

**ANNEX D**  
**GENERAL AND TECHNICAL CONSIDERATIONS FOR TESTS LISTED**  
**IN ANNEX E**

## LIST OF LABORATORY TESTS BY LEVEL

### LEVEL I

1. HIV Serology: Rapid Test
2. Hemoglobin
3. Urine Pregnancy Rapid Test
4. Urine Dipstick
5. Collection Procedure for Dried Blood Spot (DBS)
6. Chemistry: ALT and Creatinine
7. Whole Blood Glucose
8. AFB Smear
9. Malaria Smear
10. Malaria Rapid Test
11. Wet Mounts (NaCl and KOH) - Direct Microscopy
12. Rapid Syphilis Test (RST)

### LEVEL II

1. HIV Serology (for rapid test, refer to Level I; for EIA, refer to Level III)
2. CBC - Automated Differential
3. CBC – Manual
4. CSF/Body Fluid Cell Counts (refer to manual CBC)
5. CD4
6. Chemistry Panel/Whole Blood Lactate
7. AFB Smear (for AFB Smear Light Microscopy, refer to Level I)
8. Cryptococcal Antigen Test
9. India Ink Stain
10. Hepatitis B Surface Antigen and Hepatitis C Antibody Testing
11. Gram Stain
12. Malaria Smear (refer to Level I)
13. Malaria Rapid Test (refer to Level I)
14. TPPA/TPHA/RPR (for Rapid Syphilis Test, refer to Level I)
15. Type and Crossmatch
16. Urine Dipstick – Microscopy
17. Urine Pregnancy Rapid Test (refer to Level I)
18. Wet Mounts (NaCl and KOH) - Direct Microscopy (refer to Level I)

### LEVEL III

1. HIV Serology by EIA (for rapid test, refer to Level I)
2. Viral Load
3. CBC - Automated Differential
4. CD4
5. Urine Dipstick – Microscopy (refer to Level II)
6. Chemistry Panel (for Whole Blood Lactate, refer to Level II)
7. AFB Smear, Culture, and Susceptibility (for AFB Smear, refer to Level II)
8. Microbiology Smear and Culture
9. Malaria Smear (refer to Level I)
10. Malaria Rapid Test (refer to Level I)
11. Syphilis (for rapid test, refer to Level I; for TPPA/TPHA/RPR, refer to Level II)
12. Hepatitis B and C: Serology by Automated Immunoassay
13. Type and Crossmatch (refer to Level II)
14. Urine Pregnancy Rapid Test (refer to Level I)
15. Wet Mounts (NaCl and KOH) - Direct Microscopy (refer to Level I)
16. Cryptococcal Antigen Test (refer to Level II)
17. India Ink Stain (refer to Level II)

## **LEVEL I**

### **General Considerations for Laboratory Operations:**

1. A phlebotomy program outlining standards of competency must be established. Areas to address include:
  - a. Staff competency in blood collection techniques (venous and capillary)
  - b. Proper specimen labeling.
  - c. Correct specimen collection container or tube.
  - d. Proper handling of anticoagulant and other tubes.
  - e. Sufficient specimen volume obtained in anticoagulated tubes and testing requirements.
  - f. Inventory management for phlebotomy supplies.
2. A Quality Assurance (QA) program must include daily quality control (QC) evaluation and documentation, on-site assessments/inspections, inventory management (sufficient supplies within expiration date), external quality assessment (EQA) with timely feedback of results, staff competency, and equipment maintenance.
3. System needs to be developed to standardize ordering, resulting, and recording of results in the laboratory system. An adequate documentation system that is able to retrace and recreate the total testing event must be in place including date, time and person responsible at each point in the chain of events.
4. Test processing information must include the following:
  - a. Pre-analytical
    - i. Complete processing: All requested test specimens obtained are accounted for.
    - ii. Partial processing:
      1. Some requested test specimens received.
      2. Collection containers given to patient for specimens to be submitted at a later time.
      3. Specimens not obtained due to unsuccessful or incomplete phlebotomy.
      4. Specimens not obtained as patient does not meet fasting requirements for specific tests.
    - iii. Receipt of specimens
      1. Specimens submitted from a referral site or non-laboratory area.
      2. Specimens received from patients on-site.
  - b. Analytical
    - i. All tests that should be analyzed same day.
    - ii. Tests not analyzed at site, but referred with no or some additional specimen processing.
    - iii. Tests unable to be performed due to equipment or supply issues; specimen retained for later testing or referred.
    - iv. Testing of shared specimens between site and referral site.
    - v. Insufficient sample for completion of all testing.
    - vi. Unacceptable sample for testing due to analytical interference.
  - c. Post-analytical
    - i. All final results available same day.
    - ii. Some final results available same day; other final results available on subsequent days.
    - iii. Specimen results (preliminary and final) from referral laboratory are incorporated.
    - iv. Specimen follow-up to non-qualitative or quantitative results (i.e., no specimen received, quantity not sufficient, lab accident, or rejected).
5. Laboratory results should be retrievable and maintained for a minimum of two years. Results from repeated specimen analyses on the same specimen should be retained by either documenting the repeat value or retaining both equipment-generated reports.
6. Equipment logs and print-outs of maintenance (e.g., system checks, corrective action, service and QC) should be kept for a minimum of two years. Equipment inventory and service contact information should be available at the site.
7. Availability and adherence to defined processes for critical result determination, handling, and notification.

8. Availability and adherence to processes that define an unacceptable/rejected specimen (policy) and its handling (procedure).
9. Staff must understand equipment linearity, generated flags, reference ranges and how they apply to patients.
10. Staff understands specimen interferences (pre-analytical: hemolysis, lipemia, ictericia; and analytical: concentration of other analytes, over the counter and prescriptive medications) and their effects on the testing.
11. QC system must be developed so that no patient results are reported until system checks and QC results are acceptable and documented.
12. Staff must have the ability to determine QC reference ranges with control material and develop new ranges with different lots; ability to change over from current to new lots.
13. Availability and adherence to a current SOP, chemical hygiene manual (MSDS sheets), and safety manual.
14. Availability and routine use of personal protective equipment (PPE).
15. Availability of an occupational exposure plan and access to post-exposure prophylaxis.
16. Documentation that work area is cleaned and properly disinfected on a daily basis.
17. Sufficient space and resources must be available to organize work and to store specimens (e.g., test tube racks).
18. A stock of expendable parts such as bulbs and batteries must be maintained.
19. Staff must monitor and document all storage conditions for supplies.

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### **HIV SEROLOGY: RAPID TEST**

**Method:** Manual  
**Kit:** HIV Rapid Test Kits  
**Number of Samples:** <40/day

**Technical Considerations for this Procedure:**

1. Single-use format, therefore individual specimens can be analyzed without batching.
2. Patient identification, by directly labeling the cassette, should be used during testing.
3. Non-cold chain rapid test kit required.
4. A QC program should incorporate both internal and external QC performance and documentation, on-site assessment and periodic assessment of the rapid method by retesting with a different method or participation in a proficiency program.
5. If algorithm uses additional testing such as ELISA, then transportation, specimen, and result tracking must be developed.
6. Each country should use the standardized algorithm for use of validated HIV Rapid Test Kits based on the recommendations of the WHO and CDC.
7. See complete list of rapid HIV testing investigated by the WHO in the Document Reference List in **Annex J**.

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### **HEMOGLOBIN**

**General Considerations:**

1. If venous blood is used (specimen analyzed, or sharing between testing site and then referred for additional testing), care must be taken that the aliquot of sample is obtained from a well-mixed EDTA whole blood specimen.
2. Criteria for acceptable specimen must be established. Under-filled tubes affect results due to the effects and volume of the anticoagulant. Difficult draws can affect all results such as falsely decreased platelet counts. The presence of any clot in the specimen, regardless of the size, must be rejected and redrawn.

## Hemoglobinometer

**Method:** Hemoglobinometer

**Equipment:** HemoCue

### Technical Considerations for this Procedure:

1. Hand-held portable analyzer.
2. Uses capillary or venous blood: venous blood requires an additional transfer device.
3. Automatically corrects for sources of turbidity (e.g., lipemia, leukocytosis).
4. Power source can be an AC adapter or batteries. Additional back-up batteries for should be available.
5. Microcuvettes must be stored in a dry place and be stable for 3 months; since humidity affects cuvettes, container must remain closed between uses.
6. Provides highly accurate hemoglobin determination.
7. Cuvettes are expensive for routine use.
8. Proper capillary specimen technique is essential for accurate results.
9. The first two to three drops of blood should be wiped away so that results are not falsely decreased due to tissue fluid dilution.
10. Excess blood on the outside tip must be carefully wiped away without removing any of the sample.
11. Adequate blood flow is essential to fill cuvette in one continuous process; bubbles in the sample will affect analysis and will require sample recollection.
12. Microcuvettes must be treated as hazardous waste due to blood contamination.
13. Equipment maintenance is minimal: cleaning cuvette holder and optic window with cuvette cleaner and swabs.
14. Maintenance logs should be used and documented.
15. HemoCue 301 model may be more appropriate for areas with high humidity and high temperatures.
16. Different models require different system checks, calibrators, control procedures and accessories.
17. Storage of calibrators and controls appropriate for the model may require refrigeration.
18. A QC program should incorporate both internal and external QC performance and documentation.
19. Service should include the replacement of minor parts or of the defective analyzer.

## Hemoglobin Color Scale

**Method:** Hemoglobin Color Scale

**Kit/Equipment:** WHO Color Scale

### Technical Considerations for this Procedure:

1. Uses capillary or venous blood, but best suited for finger stick specimens.
2. Proper finger stick collection is required.
3. Wipe away the first two to three drops of blood and then place blood on special chromatography paper.
4. Compare to color scale shown in increments of 2g/dL.
5. Method is simple, rapid and cheap; not sensitive enough to guide administration of blood transfusions, but is used to identify potential donors.
6. Can be used as a screen with reflex to HemoCue for levels below 10g/dL.
7. No maintenance required; must keep color chart clean.

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## URINE PREGNANCY RAPID TEST

### General Considerations:

1. Can detect before first menses is missed.
2. Some products use urine only; others use urine and serum in combination.
3. Qualitative measurement only (positive or negative).
4. If quantitative results are needed, specimen transport issues would need to be addressed.

5. Prozone effects (false-negatives) can be obtained in abnormal pregnancies and in cancerous conditions.

**Test:** Pregnancy Rapid Test  
**Method:** Manual  
**Kit:** Beta Clear HCG  
**Company:** Core Diagnostics; various vendors

**Technical Considerations for this Procedure:**

1. Single-use format, therefore individual specimens can be analyzed without batching.
2. A QC program should incorporate both internal and external QC performance and documentation.
3. Some products include external QC and others require QC to be separate.
4. Occasionally, diluted or early determinations give indeterminate results that should be repeated in 48 hours or first morning void. Availability for patient to return should be considered.
5. Urine testing is preferred on first morning, clean catch specimen.

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## URINE DIPSTICK

### Urine Chemistry by Dipstick (multi-parameter)

**General Considerations:**

1. Dipstick performed on all urines as a chemical screen. Microscopy performed for positive protein, blood, nitrite or leukocyte if available.
2. Appropriate specimen cups and collection instructions must be provided for each collection. Toilette wipes allow for better specimen collection and less interference from vaginal/penile secretions. Cups must be labeled using a waterproof marker.
3. Bathroom facilities must be considered. Patients should be able to wash hands before collection.
4. Specimens should be analyzed shortly after collection or stored at refrigerated temperatures for up to four hours.
5. Culturing algorithms need to be determined which includes transportation of appropriately preserved specimen, specimen and result tracking.
6. QA program must include positive and negative controls and proficiency assessment of staff at the macroscopic and microscopic areas of testing and assess the ability to correlate macroscopic findings with microscopic.
7. Blood and leukocyte pads measure intracellular components (hemoglobin and leukocyte esterase), amount of cell lysis influences detection.

**Test:** Urine Dipstick  
**Method:** Manual  
**Kit/Equipment:** Cypress Urine, 10 Reagent Strips  
**Company:** Cypress Diagnostics; various vendors

**Technical Considerations for this Procedure:**

1. Dipsticks are light and sensitive to humidity. Container lid must be tightly screwed on to container at all times when not in use, and discarded when beginning to deteriorate.
2. Staff should ensure water-absorbing material is present in dipstick container and changed regularly.
3. Staff must be aware of interfering factors for each indicator pad.
4. Must be able to perform analysis without reagent pads mixing with one another by laying the strip onto gauze. Timer with alarm is needed for the 1- or 2-minute timing requirements.
5. Important to gently mix urine prior to dipping.
6. Reporting must be standardized using either the +++/small-mod-large system or units at the macroscopic levels, and ranges at the microscopic levels.

7. An appropriate patient identification system needs to be in place in the following areas: specimen acquisition, macroscopic testing, centrifuging aliquots, performing microscopy.
8. Bilirubin pad is difficult to visually assess. Consideration for confirmatory testing such as the Ictotest should be considered.

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## COLLECTION PROCEDURE FOR DRIED BLOOD SPOTS (DBS)

### **General Considerations:**

1. Collection procedure that allows for early HIV virological diagnosis in infants and children.
2. Issues involving pediatric venipuncture can be avoided since capillary specimen (finger stick or heel stick) collection allows for blood to be applied directly to the filter paper.
3. Since viral nucleic acids easily degrade, remote areas lacking refrigeration or experience prolonged transport times may incorporate DBS collection as an alternative solution.
4. Improper sampling collection, handling and transporting of the DBS will affect the accuracy of the test. It is important to validate the entire DBS procedure.
5. DBS can be incorporated into an EQA program. DBS collected at the time of patient testing can monitor the rapid testing program by easily transporting a specimen to the reference laboratory.
6. If an unsealed microhematocrit tube is used to apply the blood, care must be taken not to rip the filter paper. Avoid using glass microhematocrit tubes since breakage may lead to a puncture wound creating an occupational exposure incident.

### **Technical Considerations for this Procedure:**

1. Staff must demonstrate competency with capillary collection. Improper collection or site selection can result in infection or damage to the area.
2. After cleaning the area, the first drop of blood must be wiped away. Milking or squeezing the puncture site may cause hemolysis. The DBS must air-dry at room temperature for a minimum of 3 hours. In humid climates, the DBS should dry overnight.
3. DBS must be completely dried before storage or transport. Moisture resulting from a partially dried sample may cause bacterial growth, thus compromising the specimen's quality. The collection cards should be suspended or placed so that nothing touches the wet surface while drying. Do not place cards in direct sunlight or heat the cards to assist with drying. Avoid stacking cards to prevent cross-contamination.
4. Air-dried samples must be individually protected to avoid contact with other specimens during transportation.
5. A low gas-permeable zip-closure bag with desiccant packs and humidity indicators should be used to transport specimens.
6. If samples are being mailed, ensure a high quality bond envelope suitable for mailing biological specimens is used. Specimens should be enclosed in a foam or plastic cooler for transport to protect the integrity of the samples.

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## CHEMISTRY: ALT AND CREATININE

### **General Considerations:**

1. Baseline measurements should be performed prior to medication initiation and with routine monitoring of abnormal patients.
2. ALT and creatinine measurements are considered the minimum for ART monitoring. Glucose testing is required for severe malaria. Liver function tests may be required for patients with TB. Depending on staff skill set, volume, fuller testing menu needs, support turnaround time (TAT) from intermediate laboratory, and patient accessibility, larger desktop equipment may be desired.
3. Rapid point-of-care (POC) or semi-automated analyzers
  - a. Easier to use; requires less technical expertise by prompting the user with display messages.
  - b. Require minimal training.

- c. Short TAT per test.
  - d. Unable to perform dilutions on automated basis.
  - e. Limited testing menu.
  - f. May be higher in cost of reagents.
  - g. Single-use strips or testing cartridges are usually the only consumables required for patient testing for dry chemistry POC testing.
  - h. Company service may require shipment of entire model to be returned for servicing.
  - i. In-country servicing may be possible through distributors.
  - j. Do not have walk-away capability and may not be fully automated.
  - k. POC devices may require a confirmatory testing algorithm to be developed when results obtained are near the linearity endpoints or at pre-determined critical value cut-offs.
4. Automated desktop analyzers are not recommended at Level I due to the following factors:
    - a. Harder to use and maintain as more hands-on maintenance is required.
    - b. Require more advanced equipment training and laboratory expertise.
    - c. Provide more extensive chemistry menu than needed (LFT, BMP, amylase, lipid, CPK) in a profile format.
    - d. Less expensive models do not include electrolytes.
    - e. Require calibration and a better understanding of QC.
    - f. Multiple consumables needed to operate equipment.
    - g. Inventory management more extensive.
    - h. An external QC system should be available with either vendor and/or in-country oversight.
    - i. Reagent on board stability expiration times may result in wastage of reagents.
    - j. Require deionized water, pipettes and tips for calibrators, controls and/or reagents. Depending on the accuracy requirement needed for preparation, volumetric pipettes and rubber bulbs may need to be available.
  5. In-vitro changes to the specimen will affect chemistry analysis. Serum cannot remain in contact with the cells for extended periods of time (no longer than 4 hours; 1 hour is recommended). Care must be taken to either aliquot a serum sample or utilize centrifuged serum separator tubes (SST) to create a barrier. SSTs cannot be later respun since this will contaminate the earlier separated serum due to intracellular analyte leakage (potassium) or analyte consumption by the cells (glucose). If separation from cells is not possible, plasma from a grey top tube is an acceptable choice for glucose testing since it inhibits glycolysis. Always confirm specimen selection with the manufacturer's requirements or additional method validation studies.
  6. Because enzymes are measured as rate of change and temperature affects rate, they are frequently the first analytes affected by inappropriate equipment or room temperatures.
  7. Overall QC program incorporating normal and abnormal ranges would need to be developed which will require additional purchase of QC material. When determining appropriate QC material, medical decision points should be considered.
  8. If lyophilized QC material is chosen, then deionized water and correct fixed pipette volume must be available.

### Roche Reflotron

**Test:** ALT and Creatinine  
**Method:** Semi-Automated  
**Kit/Equipment:** Roche Reflotron  
**Company:** Roche Diagnostics, Switzerland  
**Number of Samples:** <50/day (< 250/week)

### Technical Considerations for this Procedure:

1. Dry chemistry strips.
2. Desktop analyzer that requires no test reagent preparation or calibration step.
3. Can use capillary or venous whole blood, serum or plasma.

4. Can obtain by finger stick, therefore specimens can always be obtained for analysis.
5. Does not have walk-away capabilities but requires staff to introduce each specimen/test.
6. Reagents required for analysis are individual test strips.
7. Equipment maintenance program consists of using Clean & Check strips and QC material (purchased separately) to monitor analyzer's performance, as well as monthly cleaning of air filter.
8. Testing menu can support 17 different analytes, including hemoglobin measurement (each purchased separately).
9. All reagent strips, except creatine kinase (CK) and uric acid, must be stored from 2 to 30°C. CK and uric acid reagent strips must be stored from 2 to 8°C. Clean & Check strips must be stored from 2 to 30°C. Controls must be stored from 2 to 8°C.
10. Reagent vials must be closed immediately, since temperature and humidity will affect strips.
11. Equipment print-out should be retained as part of laboratory's record retention.
12. Need for some type of peer review, external QC program.
13. High reagent cost per test, and temperature and humidity sensitivity should be considered.
14. Temperature must be between 15 and 34°C and humidity must be no greater than 95% to ensure proper test results.
15. May not be sensitive enough for pediatric populations.
16. Troubleshooting is limited to replacement of basic parts (e.g., fuse, bulb). Defective units must be shipped back to manufacturer or distributor for service; loaner units are available.
17. Electricity is required to operate device. Can be run from a car battery.

## **VITROS DT60 II**

**Test:** ALT and Creatinine  
**Method:** Semi-automated  
**Kit/Equipment:** VITROS DT60 II  
**Company:** Ortho-Clinical Diagnostics, Inc.  
**Number of Samples:** >50/day (>250/week)

### **Technical Considerations for this Procedure:**

1. Dry chemistry slides; reagent preparation is not required.
2. Results are printed onto a paper roll. Laboratory needs a system to retain equipment tape for records.
3. Each test cartridge is sold separately; laboratory menu can be expanded.
4. Specimens are injected into a port so vendor-specific pipette and tips are required.
5. For plasma or serum tests, centrifuge must be available.
6. Does not have walk-away capabilities, but requires staff to introduce each specimen/test.
7. To evaluate enzymes (ALT), the enzyme module (DTSC II) would be required.
8. To evaluate electrolytes, an additional module (DTE II) would be required.
9. Slides are individually wrapped. Analyzer can hold up to six slides at a time.
10. Creatinine slides must be stored at frozen temperatures; ALT slides are stored at refrigerated temperatures.
11. Slides must equilibrate to an ambient temperature (15-30 minutes) before being unwrapped and used. Once slide is unwrapped from individual packaging, it must be used immediately.
12. Results are obtained after 5 minutes; electrolytes after 3 minutes.
13. Calibration is required for every new lot number of reagent. Calibrators are reconstituted with supplied diluent and a 3ml fixed pipette. ALT and creatinine use the same calibrator material: DT Calibrator Kit. Creatinine requires the use of bottles 1, 2, 3 and 4; ALT requires the use of bottles 1, 2, and 4.
14. Specimens above linearity require dilution. Creatinine uses 7%BSA or reagent-grade water; ALT uses 7%BSA or isotonic saline.
15. Electricity is required to operate equipment.
16. Maintenance is minimal.
17. Loaners may be available if equipment must be serviced off-site.

**Other Options:**

**Test:** ALT and Creatinine  
**Method:** Semi-Automated  
**Kit/Equipment:** Humalyzer 2000 BA-88  
**Company:** Human International Mindray Medical International Ltd., CHINA  
**Number of Samples:** >15/day (>70/week)

**Technical Considerations for this Procedure:**

1. Semi-automatic, micro-processor-controlled photometer with flow cell.
2. Tests, parameters, and standard curves are stored in equipment's memory.
3. The analyzer assists the operator through use of prompts.
4. Does not have walk-away capabilities, but requires staff to introduce each specimen/test.
5. An air barrier separates specimens. Care must be taken to remove sample during air aspiration cycle.
6. Reagent kits are available that may require additional reagent preparation and/or refrigeration.
7. With each batch run, a reagent blank and QC should be performed.
8. Excellent pipetting, reagent preparation, and technical skills required.
9. Requires a water bath.

**Manual Method**

**Test:** ALT and Creatinine  
**Method:** Manual Spectrophotometry  
**Kit/Equipment:** Spectrophotometer  
**Company:** Various vendors  
**Number of Samples:** >15/day (>70/week)

**Technical Considerations for this Procedure:**

1. Requires staff to have a fundamental understanding of Beer's Law and its application to testing analysis as well as the ability to create standard calibration curves and be able to determine how to obtain an unknown concentration from a single standard.
2. Excellent pipetting, reagent preparation and technical skills are required.
3. Spectrophotometer must be verified by checking photometric linearity, wavelength accuracy (didymium filter or cobalt chloride solution), and photometric accuracy. Additional maintenance should include checking for stray light, temperature calibration, and baseline stability.
4. Staff should have a fundamental understanding of chemical reactions, reagent preparation, reaction timing, endpoint colorimetric and kinetic spectrophotometry, and dilution factor conversions.
5. A reagent blank and QC should be performed with each batch of tests.

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**WHOLE BLOOD GLUCOSE****Glucometer Method****General Considerations:**

1. Care should be taken with glucometers so as not to compare whole blood glucose levels with plasma glucose levels measured by other tests. Some glucometers do provide "plasma equivalent" estimates that can be compared with direct plasma glucose levels.
2. Care must be taken not to interchange models that internally perform a calculation to a plasma glucose value with glucometers that only report whole blood glucose values. Interchanging such manufacturer or model types may negatively impact patient management.
3. Whole blood glucose may be 10-15% lower than plasma glucose levels.

4. POC glucometers are normally recommended for use as a screening tool and for home use, but not for diagnostic use. Many rural labs rely on these as the sole diagnostic test for abnormal glucose levels.

**Method:** POC- amperometry (electrochemistry)

**Kit/Equipment (Company):** Accu-Chek Performa (Roche Diagnostics, SWITZERLAND)  
Accu-Chek Aviva (Roche Diagnostics, SWITZERLAND)  
Bayer Ascensia ELITE (Siemens USA (Bayer))

**Technical Considerations for this Procedure:**

1. Models indicated above are calibrated to deliver accurate plasma glucose results from whole blood specimens.
2. Through capillary action, the correct amount of sample is drawn into the test strip, with no additional wiping or blotting required. Under-filling the test strip will affect result accuracy.
3. Test strips must be used immediately after removing them from the original container.
4. Electronic quality check and display functionality of meter is done by either an external code chip (Bayer) or an internal integrity check (Roche). This check should be included with daily maintenance and documented. If more than one device is available at the site, then each device, specified by serial number, should have its own maintenance log.
5. Testing uses prepackaged calibrator strip (code chip or check strip test) and single-use reagent test strips. No additional calibration or reagent preparation is required.
6. Calibrator strip, specific to the test strip's reactivity, is included with each package of test strips. The calibrator strip should be retained while that lot-number of test strips is in use. Calibrator strip should be replaced when a new lot number of test strips is started.
7. Battery-powered and easy to use.
8. Additional batteries (3V lithium) should be part of inventory management.
9. Service would consist of defective device replacement.
10. Low, Normal, and High controls are available as a separate purchase.
11. Preventative maintenance of the device should include checking daily function of the character display to ensure all characters can be generated (i.e., an improper displayed "8" can appear as a "6" or a "2") and wiping the exterior surface with damp gauze.
12. Used test strips must be treated as a biohazard.
13. Neonate glucose testing may not be validated or acceptable. Check with manufacturer.
14. Glucometer testing may not be appropriate when peripheral blood flow is decreased (shock, hypotension, severe dehydration) or when hematocrit values exceed manufacturer's specified range of acceptability.

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**AFB SMEAR**

**General Considerations:**

1. Patient's clinical progress and treatment plan should be reviewed monthly (CDC, 2003; WHO, 2004).
2. Consideration must be given to proper instructions for sputum collection, safety measures for handling and transporting specimen to lab.
3. Sufficient slide storage and retrieval for QA programs.
4. QA program must include assessing the quality of each specimen.
5. Laboratory needs to establish a chemical, safety (includes use of PPE), and waste disposal program.
6. Employee surveillance program is required.
7. System to transport specimens for culture and DST must be established to ensure specimen result tracking and reporting. Laboratory accident plan should be developed that includes the transportation component. Consideration must be given to specimen storage (refrigeration) in the case of delays in transporting to the processing laboratory.

8. For slide preparation, wooden sticks or wire loops (requires technique to prevent specimen cross-contamination) by using either a sand bath and spirit lamp or disposable wooden applicators, clean slides and appropriate device to label slides.
9. Xylene and coplin jar to dip reviewed slide, removing immersion oil before storing slide.
10. Cool box must be available to transport culture specimens.
11. QC program that evaluates and documents stain performance using positive and negative control slides.
12. Sufficient workspace for slide preparation (i.e., air flow must be away from the staff), staining, drying, microscopy evaluation and reporting.
13. Sputum containers should have a screw-top lid, be unbreakable, and be transparent to view contents.
14. Waterproof markers (a system of patient identification, specimen and slide labeling must be in place and strictly adhered to).
15. Poor laboratory technique will result in aerosol production and safety issues during specimen handling and slide preparation, subsequently risking employee safety.
16. Staff must be able to investigate poor staining issues and have the ability to resolve them.
17. Reporting results must include the request form and a TB register.
18. QA program should include onsite evaluation, blind rechecking, and receipt of stained and unstained smears from the overseeing Level II laboratory. Timely feedback mechanism is essential.
19. Algorithms to address negative smear suspected patients, treatment failure or relapse and data management of such patients.
20. Laboratory surfaces must be such that they can be easily disinfected; staff should be familiar with the correct concentrations of appropriate disinfectants and how to prepare them.

#### **AFB Smear (Light Microscope)**

- Method:** Manual
- Kit/Equipment:** AFB Binocular Microscope (light microscope for low volume laboratories; fluorescent microscope for higher volumes)
- Company:** Olympus, Japan or Nikon, Japan or Carl Zeiss MicroImaging Inc.

#### **Technical Considerations for this Procedure:**

1. Microscope maintenance and supplies (e.g., lens paper, lens cleaner, spare bulbs, dust cover) must be available.
2. Allow drop of immersion oil to be free falling to prevent contamination; makeshift oils are not appropriate.
3. Availability of service for microscopes.
4. Other laboratory tests, such as a differential, do not use fluorescence. Access to light microscopes or LED light sources alternatives such as Earl Light should be considered in overall workflow patterns.

#### **AFB Smear Staining Reagents (Ziehl-Neelsen stain)**

- Method:** Manual
- Kit/Equipment:** AFB Smear Microscopy reagents (Ziehl-Neelsen stain)
- Company:** Becton, Dickinson and Company, USA; donors; various

#### **Technical Considerations for this Procedure:**

1. For staining capability, additional supplies are needed: forceps, adequately sized sink (additional sink or two basin sink for washing hands), slide staining rack, spirit lamp, water, drying rack, and timer with alarm.
2. Prepackaged stains eliminate the need to work with powder reagents that require purity evaluation, weighing, water quality and preparation of acid solutions.
3. Blotting of smears may cause cross-contamination. Allow them to air dry.
4. Reagents are light sensitive and must be stored in the dark.

### **AFB Smear Microscopy Reagents (Fluorescent Stain)**

**Method:** Manual  
**Kit/Equipment:** AFB Smear Microscopy Reagents (fluorescent stain for high volume laboratories)  
**Company:** Becton, Dickinson and Company, USA

#### **Technical Considerations for this Procedure:**

1. Fluorochrome-stained slides use a lower magnification for review, thus the scanned area is larger and more rapid. However, it is recommended that positive smears be confirmed at a higher magnification using the Ziehl-Neelsen method (WHO, 1998b).
2. For staining capability, additional supplies are needed: forceps, adequately sized sink (additional sink or two basin sink for washing hands), slide staining rack, distilled water (tap water may interfere with fluorescence), drying rack, and timer with alarm.
3. Blotting of smears may cause cross-contamination. Allow them to air dry.
4. Reagents are light sensitive and must be stored in the dark.
5. Fluorescence may fade with time, so specimens should be examined within 24 hours (WHO, 1998b).

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## **MALARIA SMEAR**

#### **General Considerations:**

1. Staff should have access to reference books with atlases and illustrated bench or job aids.
2. Laboratories should be equipped with adequate microscopes, supplies, and reagents for reliable diagnosis of malaria.
3. If species identification is warranted, then the intermediate level should perform this task.
4. Staff must be proficient in reading thick and thin smears and differentiating malaria and other hemoparasites from platelets and artifacts. Other important pathogens that can be observed on stained blood films are: borrelia, microfilariae, and trypanosomes.
5. The best malaria smears are both thick and thin smears, particularly if species identification or parasite counts are required (for test of cure).

**Test:** Microscopy and Stain  
**Method:** Manual  
**Kit/Equipment:** Wright-Giemsa Stain Pack  
**Company:** Various vendors

#### **Technical Considerations for this Procedure:**

1. Stains used for peripheral smear differentials (Wright-Giemsa) can also be used for assessment.
2. The QA system in place for differentials can easily incorporate the needs of specific blood parasite evaluations.

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## **MALARIA RAPID TEST**

**Test:** Manual  
**Method:** Malaria Rapid Test  
**Kit/Equipment (Company):** Paracheck (Orchid Biomedical Systems)  
Parabank Rapid Test for Malaria (Zephyr Biomedical Systems)  
CareStart Malaria Antigen Rapid Kit

#### **Technical Considerations for this Procedure:**

1. Rapid diagnostic tests (RDT) are an alternative to microscopy in situations where electricity or reliable microscopy is not available for reliable examination of blood films, or when laboratory personnel are unavailable.

2. RDTs can provide results in 15 to 20 minutes, while microscopic examination of a stained blood film may require 20 to 45 minutes.
3. The sensitivity of RDTs is unreliable at parasitemias of less than 200 parasites per microliter of blood. There are reports that very high parasitemias may also give false-negative readings, possibly due to a prozone effect.
4. Although RDTs are simpler than microscopy in concept, they are sensitive to high temperature and humidity. They also require some understanding of their function and very careful training before use.
5. Expert microscopy should always be used for quality control of RDTs.
6. Some RDTs are pan-specific and can detect non *P. falciparum* malaria species, but cannot differentiate between *P. vivax*, *P. ovale*, and *P. malariae*. In mixed infections that include *P. falciparum*, the tests may be positive for *P. falciparum* only.
7. Circulating malaria antigens may persist for several weeks after cure and give positive results with RDTs that detect *P. falciparum* histidine-rich protein 2 (PfHRP2).
8. See reference document list in **Annex J** for additional information from the WHO on test kits.

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### **WET MOUNTS (NaCl and KOH) - DIRECT MICROSCOPY**

#### **General Considerations:**

1. Can provide immediate evaluation of pathogenic fungi in skin and nails.
2. Used to evaluate candidosis, trichomonas and bacterial vaginosis.
3. Used in evaluation of stool and other samples for nematodes, trematodes, cestodes, and protozoa.
4. Used to evaluate protozoa and larvae directly from fecal material, particularly for primary investigation in chronic diarrheal patients.
5. Storage, transportation, container, specimen tracking and result reporting issues as well as proper specimen collection must be addressed.
6. A QA system of timely feedback between caregivers with the initial assessment performed at peripheral site will increase overall effectiveness of care.
7. Staff should have access to reference pictures and have a fundamental grasp of cellular size relationships (essential).
8. A high quality specimen is critical for diagnosis.

**Test:** Wet Mounts

**Method:** Manual Light Microscope

#### **Technical Considerations for this Procedure:**

1. Simple to perform, but requires technical expertise in interpretation.
2. Need access to sterile swab, transport tube or cup, microscope, slides, coverslips, petri dish, dropper bottles, 0.9% NaCl, and 5% w/v KOH.
3. Periodically assess KOH and NaCl fluids for contamination.
4. Aliquots of mounting fluid should be kept in a closed bottle with dropper.

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### **RAPID SYPHILIS TEST (RST)**

#### **General Considerations:**

1. RST and TPPA (or TPHA) is specific for *Treponema pallidum* (*T. pallidum*), causative agent of syphilis.
2. Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) detect non-specific treponemal antibody.
3. There are concerns that screening with a nontreponemal test can result in false-negatives if high titres of antibody are present (prozone effect) in very early infection or very late stages of disease and with concomitant HIV infection. Cases of false-negatives are apparently rare but delayed seroreactivity

may be of clinical concern in HIV positive patients given the potential for rapid progression of disease. May consider use of TPPA and VDRL/RPR in high HIV prevalence areas.

4. Because antibodies are present even after treatment, test is unable to distinguish between active disease and successfully treated disease. All positive results should be confirmed with an RPR to distinguish active versus treated.
5. RST/TPPA is not appropriate to monitor response to treatment.
6. RPR is a nontreponemal test; false-positives may be seen in other tissue-damaging states (WHO, 2006a, Appendix 1). Confirmation specific to *T. pallidum* is recommended with reactive RPR results when diagnosing syphilis.
7. When quantitated, RPR results are appropriate to monitor treatment success.
8. In syphilis with treponemal RDTs, the test may remain positive years after successful treatment.
9. Rural conditions (e.g., dust, temperature, skill level) may decrease RPR tests' sensitivity and specificity (Montoya et al., 2006) (West et al., 2002).

### **Rapid Syphilis Test**

**Test:** Rapid Syphilis Test

**Method:** Manual

**Kit (Company):** Determine Syphilis TP (Abbot Laboratories, USA)  
Syphilis Fast (DIESSE Diagnostica, ITALY)  
Espline TP (Fujirebio Inc., JAPAN)  
Syphicheck- WB (Qualpro Diagnostics, INDIA)  
SD Bioline Syphilis (Standard Diagnostics, Inc., KOREA)  
VisiTect Syphilis (Omega Diagnostics Group PLC, UK)

### **Technical Considerations for this Procedure:**

1. Usually in diskette or cassette form.
2. Can use whole blood, serum or plasma.
3. If RST is used within a testing algorithm, it is set up to automatically order and do (reflex) RPR after a positive RST must be devised (WHO, 2003b, 2006).
4. See reference document list in **Annex J** for additional information from the WHO on test kits.

## **LEVEL II**

### **General Considerations for Laboratory Operations:**

1. All general considerations from Level I are considered good laboratory practice and are applicable to Level II.
2. A referral system of specimens received from less automated Level I laboratories (or those experiencing technical difficulties) needs to be established including the following:
  - a. Reliable transportation system established that includes safety issues with specimen transport.
  - b. Receipt and handling of specimens from several locations.
  - c. Test tracking mechanism.
  - d. Order entry system that correctly identifies patient, ordering location, and tests requested.
  - e. Result delivery to correct facility.
  - f. Results provided in timeframe that meet the needs of clinician for patient care.
  - g. Protocol for handling unacceptable specimens or critical tests with Level I.
  - h. Handling of additional test requests from Level I once specimen has been transported.
  - i. Reflex testing/algorithms established.
  - j. SOP written that addresses referral test processing and testing.
3. Establish back-up manual or automated methods or a system of specimen referral so that equipment issues do not impact patient care.
4. Inventory management must include testing volume from site and referral sites.
5. Monitoring and organizing work and storage areas.
6. Documentation of an annual review of all policies and procedures.
7. QA system to monitor and address issues that includes immediate corrective action and follow-up reassessment.
8. Active communication between Level II and Level I sites with roles assigned.
9. Active communication between staff members and supervisors to create a productive and quality environment.
10. Work schedules and task assignments at each workstation must be defined.
11. A training program that documents new employee orientation and periodic competency evaluations must be established.
12. Ensure all staff participate in EQA on a rotational basis.
13. Maintain a record system consistent with national procedures.
14. Create, review and submit reports regarding laboratory operations per national procedures.
15. Adhere to waste management processes according to established policies and procedures.
16. Ensure proper storage of chemicals and perform a chemical inventory.
17. Maintain an equipment inventory that includes service information, date of purchase, condition, and serial number.
18. Monitor all ancillary equipment needed for operations such as pipette calibration, centrifuge maintenance, biologic safety cabinet maintenance, and microscope maintenance.
19. Reagent management system must be consistent and incorporate:
  - a. Received date.
  - b. Manufacturer's expiration date.
  - c. Opened or reconstituted date.
  - d. On-board or in-use expiration date.

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## **HIV SEROLOGY**

**HIV Rapid Test: *Refer to HIV Serology section in Level I for details.***

### **HIV Serology by EIA**

### **General Considerations**

1. HIV EIA could be considered at Level II if volume and technical capabilities support it.

**Refer to HIV Serology section in Level III for details.**

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## **CBC - AUTOMATED DIFFERENTIAL**

### **Automated Complete Blood Count with Automated Differential (3-part)**

#### **General Considerations:**

1. Automated testing is preferred for complete blood count (CBC)/full blood count as manual testing is not as accurate or precise and requires a high level of technical skill.
2. The 3-part electronic differential consists of granulocytes, lymphocytes, and monocytes.
3. Hematology control and calibrator material has a short shelf life (usually 4-6 weeks). Since frozen aliquots are not an acceptable alternative, procurement by means of a monthly standing order may address the needs of the QC program.
4. Equipment that does not include a QC data maintenance program requires the staff to manually plot and assess numerous parameters from several levels of QC material. This is a laborious and time consuming task that makes QC assessment difficult to perform.
5. Maintain the appropriate tool kit that includes specific maintenance tools and expendable parts such as probes, tubing and fuses.
6. Ensure regular preventative maintenance and timely repairs are performed and documented in the appropriate logs.
7. Review and address any manufacturer's update notification included those for reagents, controls and calibrators.
8. Calculate and apply observed QC means and ranges specific to the analyzer as part of QC program and do not rely solely on package inserts to monitor method stability.
9. Participate in an EQA program.
10. Verify the acceptable performance when new reagent is placed onto the analyzer by either performing QC or analyzing previously run patient samples that perform within predetermined limits of acceptability.
11. Create a schedule of system function checks and QC according to the operator's manual.
12. Monitor all logs (e.g., maintenance, QC, corrective action, service) for completeness and acceptability.
13. Track trends and shifts in QC that impact precision and accuracy. Take corrective action when required.
14. Perform basic troubleshooting as outlined in the operator's manual and document all troubleshooting activities performed.
15. Have the customer service information readily available.
16. Staff should familiarize themselves with vendor-supplied information from the operator's guide and the reagent/calibrator package inserts for optimal performance. Reliance on training sessions alone is insufficient.
17. Establish a QC and maintenance program that ensures back-up methods are available when needed.
18. If venous blood is used (specimen analyzed, or sharing between testing site and then referred for additional testing), care must be taken that the aliquot of sample is obtained from a well-mixed EDTA whole blood specimen.
19. Criteria for acceptable specimen must be established. Under-filled tubes affect results due to the effects and volume of the anticoagulant. Difficult draws can affect all results such as falsely decreased platelet counts. The presence of any clot in the specimen, regardless of the size, must be rejected and redrawn.
20. Criteria must be developed for peripheral smear review based upon CBC and electronic differential results and equipment flags.

As part of their testing menu, the following equipment perform automated CBC and electronic differentials required for ART monitoring. All can perform >25/day.

<u>Company</u>	<u>Equipment</u>	<u>Number of Samples</u>
Abbott Laboratories, USA	Cell-Dyn Series	>25/day
Beckman Coulter, USA	Coulter A <sup>c</sup> •T diff II	>25/day
Sysmex, Japan	Sysmex KX-21N	>25/day
HORIBA ABX, France	ABX Micros 60	>25/day

**Technical Considerations for this Procedure:**

- Automation requires staff training and periodic retraining.
- Equipment requires a reliable source of electricity and should be protected from electrical surges.
- Manufacturers provide several model options within their analyzer series. The chosen analyzer must be capable of handling the service needs of the program.
- Open system is preferred but may be subject to uneven quality of reagents and the need for extensive validation.
- QA program would need to include internal and external QC; many vendors offer external controls for use.
- Staff must be able to perform basic troubleshooting skills.
- At a minimum, an understanding and interpretational assessment of calibration and QC procedures.
- Calibrators and controls require refrigeration; temperatures should be monitored and documented.
- Certain analyzers have limited QC capabilities and may require development of paper charts to assess QC.
- Clot detection must be either assessed by equipment or rimmed with two applicator sticks using gauze to remove lids prior to analyzing.
- If printer is used for reporting, then sufficient paper and cartridges need to be on hand and there needs to be in place a system to report results if printer is malfunctioning.
- Understanding between on-board stability of reagents and storage versus manufacturer's expiration date and storage of stock reagents must exist.
- Development of a system to determine when on-board stability has been exceeded.
- Reviewing manufacturer's update notifications and how to incorporate them, if applicable.
- Quality is dependent on staff's ability to utilize operator's manual and package insert information.
- Reagent inventory and waste management system is needed.
- Routine daily and monthly maintenance plan with documentation is needed. Bleach is often needed for maintenance and troubleshooting procedures.
- Establish how to address specimens that exceed the equipment's linearity and the other parameters affected.
- Establish how to address specimens with interfering factors such as lipemia.

**Automated CBC**

**Method:** Automated  
**Kit/Equipment:** Cell-Dyn 1600CS; Cell-Dyn 1700; and Cell-Dyn 1800  
**Company:** Abbott Laboratories, USA  
**Number of Samples:** 60/hour

**Technical Considerations for this Procedure:**

- QC and calibration software package.
- Automatic start-up and shutdown capabilities.
- Provides RBC, WBC, and PLT histogram.
- Integrated data station does not require an additional desktop computer.
- Model 1600CS has open and closed mode capabilities.

6. Model 1700 has an optional closed mode sampling.
7. Model 1800
  - a. Uses Cyanide-Free lyse, making waste disposal easier.
  - b. QC package includes Levey-Jennings graphs, Westgard rules and X-B moving average.
  - c. Has an open sampling system.

**Method:** Automated  
**Kit/Equipment:** Coulter A•T diff II  
**Company:** Beckman Coulter, USA  
**Number of Samples:** 50/hour

**Technical Considerations for this Procedure:**

1. Open and closed mode capability.
2. QC management is available, but basic. Capability to store current QC means, ranges, and files; provides QC interpretative flags. Does not have charting capability and would require manual charting of QC.
3. Requires 3 reagents, Diff Pack (Isoton and Lyse) and cleaner.
4. Data station does not require an additional desktop computer.

**Method:** Automated  
**Kit/Equipment:** Sysmex KX-21, KX-21N  
**Company:** Sysmex, Japan  
**Number of Samples:** 60/hour

**Technical Considerations for this Procedure:**

1. Optional built-in thermal printer.
2. Reports the granulocyte category as neutrophils (% and #).
3. Does not have walk-away capability.
4. KX-21 has built-in data storage for 240 results and 6 QC files.
5. KX-21N has built-in data storage for 300 results with histograms and 6 QC files.

**Method:** Automated  
**Kit/Equipment:** ABX Micros 60  
**Company:** Horiba ABX, France  
**Number of Samples:** 55/hour

**Technical Considerations for this Procedure:**

1. Open and closed mode capability.
2. Optional automated smart cards used for QC and patient data storage.

**CBC - MANUAL**

**CBC (Manual) – Used as backup for automated procedures.**

**Method:** Manual  
**Test:** CBC: Manual comprised of hemoglobin and/or hematocrit, WBC and differential  
**Number of Samples:** < 15/day

**Technical Considerations for this Procedure:**

1. QA program should periodically evaluate accuracy of manual method results obtained by staff.
2. Manual methods can provide backup methods while automated analyzers are being serviced.

3. Even though either hemoglobin or hematocrit can provide assessment of anemia, performing both tests provides an additional quality measure due to the relationship between the results ( $Hgb \times 3 = HCT \pm 3$ ).
4. Improperly filled chambers (over-filled/under-filled) cannot be used for counting.
5. Both chamber sides should agree within 10% before proceeding with total WBC calculation.
6. Hemacytometers have two chambers for duplicate testing. It is not an acceptable practice to fill sides from two patients.
7. Raw counts are multiplied by a diluting factor. Staff must be able to perform calculation.
8. Counts should always be verified by peripheral smear regardless if a differential has been requested.
9. Counts above 30 ( $\times 10^3/mm^3$ ) or below 3 ( $\times 10^3/mm^3$ ) should be repeated using a different dilution factor.
10. Additional supplies to meet daily volume should be available: each result requires one pipette, standardized coverslip, and hemacytometer.
11. Staff needs to recognize that nucleated RBCs will not lyse and will be easily counted as WBCs.
12. Disposable hemacytometers are available, but expensive.
13. Manual cell counts may be performed on CSF and body fluids by hemacytometer using modified diluents.

#### **Differential (Manual)**

**Test:** Differential  
**Method:** Manual  
**Kit/Equipment:** Wright-Giemsa Stain Pack  
**Company:** Various vendors  
**Number of Samples:** <25/day

#### **Technical Considerations for this Procedure:**

1. Manual differentials are used to assess the accuracy of automated differentials and for patients with abnormalities, which create flags or the inability to perform the automated differential.
2. WBC estimate is a good quality check on the total WBC.
3. Advanced training is usually required for morphologic interpretations.
4. Differentiates WBC into type of cell present in the peripheral blood in percent.
5. RBC morphology and inclusions can be evaluated.
6. Platelet estimate (e.g., adequate, decreased, markedly decreased) can be performed to verify the automated platelet count.
7. Can obtain absolute counts for lymphocytes and other cell types.
8. If platelet count is needed to evaluate thrombocytopenia, platelets can be performed by a manual hemacytometer method or by automated CBC instrument.
9. Staff must be proficient in making wedge smears and in Wright's staining technique.
10. QA system must evaluate stain quality and staff proficiency for performing all aspects of the differential count.
11. System needs to be considered on how to address suspicious or abnormal cells beyond staff's capability. Next tier review criteria should be considered. If slides are transported, slide holders must be used to prevent scratching and breakage.
12. Reference material and atlases should be available to assist in cell identification.

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### **CD4**

#### **General Considerations:**

1. Requires excellent laboratory skills to perform manual or automated methods properly.
2. QA should include assessment of manual method by retesting with automated method.
3. Where specimen transport is not possible, POC technology or manual methods may be considered for use at Level I. Laboratory must be incorporated into a QA network with a higher level laboratory.

4. Refer to the WHO Recommendations on.
5. See reference document list in **Annex J** for additional information from the WHO on equipment for CD4 testing.

#### **Semi-Automated (FACSCount)**

**Method:** Semi-Automated  
**Kit/Equipment:** FACSCount  
**Company:** Becton, Dickinson and Company, USA  
**Number of Samples:** >50/day

#### **Technical Considerations for this Procedure:**

1. Can use EDTA preservative whole blood tubes only.
2. Whole blood stability 48 hours after draw when kept at room temperature (20°-25°C); if over this ambient temperature range, temperature-controlled boxed must be used for transport.
3. Sample stability 48 hours after sample preparation at room temperature (20°-25°C).
4. Currently cannot calculate CD4% for pediatric patients.
5. Closed system with proprietary power on reagents and controls.
6. Capacity for enumerating CD4, CD3 and CD8 T lymphocytes.
7. Self-contained system that incorporates equipment, reagents and controls.
8. Excellent pipetting and technical skills are required.
9. Ease of sample preparation requires minimal technical skill.
10. Has software algorithm that automatically identifies lymphocyte populations of interest.
11. Two monoclonal reagent /software versions: One for enumerating CD3, CD4 and CD8 (two reagents tubes) and another for enumeration of CD3 and CD4 (one reagent tube).
12. The single monoclonal/population version is lower in cost.
13. New FACSCount version and POC devices will provide CD4%. Current version does not provide CD4% for infant monitoring.
  - a. Sheath Fluid can be costly.
  - b. Daily and monthly maintenance is critical for proper equipment performance.
  - c. Equipment must be placed in a well-ventilated area.
  - d. Ideally, equipment should be placed in an air-conditioned room away from a window.
  - e. Equipment is very sensitive to voltage fluctuations. An APC/uninterrupted power supply (UPS) unit should be provided upon purchase. Units are needed for all equipment in service.
  - f. List price of reagents is USD\$970 per 50 CD4/CD8 test.

#### **Automated (CyFlow Counter)**

**Method:** Automated  
**Kit/Equipment:** CyFlow Counter  
**Company:** Partec, Germany  
**Number of Samples:** 250/day

#### **Technical Considerations for this Procedure:**

1. Ultra-compact and fully equipped mobile/portable equipment.
2. Forward and side scatter analysis with up to three color fluorescence parameters depending on the model.
3. Real-time data acquisition and analysis.
4. Absolute CD4, CD8, CD3 and CD4%.
5. Can be operated with 12V DC battery (car battery).
6. List price of reagents is €1.75 per CD4 test and €2.50 per CD4% test.

#### **Automated (EasyCD4)**

**Method:** Automated

**Kit/Equipment:** EasyCD4 Assay, EasyCD4% Assay  
**Company:** Guava Technologies, USA  
**Number of Samples:** 80/day

**Technical Considerations for this Procedure:**

1. Performs like a mini-flow cytometer.
2. Self-aligning user replaceable flow cell.
3. Enables direct absolute cell counts without reference beads.
4. Minimizes service calls.
5. Operator can set the lymphocyte population gate to maximize cell capture.
6. Absolute CD4, CD8, and CD4% can be resulted.
7. Equipment can also perform apoptosis and cell viability tests with different software, reagents.
8. Micro centrifuge format.
9. Requires less reagent and sample.
10. Needs only 10ul of blood to run assay.
11. Dual sample loader enables running one sample while preparing the next.
12. Small enough to fit into small lab spaces: Less than 1.5 sq. ft.
13. Low cost.
14. Uses minimal sheath fluid.
15. Upgrade to software includes autogating, which would reduce the technical skills required to perform analysis.

**Automated (PointCare NOW)**

**Method:** Automated  
**Kit/Equipment:** PointCare NOW  
**Company:** PointCare Technologies, USA  
**Number of Samples:** 45/day

**Technical Considerations for this Procedure:**

1. Ultra compact and fully equipped mobile/portable equipment designed to serve patients where they are located.
2. A single platform that measures essential hematology parameters and both absolute and lymphocyte percentage CD4 t-cell results.
3. Test result in less than 8 minutes.
4. Fully automated from start-up to shutdown.
5. Closed-cap sample handling-containment system complies with the highest industrial bio-safety standards.
6. Reagents are heat stable to 30°C.
7. Cold chain independent controls available pending FDA review.
8. No calibration required.
9. FDA approved.

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**CHEMISTRY PANEL/WHOLE BLOOD LACTATE**

**General Considerations:**

1. Automated benchtop analyzer's test menu includes: renal, liver, lipid, and electrolyte panels, amylase, and glucose. Lactate may or may not be included.
2. In vitro changes to the specimen will affect chemistry analysis. Serum cannot remain in contact with the cells for extended periods of time (no longer than 4 hours; 1 hour is recommended). Care must be taken to either aliquot a serum sample or utilize centrifuged SST to create a barrier. SSTs cannot be later respun since this will contaminate the earlier separated serum due to intracellular analyte leakage (potassium) or analyte consumption by the cells (glucose). If separation from cells is not

possible, plasma from a grey top tube is an acceptable choice for glucose testing since it inhibits glycolysis. Always confirm specimen selection with the manufacturer's requirements or additional method validation studies.

3. Because enzymes are measured as rate of change and temperature affects rate, they are frequently the first analytes affected by inappropriate equipment or room temperatures.
4. Overall QC program incorporating normal and abnormal ranges would need to be developed which will require additional purchase of QC material. When determining appropriate QC material, medical decision points should be considered.
5. If lyophilized QC material is chosen, then deionized water and correct fixed pipette volume must be available.
6. Maintain the appropriate tool kit for each analyzer that includes specific maintenance tools and expendable parts such as probes, tubing and fuses.
7. Perform system backups as needed to retain records and system parameters.
8. Make sure regular preventative maintenance and timely repairs are performed and documented in the appropriate logs.
9. Perform parallel testing of new lot with current lot of reagents to ensure no clinical disparity with change over.
10. Review and address any manufacturer's update notification included with reagents, controls and calibrators.
11. Calculate and apply observed QC means and ranges specific to the analyzer as part of QC program. Do not rely solely on package inserts to monitor method stability.
12. Participate in an EQA program for all analytes.
13. Verify the acceptable performance when new reagent is placed onto the analyzer by either performing QC or analyzing previously-run patient samples that perform within predetermined limits of acceptability.
14. Create a schedule of system checks and QC according to the operator's manual. The staff must perform the activities as scheduled to achieve quality results, reduce frequency and length of down-times, and extend the operational life of the analyzer.
15. Monitor all logs (e.g., maintenance, QC, corrective action, service) for completeness and acceptability.
16. Track trends and shifts in QC that impact precision and accuracy and take corrective action when required.
17. Perform basic troubleshooting as outlined in the operator's manual and document all troubleshooting activities performed.
18. Have the customer service information for service/hot-line readily available.
19. Establish a QC and maintenance program that ensures backup methods are available when needed.
20. User-defined menu will determine which testing reagents need to be purchased.
21. Testing volume will determine inventory needs.
22. Equipment consumables (e.g., cups, trays, wash) are a separate purchase.
23. Analyzers require consumables that must be included in the inventory management system.
24. For optimal performance, staff should familiarize themselves with vendor-supplied information from the operator's guide and the reagent/calibrator package inserts. Reliance on training sessions alone is insufficient.
25. Areas to consider when evaluating automated chemistry analyzers:
  - a. Level of technical expertise to:
    - i. Operate and performance maintenance on the analyzer.
    - ii. Interpret data from results, error codes, QC, calibration, and system checks.
    - iii. Prepare and handle reagents.
    - iv. Manage inventory of stored supplies and on-board supplies.
    - v. Operate the computer data management system.
  - b. Equipment has the following capabilities:

- i. On-board reagent compartment sufficient to hold all necessary reagents for desired test menu at the correct temperature.
- ii. On-board inventory management.
- iii. Calibration curve/QC program capability.
- iv. Automatic generation of flags for abnormal or invalid results.
- v. Continuous load and unload capability (walk-away capability of batch or load list).
- vi. STAT capability.
- vii. Primary tube sampling with clot detection.
- viii. Data management system and printer that can include patient identification information, reference ranges, and units.
- ix. Manufacturer's linearity/reproducibility/carry-over limits that match method selection criteria.
- x. Reaction and specimen tray capability that supports testing volume.
- xi. Throughput capacity that matches testing volume.
- c. Other equipment capabilities that may be desirable:
  - i. Automatic dilution capability.
  - ii. Automatic rerun capability.
  - iii. Open system.
- d. On-site requirements:
  - i. UPS.
  - ii. Water requirements, many require deionized water (CLSI Type I).
  - iii. Room temperature and humidity requirements specific to manufacturer.
  - iv. Biohazard waste requirements for solid (consumables) and liquid (drain may be required) as specified by manufacturer.
  - v. Sufficient space for analyzer, computer, and printer.
  - vi. Sufficient clearance space so fans and air vents are not blocked.
  - vii. Sufficient workspace.
  - viii. Proper equipment for processing samples (serum/plasma requires a centrifuge).
  - ix. Proper supplies for reagent/calibrator/control handling and preparation (volumetric pipettes may be required to ensure desired level of accuracy).
  - x. Storage capacity (e.g., consumables, reagents, specimen retention) and requirements (e.g., room temperature, refrigerated, frozen).
- e. Other considerations:
  - i. Cost per test: testing volume includes reagents for QC and calibration tests and reagent waste (reagent not consumed before on-board expiration occurs).
  - ii. Heat output and its overall effect on the current space.
  - iii. Vendor support.
  - iv. Technical time to perform testing, staffing requirements.
  - v. Wet versus dry reagent systems. Systems that utilize pre-packaged, ready-to-use reagents (prepared by manufacturer, such as slides, cassette packs) may be more expensive, but eliminate errors with reagent preparation. Preparation errors create waste (e.g., loss of reagent, increased calibration and controls for troubleshooting, inaccurate and unreliable results, reducing available inventory for testing, inefficient use of technical time and personnel spent on troubleshooting and not patient testing), which results in increased operational expense and inefficiency.

### Chemistry Panel

The following benchtop equipment performs chemistry panels required for ART monitoring as part of their testing menu on an automated equipment platform. All can perform >15/day or >70/week.

**Method:** Automated

**Kit/Equipment (Company):** Fully (Biochemical Systems International, ITALY)  
cobas c 111, COBAS INTEGRA 400 plus (Roche Diagnostics, SWITZERLAND)

IL 300+ (Instrumentation Laboratory, Distributed in conjunction w/Coulter)  
HumaStar 300 (Human International)  
BS-120, BS-200 (Mindray Medical International Ltd., CHINA)  
ABX Pentra 400 (Horiba, FRANCE)

**Technical Considerations for this Procedure:**

1. Equipment must have a reliable source of electricity and should be protected from electrical surges. Other factors must include availability of refrigeration for reagents and the quality of water.
2. Automation requires staff training and periodic retraining.
3. Different systems have various calibration requirements.
4. To increase control and calibrator stability once reconstituted, frozen aliquots may be needed (need a non-defrosting freezer, aliquot tubes, system of labeling).
5. Reagent management system must be consistent that incorporates:
  - a. Received date.
  - b. Manufacturer's expiration date.
  - c. Opened or reconstituted date.
  - d. On-board or in-use expiration date.
6. Understanding between on-board stability of reagents and storage versus manufacturer's expiration date and storage of stock reagents must exist.
7. Open system is preferred, but may be subject to uneven quality of reagents and the need for extensive validation.
8. Development of a system to determine when on-board stability has been exceeded.
9. ISE module electrodes must remain wet. If they become dry, electrodes must be replaced. An uninterrupted supply of reference solution must be available.
10. Reviewing manufacturer's update notifications and how to incorporate them, if applicable.
11. Quality is dependent on staff's ability to utilize operator's manual and package insert information.
12. If printer is used for reporting, then sufficient paper and cartridges need to be on hand and a system to report results if the printer is malfunctioning.
13. QA program would need to include internal and external QC; many vendors offer external.
14. Reagent inventory and waste management system is needed.
15. Routine daily and monthly maintenance plan with documentation is needed.
16. Understanding and interpretational assessment of calibration and QC procedures.
17. Understanding statistics applicable to performance assessment (mean, SD, range, and %CV).
18. Backup procedures (e.g., manual, transporting of specimen) during down-times must be developed.
19. Storage capacity for equipment records must exist.
20. Some analyzers have limited QC capability and may require development of paper charts to assess QC.
21. Dilution protocols need to be developed, especially for enzymes and results above equipment linearity (pipette, tips, appropriate diluent, gauze, test tube). There needs to be an understanding of dilution factors and knowledge of how to report results.
22. Awareness of interfering factors such as lipemia, icterus, and hemolysis and development of policies for acceptance or rejection of these samples.
23. Small benchtop spectrophotometer may be used with appropriate reagents as a manual back-up system.

**Automated Chemistry Panel**

**Method:** Automated  
**Kit/Equipment:** Fully  
**Company:** Biochemical Systems International, Italy  
**Number of Samples:** 100/hour

**Technical Considerations for this Procedure:**

1. Random access, benchtop analyzer which can be programmed for up to 54 samples at a time for walk-away convenience.
2. 20 position reagent tray.
3. Open system capability.
4. Built-in PC computer for data management.
5. Built-in thermal printer and additional output for external connection.

**Automated Chemistry Panel**

**Method:** Automated

**Kit/Equipment (Samples):** cobas c 111 (180/hour)  
COBAS INTEGRA 400 plus (400/hour)

**Company:** Roche Diagnostics, SWITZERLAND

**Technical Considerations for this Procedure:**

1. Random access, benchtop analyzer with continuous loading.
2. Optional ISE module on c 111.
3. Uses reagent bottles (c 111) or reagent cassettes (400 plus), reagent composition is the same.
4. Data management screen integrated in c 111 analyzer, but separate from analyzer for the 400 plus.

**Automated Chemistry Panel**

**Method:** Automated

**Kit/Equipment:** IL 300+

**Company:** Diagnostic Instruments, Inc.

**Number of Samples:** 200/hour

**Technical Considerations for this Procedure:**

1. Random access, desktop analyzer with continuous loading.
2. Optional ISE module.
3. Data management system separate from analyzer.
4. Some reagents are lyophilized and require preparation; others are ready-to-use.

**Automated Chemistry Panel**

**Method:** Automated

**Kit/Equipment:** HumaStar 300

**Company:** Human International

**Number of Samples:** 300/hour

**Technical Considerations for this Procedure:**

1. Random access, benchtop analyzer with continuous loading.
2. Data management system separate from analyzer.

**Automated Chemistry Panel**

**Method:** Automated

**Kit/Equipment (Samples):** BS-120 (100/hour); and BS-200 (200/hour)

**Company:** Mindray Medical International Ltd., CHINA

**Technical Considerations for this Procedure:**

1. Random access, benchtop analyzer with continuous loading.
2. Data management system separate from analyzer.

### **Automated Chemistry Panel**

**Method:** Automated  
**Kit/Equipment:** ABX Pentra 400  
**Company:** Horiba, France  
**Number of Samples:** 420/hour

#### **Technical Considerations for this Procedure:**

1. Benchtop analyzer with continuous load capability.
2. Up to 55 chemistries can be performed.
3. Optional ISE module.
4. 52 reagent positions on board: 44 in a closed refrigerated area; 8 at room temperature.
5. Bar-coded, cassettes reagents.
6. Data management system integrated into analyzer using a touch screen.

### **Whole Blood Lactate**

**Method:** POC - colorimetry  
**Kit/Equipment:** Accutrend Lactate System  
**Company:** Roche Diagnostics, Switzerland

#### **Technical Considerations for this Procedure:**

1. Uses whole blood (capillary) and lactate test strips, no additional reagent or calibration preparation is required, calibration strip included.
2. Easy to use, hand-held, battery-operated device, additional batteries should be available on-site.
3. Alternative method if general chemistry analyzer does not include lactate in its testing menu or if additional POC service is desired.
4. Results are available within 60 seconds.
5. Reportable measurement range for whole blood is 0.7mM – 22mM.
6. Service would consist of defective device replacement.

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## **AFB SMEAR**

#### **General Considerations:**

1. AFB smear microscopy should be routinely available by light and fluorescent microscopy.
2. Culture investigation and identification could be considered at this level if volume and capabilities support it.
3. Drug Susceptibility Testing could be considered at this level if volume and capabilities support it.

**AFB Smear Light Microscopy– Refer to Level I for details.**

### **AFB Smear Microscopy Reagents (Fluorescent Stain)**

**Method:** Manual  
**Kit/Equipment:** AFB Smear Microscopy reagents (fluorescent stain for high volume laboratories)  
**Company:** Becton, Dickinson and Company, USA

#### **Technical Considerations for this Procedure:**

1. Fluorochrome stained slides use a lower magnification for review, so the scanned area is larger and more rapid. However, it is recommended that positive smears be confirmed at a higher magnification using the Ziehl-Neelsen method (WHO, 1998b).
2. For staining capability, additional supplies are needed: forceps, adequate sized sink (additional sink or two basin sink for washing hands), slide staining rack, distilled water (tap water may interfere with fluorescence), drying rack, and timer with alarm.
3. Blotting of smears may cause cross-contamination. Allow them to air dry.

4. Reagents are light sensitive and must be stored in the dark.
5. Fluorescence may fade with time. Specimens should be examined within 24 hours (WHO, 1998b).

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## CRYPTOCOCCAL ANTIGEN TEST

### General Considerations:

1. Diagnosis of bacterial, fungal and parasitic opportunistic infections could be considered at this level if volume, infrastructure, and staff capabilities support it.
2. CSF and other body fluids may be tested.
3. Higher technical skill level is required for antigen testing.

### **Cryptococcal Antigen Test (latex agglutination-CRAG)**

**Test:** Cryptococcal Antigen Test

**Method:** Manual

**Kit/Equipment:** (Latex agglutination-CRAG) for determination of *C. neoformans* in CSF and serum.

**Company:** Wampole Labs, USA

### Technical Considerations for this Procedure:

1. Can be used with serum or CSF.
2. Specimens must be centrifuged; serum requires transfer into a sterile glass tube (plain red top tube with no clot activator could be used).
3. Specimens must be heat inactivated by using a water bath at 56°C (serum and CSF) or boiled using a hot plate/beaker of water (CSF only).
4. Specimens and kit must be stored at refrigerated temperatures.
5. Qualitative or quantitative testing can be performed.
6. Additional supplies for qualitative testing are 50ul pipette, tips, slide rotator, water bath, timer with alarm, disposable stirrers, and centrifuge.
7. For quantitative a second slide, 12 x 75mm test tubes, 250ul pipette, and tips will also be needed.
8. Slides require thorough cleaning between testing using isopropyl alcohol, brush, water, and absorbent tissue.
9. Factors affecting testing are latex suspension consistency, reagent volumes, and rotation speed. Therefore, part of the overall QC program must not only include controls (supplied with kit) but also periodic pipette calibration and rotational speed.
10. Grossly hemolyzed specimens will give inaccurate results, so care must be taken with specimen collection of CSF and serum.
11. Pronase must be used to eliminate interfering rheumatoid factor in serum.
12. Test should be evaluated in-country and by the proposed level of staff prior to introduction.

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## INDIA INK STAIN

### **Presumptive Identification for Cryptococcus (India Ink Stain)**

**Test:** Presumptive Identification for Cryptococcus

**Method:** Manual

**Kit/Equipment:** India ink for staining *C. neoformans* in CSF. Note: microscope needed for reading assay

**Company:** Becton, Dickinson and Company, USA; various vendors

### Technical Considerations for this Procedure:

1. Can be used on exudates, sputum, and CSF sediment.
2. Broken ampule is good for one day's testing, but should not be retained longer.

3. QC program should be established using control organisms (*C. albicans* and 48-hour culture of *C. neoformans*). If culture capability is not available, saline suspensions can be prepared by the Level III laboratory and sent to Level II.
4. Additional supplies needed are centrifuge (obtain sediment), transfer loops, microscope slides, coverslips, sterile saline, transfer pipette and microscope with 10x, 40x or oil immersion lens.
5. Staff must be technically proficient to differentiate lymphocytes and fat droplets.
6. HIV patients may not produce the polysaccharide capsule, so they may not test positive.
7. India ink provides a presumptive diagnosis only; definitive diagnosis should be supplied by either culture or antigenic methods.
8. Staff should have access to reference material and atlases.

## HEPATITIS B and C

### Hepatitis B Surface Antigen and Hepatitis C Antibody Testing:

**Methodology:** EIA Kits (see list below)

#### General Considerations:

1. Hepatitis B surface antigen and anti Hepatitis C antibodies may be performed by quick tests, standard EIA testing, or by automated immunochemistry systems. Automated immunochemistry systems are only practical (economically) if the laboratory already has such a system for other testing.
2. Quick tests will most likely not be practical at this level where high volume is expected.
3. Consideration as to whether additional confirmatory or supplemental testing for these tests will be done at this level laboratory should be considered.

#### Technical Considerations for Hepatitis B and C testing:

1. Instrumentation is a concern here. Manufacturer should be chosen so that the same reader can be used for both assays. Alternatively, manufacturer should be contacted regarding compatibility of laboratory's existing EIA reader and their assay.
2. An evaluation of 18 Hepatitis surface B kits was performed by WHO; performance as well as technical ease in a rural laboratory setting was evaluated. It can be found here: [http://www.who.int/diagnostics\\_laboratory/evaluations/hepb/en/](http://www.who.int/diagnostics_laboratory/evaluations/hepb/en/)

Some possible sources for Hepatitis B surface Antigen and anti Hepatitis C antibody test kits:

**Abbott Laboratories, USA;** Website: [www.abbott.com](http://www.abbott.com)

**Bionike Inc., USA;** Website: [www.bionike.com](http://www.bionike.com)

**bioMérieux sa, FRANCE;** Website: [www.biomerieux.com](http://www.biomerieux.com)

**Chembio Diagnostic Systems Inc., USA;** Website: [www.chembio.com](http://www.chembio.com)

**Dade Behring, Inc., USA,** Website: [www.dadebehring.com](http://www.dadebehring.com)

**Equipar Diagnostici, ITALY;** Website: [www.equipar.it](http://www.equipar.it)

**Fujirebio Inc., JAPAN,** Website: [www.fujirebio.co.jp](http://www.fujirebio.co.jp)

**Genelabs Diagnostics Pte Ltd., SINGAPORE,** Website: [www.genelabs.com.sg](http://www.genelabs.com.sg)

**Green Cross Life Science Corp, KOREA,** Website: [www.greencross.com](http://www.greencross.com)

**J. Mitra & Co. Ltd., INDIA,** Website: [www.jmitra4u.com](http://www.jmitra4u.com)

**Organon Teknika,** see bioMérieux

**Organics, ISRAEL,** Website: [www.orgenics.com](http://www.orgenics.com)

**Trinity Biotech plc, IRELAND,** Website: [www.trinitybiotech.ie](http://www.trinitybiotech.ie)

## GRAM STAIN

### Microbiology Smear (Gram Stain)

**Test:** Microbiology Smear; Initial Assessment of Infection

**Method:** Manual

**Kit/Equipment:** Gram Stain

**Company:** Becton, Dickinson and Company, USA; various vendors

**Technical Considerations for this Procedure:**

1. Gram stain at this level can be very useful in guiding clinician interventions. If performed, it is important to define specimens such as post-operative wound swab and exudates, swabs from other “sterile” sites such as the eye and post-partum high vaginal swabs that require gram stain. It is also important for laboratory workers to properly interpret results (i.e., Gram-positive cocci in clusters may be indicative of *staphylococci* rather than *streptococci*).
3. For staining capability, additional supplies are needed: forceps, adequate sized sink (additional sink or two basin sink for washing hands), slide staining rack, water, drying rack, spirit lamp or methanol (to fix slides), microscope, slides, storage containers, immersion oil, loops or swabs, and timer with alarm.
4. Prepackaged stains eliminate the need to work with powder reagents that require purity evaluation, weighing, water quality and preparation of acid solutions.
5. Blotting of smears may cause cross-contamination, so allow them to air dry.
6. Positive (*S. aureus*) and negative (*E. coli*) controls should assess staining performance. If culture capability is not available, unstained control slides can be prepared by the Level III laboratory.
7. Staff must be well trained and should have access to reference material and atlases.
8. If further investigation is needed, then culturing algorithms need to be determined which includes transportation of appropriately preserved specimens, as well as specimen and result tracking.

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**MALARIA SMEAR**

Refer to Level I for details.

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**MALARIA RAPID TEST**

Refer to Level I for details.

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**TPPA/TPHA/RPR**

*For Rapid Syphilis Testing, refer to Level I for details.*

**TPPA or TPHA**

**Test:** *T. pallidum* Particle Agglutination Assay (TPPA) or Hemagglutination (TPHA)

**Method:** Manual

**Kit/Equipment:** TPPA or TPHA

**Company:** Fujirebio Diagnostics, Inc., Japan  
Omega Diagnostics Ltd., UK

**Technical Considerations for this Procedure:**

1. Additional supplies for particle agglutination: microplate, 25ul and 100ul micropipettes, 1.0ml pipette and tips, timer.
2. Additional supplies for hemagglutination: microplate, 25ul and 75ul micropipettes and tips, timer.
3. Kits must be stored at refrigerated temperatures.
4. Requires some reconstitution and interpretation of results in wells by user.
5. Serum or plasma may be used; therefore specimen acquisition should occur within a well-defined phlebotomy program.
6. Centrifugation is needed; periodic centrifuge maintenance and rotation speed should be performed.
7. Reactive and non-reactive controls are included. Documentation of QC performance should be included in the overall QC program.

8. If TPPA is used within a testing algorithm, data management of specimens reflexed for a TPPA after a positive RPR must be devised.
9. Some reagents contain sodium azide, requiring proper disposal/waste management.

## **RPR**

Test: RPR

Manual: Manual

Kit/Equipment (Company): Macro-Vue RPR Card Test Kit No. 104 (Becton, Dickinson and Company)  
Immutrep RPR (Omega Diagnostics Ltd.)

### **Technical Considerations for this Procedure:**

1. Kit includes cards; cards must be stored so that finger oils and dust do not interfere.
2. Antigen requires refrigeration. All other components must be stored in a dry place.
3. Additional supplies (rotator, pipette and tips, timer, 0.9% saline for quantitative testing) are needed.
4. To prevent evaporation during rotation, card should be covered.
5. Documentation of correct rotation speed is required before testing.
6. Periodic accuracy of pipettes by calibration should be performed.
7. If RPR is used within a testing algorithm, data management of specimens reflexed for a RPR after a positive RST must be devised.
8. Serum or plasma may be used, therefore specimen acquisition should occur within a well-defined phlebotomy program.
9. Centrifugation is needed; periodic centrifuge maintenance and rotation speed should be performed.
10. QC control card is purchased separately. Documentation of QC performance should be included in the overall QC program on a daily or per batch run.
11. Verification of correct antigen delivery by needle is a component of a good QC program for this test.
12. Antigen contains mercury, and must be handled as hazardous waste.
13. See reference document list in **Annex J** for additional information from the WHO on recommended test kits.

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## **TYPE AND CROSSMATCH**

### **General Considerations:**

1. Transfusion services do not only provide results, but they provide products. Therefore, the QA commitment is more extensive and rigorous.
2. Temperature storage and stability of reagents and products must be meticulously monitored.
3. Incubation temperature must be meticulously monitored.
4. Reagents must equilibrate to ambient temperature (15-30 minutes) before being used.
5. Controls must be run on day of reagent use.
6. Requires glass test tubes and method to label them, pipettes, 37°C incubator, table top centrifuge, and saline. Single-use test tubes or meticulous washing/rinsing of test tubes needed. Slide testing for ABO/Rh may be acceptable.
7. Criteria for acceptable specimen must be established. Serum or EDTA acceptable.
8. A QC program should incorporate both internal and external QC performance and documentation; on-site assessment and periodic assessment of the methods by retesting with a different method or participation in a proficiency program.
9. Service may be required on refrigerator, centrifuge and incubator.
10. Periodic centrifuge maintenance and rotation speed should be performed
11. Algorithm for additional testing such as antibody identification is necessary, so a tracking system of transportation, specimens, and results must be developed.
12. Crossmatch procedure would be immediate spin for patients with a negative antibody screen.

Note: Weak D testing not recommended at this level.

## **ABO/Rh Group and Type, Antibody Screen, Direct Antiglobulin Test and Immediate Spin Crossmatch**

<b>Test:</b>	Type and Cross match
<b>Method:</b>	Manual
<b>Kit/Equipment:</b>	Reagents
<b>Company:</b>	Ortho-Clinical Diagnostics, Inc. Medion Diagnostics GmbH, GERMANY SANYO, JAPAN

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## **URINE DIPSTICK - MICROSCOPY**

<b>Test:</b>	Urine Dipstick and Microscope
<b>Method:</b>	Manual
<b>Kit/Equipment:</b>	Multi-parameter reagent strips
<b>Company:</b>	Cypress Diagnostics; various vendors

### **Technical Considerations for this Procedure:**

1. Microscopic examination of urine is used to identify red and white blood cells, casts, squamous epithelial cells (correlates with specimen quality if performing culture), casts, bacteria (suggestive of bacterial infection), yeast, *Schistosoma haematobium* and other cellular components.
  2. Dipsticks are light and humidity sensitive, so container lid must be tightly screwed on to container at all times when not in use, and discarded when beginning to deteriorate.
  3. Staff should ensure water-absorbing material is present in dipstick container and changed regularly.
  4. Staff must be aware of interfering factors for each indicator pad.
  5. Must be able to perform analysis without reagent pads mixing with one another by laying the strip onto gauze. Timer with alarm is needed for the 1- or 2-minute timing requirements.
  6. Important to gently mix urine prior to dipping.
  7. Reporting must be standardized using either the +++/small-mod-large system or units at the macroscopic levels, and ranges at the microscopic levels.
  8. Microscopy requires a centrifuge, aliquot tubes, waterproof markers, slides, coverslips, a microscope, plastic pipettes to resuspend sediment, and a sink to discard supernatant.
  9. An appropriate patient identification system needs to be in place at the following areas: specimen acquisition, macroscopic testing, centrifuging aliquots, performing microscopy.
  10. Bilirubin pad is difficult to visually assess. Consideration for confirmatory testing such as the Ictotest should be considered.
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## **URINE PREGNANCY RAPID TEST**

*Refer to Level I for details.*

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## **WET MOUNTS (NaCl and KOH) - DIRECT MICROSCOPY**

*Refer to Level I for details.*

## **LEVEL III**

### **General Considerations for Laboratory Operations:**

1. All general considerations from Levels I and II are considered good laboratory practice and are applicable to Level III.
2. Define clear lines of authority and responsibilities for each position including the designation of a supervisor and QA manager.
3. Active communication between Level III and Level II sites is required and roles must be defined.
4. A referral system for specimens received from other laboratories needs to be established that includes reporting directly to the originating laboratory at Levels I and II.
5. Established back-up methods suitable for testing level must be in place.

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## **HIV SEROLOGY**

**HIV Rapid Test:** *Refer to HIV Serology section in Level I for details.*

### **HIV Serology by EIA**

**Method:** EIA

**Kit/Equipment:** EIA Kit/plate washer; EIA Reader/Incubator

**Number of Samples:** >50/day

### **General Considerations:**

1. EIA methodology requires excellent laboratory skills and method understanding. Use of this method should be limited to areas that can provide quality results; therefore, a specimen referral system needs to be established.
2. In addition to HIV, hepatitis, CMV, HVZ, toxoplasmosis, and other tests can be investigated using this methodology and equipment. Multiple use platforms are valuable and cost-effective.
3. Dust and other environmental factors may impact performance.
4. For optimal performance, staff should familiarize themselves with vendor-supplied information from the operator's guide and the reagent/calibrator package inserts. Reliance on training sessions alone is insufficient.
5. Provide data for surveillance information.
6. Water quality must be adequate.
7. Storage conditions for kits at 4°C must be available.
8. Good micropipette technique is important.
9. See reference document list in **Annex J** for additional information from the WHO on HIV EIA test kits.

### **EIA Reader**

**Kit/Equipment:** EIA Reader

**Company:** Fisher Scientific or BioTek Pharmaceuticals

**Number of Samples:** >50/day

### **Technical Considerations for this Procedure:**

1. Equipment's performance must be periodically evaluated using absorbance test plates or some other method.
2. A separate purchase of a printer may be required with reader. It is important to ensure printer compatibility with the reader.
3. Requires the user to have an understanding of calibration, accuracy, precision, and optics utilized in the verification process.
4. Additional consumables are required (e.g., tips, vials, pipettes).

5. Other equipment required: EIA Plate Washer, EIA Incubator.

#### **EIA Plate Washer**

**Kit/Equipment:** EIA Plate Washer  
**Company:** Fisher Scientific or BioTek Pharmaceuticals  
**Number of Samples:** >50/day

#### **Technical Considerations for this Procedure:**

1. Uniform of washing of each well is critical to reproducible results. Operator training is critical.
2. Washer requires verification of accuracy and consistency of deionized water delivery.
3. Requires maintenance of tubing and pumps for vacuum, waste and dispensing.

#### **EIA Incubator**

**Kit/Equipment:** EIA Incubator  
**Company:** Fisher Scientific or BioTek Pharmaceuticals  
**Number of Samples:** >50/day

#### **Technical Considerations for this Procedure:**

1. Temperature verification is required.

**Sources of EIA Kit** – See reference document list in **Annex J** for additional information from the WHO on EIA test kits.

**Kit (Company):** Vironostika Uniform II, Plus (BioMerieux, FRANCE)  
Murex HIV EIA 1.2.0 (Murex/Abbott Laboratories, USA)  
Enzygnost (Siemens USA)

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## **VIRAL LOAD**

#### **General Considerations:**

1. Laboratory layout and workflow processes must be established so that contamination does not occur.
2. A referral system of specimens received from other levels needs to be established.
3. Equipment is capable of analyzing >50 samples per day.
4. Viral loads are used to monitor HIV disease progression.
5. HIV viral detection is required in newborns since HIV antibody detection is unable to differentiate between primary infection and passive maternal transmission.

**Kit/Equipment (Company):** ExaVir Load Version 3 (Cavidi AB, SWEDEN)  
NucliSens EasyQ with MiniMAG NA Purification System (BioMerieux, FRANCE)  
Versant HIV RNA 3.0 Assay (bDNA) (Siemens, USA)  
Amplicor Monitor V 1.5 Roche with manual extraction (Roche Diagnostics, SWITZERLAND)  
Roche AmpliPrep System with COBAS AMPLICOR (Roche Diagnostics, SWITZERLAND)

#### **Technical Considerations for this Procedure:**

1. AMPLICOR Monitor V1.5 (Roche Diagnostics, Switzerland) with manual extraction should only be considered in Level IV laboratories.
2. Roche AmpliPrep System with COBAS AMPLICOR, with less technically complex, automated extraction, is recommended for Level IV laboratories. Could possibly be considered for Level III laboratories, but consideration must be given to supportive equipment. This assay and equipment should be limited to personnel trained in techniques of PCR procedures.
3. NucliSens EasyQ with MiniMAG NA Purification System, with automated extraction, is recommended in Level IV laboratories. Could also be considered for Level III laboratories, but

consideration must be given to supportive equipment, and should be limited to personnel with training in techniques of PCR procedures.

4. Equipment needs a dedicated uninterrupted electrical source.
5. Reliable results are dependent on specimen collection, transport, storage and processing procedures.

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## **CBC - AUTOMATED DIFFERENTIAL**

### **General Considerations:**

All general considerations from Levels I and II, and technical considerations are applicable.

1. Complete blood count with 5-part automated differential (multiparameter) is provided at this level.
2. The 5-part electronic differential consists of granulocytes (categorized by type: neutrophils, eosinophils and basophils), lymphocytes, and monocytes.
3. Criteria for performing manual differentials must be established.
4. Perform system back-ups as needed to retain records and system parameters.
5. Automated sample processing provides walk-away capability allowing the staff to perform other tasks while sampling and analyzing.
6. In highly automated systems if workload warrants a second analyzer without autoloading features may be appropriate as the back-up system.

### **CBC with 5-Part Automated Differential**

The following equipment perform an automated CBC and 5-part electronic differentials required for ART monitoring as part of their testing menu:

**Test:** CBC

**Method:** Automated

**Kit/Equipment (Company):** ABX Pentra series (HORIBA, FRANCE)  
XS, XT, or XE Series (Sysmex, JAPAN)  
Cell-Dyn series (Abbott Laboratories, USA)

**Number of Samples:** > 25-50/day

**Method:** Automated

**Kit/Equipment (Samples):** ABX Pentra 60C (60/hour); and ABX Pentra 80, XL80 (80/hour)

**Company:** HORIBA, FRANCE

### **Technical Considerations for this Procedure:**

1. ABX Pentra 60 C
  - a. Does not have walk-away capability.
  - b. Closed mode only.
  - c. Data management system on stand-alone PC.
2. ABX Pentra 80 Series
  - a. Open and closed mode capability.
  - b. 100 sample autoloader.
  - c. Built-in data management system.

**Method:** Automated

**Kit/Equipment (Samples):** XS Series 1000i (60/hour)  
XT Series 1800i, 2000i (80/hour)  
XE Series 2100 (150/hour)

**Company:** Sysmex, JAPAN

### **Technical Considerations for this Procedure:**

1. XS Series
  - a. Appropriate for small to medium laboratories.

- b. Optional 20 tube autoloader.
- c. Separate data management system including patient storage and QC
- 2. XT and XE Series
  - a. Walk-away capability.
  - b. Separate data management system, including parallel QC analysis (current and new), cumulative patient data, delta check, internal (Xbar M) and external QC platform.
  - c. The XT 2000i has retic capability.
  - d. The XE series has retic and NRBC capability.

**Method:** Automated  
**Kit/Equipment:** Coulter A•T 5diff  
**Company:** Beckman Coulter, USA  
**Number of Samples:** CP and OV 60/hour  
 AL 80/hour

**Technical Considerations for this Procedure:**

- 1. Cap Pierce Model (CP)
  - a. Includes a QC data management system as a separate unit to set up files for control storage, Levey-Jennings charts and patient storage.
  - b. Does not have walk-away capability.
  - c. Closed mode system.
- 2. Open vial Model (OV)
  - a. Has a built-in data station (does not require an additional desktop computer); however the data management does not include QC capability or data storage.
  - b. Does not have walk-away capability.
- 3. Autoloader Model (AL)
  - a. Can accommodate 100-position sample loader system.
  - b. Has a separate date management system unit to set up files for control storage, Levey-Jennings charts and patient storage.

**Method:** Automated  
**Kit/Equipment (Samples):** Cell-Dyn 3200 (71/hour); and Cell-Dyn 3700 (90/hour)  
**Company:** Abbott Laboratories, USA

**Technical Considerations for this Procedure:**

- 1. Full data management with data storage.
- 2. Walk-away capability.
- 3. Model 3200 has a single-tube closed sampling system and optional 50-position sample loader.
- 4. Model 3700 has an optional 100-position sample loader system.

**CD4**

**Automated or Semi-Automated**

**Method:** Semi-Automated/Automated  
**Kit/Equipment:** FACSCalibur  
**Company:** Becton, Dickinson and Company, USA  
**Number of Samples:** >50/day

**Technical Considerations for this Procedure:**

- 1. BD FACSCalibur equipment provides a single platform for determination of total lymphocyte, CD3, CD4 and CD8 T-subsets.

2. Absolute counts and percents.
3. Leukemia and lymphoma studies can be done.
4. DNA analysis.
5. Cell sorting and transplantation research can be done; cell sorting can only be done on equipment with sorting capacity.
6. The FACSCalibur system consists of a flow cytometer, a computerized workstation, and optional automated sample loader.
7. Fluorescence for four different colors can be detected.
8. Reagents needed:
  - a. Multitest or tritest reagents (Antibody stain).
  - b. TruCount tubes (Reference beads) only if single platform testing.
  - c. Calibrite 3 and APC beads (calibration beads).
  - d. Multicheck controls - low and high.
  - e. FACSCalibur lysing buffer.
  - f. Sheath fluid (FACSFlow).

#### **Semi-Automated CyFlow Counter**

**Method:** Semi-Automated  
**Kit/Equipment:** CyFlow SL  
**Company:** Partec, Germany  
**Number of Samples:** >25/day

#### **Technical Considerations for this Procedure:**

1. Small size.
2. Three color fluorescence.
3. CD4, CD8, CD3.
4. DNA analysis.
5. Leukemia research.
6. Can be operated with 12V DC battery (car battery).

#### **Automated or Semi-Automated**

**Method:** Automated  
**Kit/Equipment:** EPICS XL, XL-MCL (Multi Carousel Loader)  
**Company:** Beckman Coulter, USA  
**Number of Samples:** >50/day

#### **Technical Considerations for this Procedure:**

1. Four color analysis.
2. CD4, CD3, CD8, CD4% and absolute count.
3. DNA analysis.
4. Reticulocyte enumeration.
5. Optional Multi Carousel Loader.
6. XL II [automated S\software.]

### **URINE DIPSTICK - MICROSCOPY**

*Refer to Level II for details.*

### **CHEMISTRY PANEL/WHOLE BLOOD LACTATE**

#### **General Considerations:**

1. All general considerations from Levels I and II, and technical considerations where applicable.

2. Automated stand-alone analyzer's test menu includes: renal, liver, lipid, and metabolic panels, amylase, and CPK. Lactate may or may not be included.
3. Establish and maintain automated or semi-automated backup methods so that equipment issues do not impact patient care.
4. Areas to consider when evaluating automated chemistry analyzers in higher volume settings:
  - a. LIS capability.
  - b. Delta-check capability.
  - c. Patient bar-coding.

### Chemistry Panel

The following stand-alone equipment perform chemistry panels required for ART monitoring as part of their testing menu on an automated equipment platform. In addition, they perform many other types of tests for patient care. All are high-volume, high throughput models with ISE modules and separate data management systems.

**Method:** Automated

**Kit/Equipment (Company):** Hitachi 902, cobas c501 (Roche Diagnostics, SWITZERLAND)  
 SYNCHRON CX5 (Beckman Coulter, USA)  
 HumaStar 600 (Human International)  
 BS-300, BS-400 (Mindray Medical Intl. Ltd., CHINA)  
 ADVIA 1200 (Siemens USA)  
 VITROS 250, 350 (OrthoClinical Diagnostics)

### Chemistry Panel

**Method:** Automated

**Kit/Equipment:** Hitachi 902

**Company:** Roche Diagnostics, Switzerland

**Number of Samples:** 300/hour with ISE

**Technical Considerations for this Procedure:**

1. Up to 36 tests on-board.
2. Open reagent system.
3. Touch screen monitor.
4. Requires deionized or distilled water to operate.

### Chemistry Panel

**Method:** Automated

**Kit/Equipment:** cobas c 501, part of 6000 analyzer series

**Company:** Roche Diagnostics, Switzerland

**Number of Samples:** 600/hour

**Technical Considerations for this Procedure:**

1. Ready-to-use reagent cartridges; can be changed during operation.
2. Can be combined with the e 601 module for infectious disease testing.
3. Requires deionized water to operate.
4. Automatic rerun and clot detection capability.
5. 60 reagent slots.

### Chemistry Panel

**Method:** Automated

**Kit/Equipment:** SYNCHRON CX5

**Company:** Beckman Coulter

**Number of Samples:** 600/hour

**Technical Considerations for this Procedure:**

1. 1 minute STAT testing capability.
2. Open reagent system.
3. 29 on-board chemistries with electrolytes.
4. Primary tube sampling option.
5. Non-disposable, self-cleaning reaction cuvettes.
6. Up to 90-day calibration stability for most chemistries.
7. Requires deionized water to operate analyzer.

**Chemistry Panel**

**Method:** Automated

**Kit/Equipment:** HumaStar 600

**Company:** Human International

**Number of Samples:** 770/hour with ISE

**Technical Considerations for this Procedure:**

1. Limited release for 2008 in Africa due to complexity of equipment
2. 72 reagent tray compartment

**Chemistry Panel**

**Method:** Automated

**Kit/Equipment (Samples):** BS-300 (480/hour with ISE); BS-400 (640/hour with ISE)

**Company:** Mindray Medical International Ltd., CHINA

**Technical Considerations for this Equipment:**

1. 4 ions and 50 on-board chemistries (BS-300); 77 on-board chemistries (BS-400).
2. Automatic sample dilution.
3. Requires deionized water to operate analyzer.
4. Open reagent system.

**Chemistry Panel**

**Method:** Automated

**Kit/Equipment:** ADVIA 1200

**Company:** Siemens USA

**Number of Samples:** 1200/hour with ISE

**Technical Considerations for this Procedure:**

1. Refrigerated reagent compartment for 41 reagents.
2. Automatic dilution and repeat capability.
3. Calibration stability is 14 days.
4. Deionized water to operate analyzer; drain requirements.

**Chemistry Panel**

**Method:** Automated

**Kit/Equipment (Samples):** VITROS 250 (250/hour); and VITROS 350 (300/hour)

**Company:** OrthoClinical Diagnostics

**Technical Considerations for this Procedure:**

1. Bar-coded slide cartridges (dry-chemistry) with no additional reagent preparation.
2. Up to 6-month calibration curves.
3. No plumbing or drain requirements.

4. Touch screen monitor.

**Whole Blood Lactate: *Refer to Level II for details.***

**Method:** POC - colorimetry

**Kit/Equipment:** Accutrend Lactate System

**Company:** Roche Diagnostics, Switzerland

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## AFB SMEAR, CULTURE, AND SUSCEPTIBILITY

**General Considerations:**

1. AFB smear, culture investigation and identification, and susceptibility should be available.
2. Decontamination, digestion and concentration procedures (WHO, 1998c).
3. MOTT capability.
4. Consideration to using liquid TB media and automated readers if volume and capabilities support it (WHO, 2007).
5. Drug susceptibility testing including resistance investigation.
6. Provide data for surveillance information.
7. Must determine if additional procedures will be applicable to smear negative, relapse, treatment failures only or if it will include initial diagnosis patients (CDC, 2003; WHO, 1998a).
8. Purchased media eliminates issues encountered with on-site prepared media: carefully controlling the temperature for protein coagulation needed for egg-based media, or the need for a large stock of reagent grade chemicals and its inventory management.
9. Examination schedule for cultures is required.
10. Niacin, nitrite reduction, catalase, PNB media are used to identify *M. tuberculosis*.
11. Interim and final culture reports will be required. Information flow back to collection site must be incorporated and documented.
12. Laboratory layout must be designed to control airflow (inward airflow and ventilating system).
13. Isolation from general laboratory traffic. Biosafety level 2 cabinet required.
14. Anteroom with double door entry.
15. Must adhere to BSL 3 requirements.
16. Establish procedures for personnel safety monitoring.

**AFB Smear –*Refer to Levels I and II for details.***

**AFB Culture and Susceptibility (Manual)**

**Method:** Manual

**Method:** Culture and Susceptibility

**Kit/Equipment:** Culture- Middlebrook and Cohn 7H10 Agar, Ogawa, Lowenstein-Jensen  
Digestion/Decontamination- MycoPrep™ Specimen  
Digestion/Decontamination Kit  
Susceptibility- Sensi-Disc™ Antimycobacterial Discs for Use in Culture Media

**Company:** Becton, Dickinson and Company, USA; various vendors

**Technical Considerations for this Procedure:**

1. Biological safety cabinet class to be considered should be at minimum Class II, Type B.
2. BSC numerous sources.
3. Confirmatory reagents placement and room accessibility needs to be determined.
4. Validation once installed and annual certification.
5. CO2 and non-CO2 incubators will be required and monitored.
6. QC and documentation before use with patient samples.
7. Appropriate media and QC for media must be available.
8. Susceptibility reagents must be available.

9. Additional supplies such as plate sleeves, culture tube racks, culture boxes, Bunsen burners, wire loops and needles, and baskets will be needed need to stick with the tube/bottle method (Lowenstein-Jensen slopes).
10. A system for autoclaving waste and monitoring its effectiveness will need to be established.
11. Centrifuge requires sealed buckets and must achieve relative centrifugal forces of 3000x g to be contained into the sediment pellet (WHO, 1998c).
12. Establish susceptibility guidelines and reporting methods.
13. Appropriate collection devices and technique for a wide variety of specimen types.
14. Transporting of specimens must be packaged to prevent leakage, remain cool, and protected from sunlight.

### **Liquid AFB Culture and DST (Automated)**

**Method:** Automated

**Method:** Liquid TB Culture and DST

**Kit/Equipment:** BACTEC 460 TB; and BACTEC MGIT 960

**Company:** Becton, Dickinson and Company, USA

#### **Technical Considerations for this Procedure:**

1. Should be developed in relationship to a detailed and comprehensive country plan for TB laboratory capacity strengthening.
2. Equipment requires dedicated source of electrical power.
3. BACTEC 460 may no longer be supported. BACTEC 960 would be required.
4. Equipment produces heat and this must be addressed to achieve an ambient room temperature.
5. Current guidelines recommend simultaneous culturing on solid media and any practices to discontinue routine solid media culturing would require method validation to support this practice.
6. Solid media will be required for positive specimens processing and for a backup method in equipment failure situations.

## **MICROBIOLOGY SMEAR AND CULTURE**

### **General Considerations:**

1. Diagnosis of bacterial, fungal and parasitic infections. Perform all microbiologic methods listed under Levels I and II with the addition of culture, ID, and susceptibility. Refer to Levels I and II for details on microbiologic procedures.

### **Culture (Manual)**

**Method:** Manual

**Method:** Culture

**Kit/Equipment:** Prepared Packaged Media

**Company:** Becton, Dickinson and Company, USA; and various vendors

#### **Technical considerations for this Procedure:**

1. Gram stain report should be available within one day upon receipt of specimen to provide a rapid indication to the nature of the infection.
2. Culture and susceptibility report should be available within three to five days upon receipt of specimen.
3. Specimens should be transported to Level III labs as soon as possible. If delays occur, properly sealed specimens (except CSF or other body fluids, Cryptococcus isolation or blood cultures) can be refrigerated or inoculated into the appropriate transport media.
4. Access to fresh blood is required. Safety is a concern and fresh blood may be difficult to obtain.
5. Techniques for identification and susceptibility include:
  - a. Latex agglutination tests for meningitis.

- b. Biochemical tests for identifying enteropathogens.
  - c. API strips for a range of organisms.
  - d. Kirby-Bauer or other sensitivity methods.
6. Equipment and supplies for the culture and identification of microaerophilic and anaerobic pathogens must exist.

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#### MALARIA SMEAR

*Refer to Level I for details.*

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#### MALARIA RAPID TEST

*Refer to Level I for details.*

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#### SYPHILIS

*For Rapid Syphilis Testing, refer to Level I for details.*

*For TPPA/TPHA/RPR, refer to Level II for details.*

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#### HEPATITIS B AND C: Serology by Automated Immunoassay

*Refer to Level II for test details on other methods.*

**Methodology:** Automated EIA

**Methodology:** Automated immunochemistry platform (cobas 601 – Roche Diagnostics, Switzerland); Access - Beckman Coulter, Immulite 1000 - Siemens; Centaur XP -Siemens USA; ECI-Ortho Diagnostics

**Technical Consideration:**

1. Automated immunoassay instruments provide highly accurate and efficient automated methods for many EIA tests including Hepatitis B, HIV 1/2, and other assays.
2. Level III Laboratories with high volumes of these tests (over 50 /day) may want to consider one of the immunoassay instruments listed below.
3. All of the below instruments offer high throughput, walk-away models; test menus available on each instrument vary therefore test menu must meet user requirements. Of the instruments listed, ECI and Centaur only have FDA approval for automated Hepatitis C Antibody and HIV Antibody.

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#### TYPE AND CROSSMATCH

*Refer to Level II for details.*

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#### URINE PREGNANCY RAPID TEST

*Refer to Level I for details.*

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#### WET MOUNTS (NaCl and KOH) - DIRECT MICROSCOPY

*Refer to Level I for details.*

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#### CRYPTOCOCCAL ANTIGEN TEST

*Refer to Level II for details.*

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#### INDIA INK STAIN

*Refer to Level II for details.*