality Control & Cut-Off Interpretation: Daily calibration and 2-level QC (Negative and Positive controls) are performed prior to clinical sample processing. Results are evaluated using Cut-off Index (COI) / Signal-to-Cutoff (S/CO) thresholds:

- **Non-Reactive (COI < 0.90):** Declared Negative / Normal.
- **Borderline / Grey Zone ($0.90 \leq \text{COI} < 1.00$):** Re-tested in duplicate.
- **Reactive (COI ≥ 1.00):** Re-tested in duplicate. Repeat reactive samples are documented as Positive.

4.2 Syphilis Serology (VDRL Screening & TPHA Confirmation)

Principle: Carbon Antigen Non-Treponemal Flocculation Test (VDRL/RPR) for screening, followed by Treponema Pallidum Hemagglutination Assay (TPHA) for specific treponemal antibody confirmation.

Methodology Protocol:

- **VDRL/RPR Screening:** 50 µL of unheated serum is mixed with 16 µL of modified VDRL carbon antigen suspension on a rotative test card. Rotated at 100 RPM for 8 minutes. Macroscopic flocculation indicates reactivity.
- **TPHA Confirmation:** All reactive VDRL samples undergo TPHA testing. Diluted serum is mixed with avian erythrocytes sensitized with *Treponema pallidum* antigens. Hemagglutination mat formation in microtiter wells confirms specific antibodies.

Interpretation: Positive TPHA test confirms specific treponemal reactivity.

4.3 Blood Parasitology (Malaria & Microfilaria Microscopy)

Principle: Microscopic identification of hemoparasites in peripheral blood smears using Giemsa staining.

Equipment: High-resolution Binocular Medical Microscope with 100x Oil Immersion Objective Lens.

Methodology Protocol:

- **Thick Film:** 2 drops of EDTA whole blood are de-hemoglobinized and stained with 10% Giemsa solution (pH 7.2) for 15 minutes. Examined under 100x oil immersion across at least 200 microscopic fields to detect *Plasmodium* species or microfilariae.
- **Thin Film:** Single blood drop spread at 45° angle to form a monolayer feather edge. Fixed with methanol (100%) for 1 minute and stained with Giemsa for species identification (*P. falciparum*, *P. vivax*).

4.4 Complete Blood Count (CBC) & Hemoglobin

Principle: Electric Impedance Method for cell counting and sizing; Sodium Lauryl Sulfate (SLS) / Cyanmethemoglobin method for Hemoglobin determination.

Equipment: Fully Automated 5-Part Differential Hematology Analyzer.

Methodology Protocol:

- Whole blood (EDTA) aspirated and diluted automatically. Red blood cells and platelets counted via electrical impedance pulses.
- Hemoglobin measured photometrically at 555 nm following SLS reaction.

4.5 Clinical Biochemistry (RBS, Serum Creatinine, Liver Enzymes)

Principle: Photometric Enzymatic and Kinetic Colorimetric reactions on fully automated clinical biochemistry analyzers.

Equipment: Fully Automated Chemistry Analyzer.

Methodology Protocols:

- **Random Blood Sugar (RBS):** Glucose Oxidase - Peroxidase (GOD-PAP) enzymatic colorimetric test, measured at 500 nm wavelength.
- **Serum Creatinine:** Jaffe's Kinetic Method (alkaline picrate reaction) or Enzymatic Method, measured at 510 nm. Estimated Glomerular Filtration Rate (eGFR) calculated automatically using standard equations.
- **Alanine Aminotransferase (ALT/SGPT) & AST:** IFCC method without pyridoxal phosphate. Kinetic UV rate measurement at 340 nm.

4.6 Urine Toxicology Screening & Routine Urinalysis

Principle: Reflectance Photometry for urinalysis reagent strips; Competitive Lateral Flow Chromatographic Immunoassay for Urine Drug Screening.

Methodology Protocol (Urine Drug Screen):

- Fresh, unadulterated urine collected under supervised chain-of-custody protocols.
- 3-Panel Drug Test Strip inserted into fresh urine specimen:
 - **Opiates (Morphine/Heroin):** Cut-off 300 ng/mL.
 - **Cannabis (THC):** Cut-off 50 ng/mL.
 - **Amphetamines (AMP):** Cut-off 1000 ng/mL.
- Results evaluated at 5 minutes. Presence of control line with absence of test line indicates a POSITIVE finding for the respective parameter.

4.7 Digital Chest Radiography (Radiology Protocol)

Principle: High-frequency Direct Digital Radiography (DR) system producing DICOM images.

Equipment: Floor-mounted Digital Radiography System (DR $\geq$ 200mA) with high-resolution acquisition workstation and Picture Archiving and Communication System (PACS).

Methodology Protocol:

- Candidate positioned in standing Postero-Anterior (PA) view with full inspiration.
- Lead shielding applied for radiation safety according to ALARA principles. Technicians wear dosimeter (TLD) badges.
- Digital image processed and evaluated by Licensed Consultant Radiologist for pulmonary lesions, active infection, cavitation, fibrotic scarring, or pleural effusion.

Quality Endorsement & Authorization:

This Standard Operating Framework and diagnostic technical manual reflects the active operational standard of Evergreen Medical Center. All procedures are subject to continuous quality monitoring and internal review.

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