

Consultation on Technical and Operational Recommendations for Clinical Laboratory Testing Harmonization and Standardization

*22-24 January 2008
Maputo, Mozambique*

Helping to Expand Sustainable Quality Testing to Improve the Care and Treatment of People Infected with and Affected by HIV/AIDS, TB and Malaria



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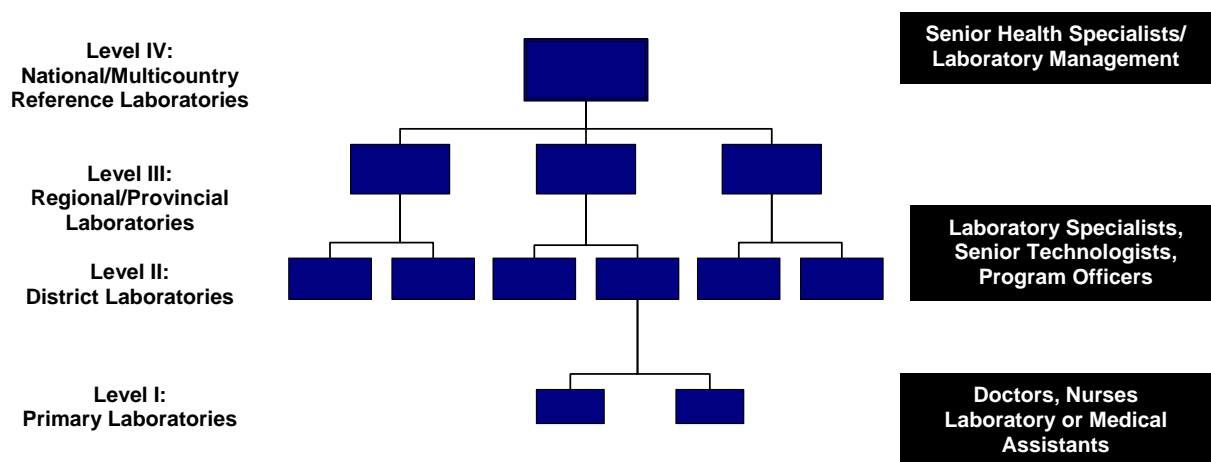
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I. Introduction

A consensus meeting of major stakeholders who were charged with making recommendations on laboratory testing standardization and harmonization in three major areas was held on 22-24 January 2008 in Maputo, Mozambique. The three areas discussed were: 1) testing needed at each level of a tiered, integrated laboratory network; 2) standardization of laboratory equipment and supplies at each level of a tiered laboratory network; and 3) key considerations to guide maintenance and service contracts for equipment at each level of a tiered laboratory network.

This effort seeks to strengthen laboratory capacity in resource-limited settings. It is believed that the best way to do this is by building sustainable laboratory capabilities that will provide access to high quality, rapid, and affordable diagnostic tests for the care, treatment, prevention and surveillance of HIV/AIDS, tuberculosis (TB) and malaria. A tiered, integrated laboratory network (see **Figure 1**) may provide the best model for service delivery across various levels of the public health system in resource-limited settings.

Figure 1: The Tiered, Integrated Laboratory Network



Accurate and reliable clinical laboratory testing is an important component of a public health approach to disease management in resource-limited settings. Laboratory data are essential for clinicians to accurately assess the status of patients' health, make accurate diagnoses, formulate treatment plans, and subsequently monitor the effects of treatment. The clinician must be able to trust the test results from the laboratory in order to use them for clinical diagnosis and treatment. As a result, the results must be accurate, reliable and timely.

In order for laboratories to provide high quality test results, the following systems must be available:

1. **Human capacity** - Competent staff who are adequately trained; effective supervision by managerial staff. Recruitment and retention programs are required to maintain trained personnel. Formal, pre-service training programs as well as orientation, performance appraisals, and in-service training systems must also be available.

2. **Infrastructure** - A safe and suitable physical environment with ample space, power, climate control, water and transport access. There should be an uninterruptible power supply (UPS) supporting laboratory equipment in case of power surges. Sufficient light, bench space, mains or bore hole water, and distilled water are also required. In place must be high quality, functioning laboratory equipment and a supply chain management system to provide adequate supplies of reagents, consumables and quality control (QC) materials. The laboratory environment should have enough space to perform day-to-day operations safely and efficiently and to store cold chain and non-cold chain supplies.

3. **Management of Quality Systems** - Effective laboratory quality systems, including well-written policies and procedures, a QC system, quality improvement (QI), external quality assessments (EQA), and accreditation standards should exist. Standard operating procedures (SOPs) must be understood and implemented to ensure overall test reliability, which includes test accuracy and precision. Laboratory professionals should routinely perform QC testing to guarantee that the test methods and equipment perform according to the established standards. Laboratory professionals must participate in EQA/proficiency testing (PT) programs in order to demonstrate that they have acceptable systems and that specimens are collected and processed appropriately.

II. Introduction to the Tiered Laboratory Network

The Laboratory Network in Resource-Limited Settings

A tiered laboratory network is an integrated system of laboratories organized in alignment with the public health delivery network in a country. In resource-limited settings, the following four levels of laboratories are desirable to best deliver services in a national laboratory network:

1. **Level I-Primary:** Health post and health center laboratories that primarily serve outpatients.
2. **Level II- District:** Laboratories in intermediate referral facilities (e.g., district hospitals).
3. **Level III-Regional/Provincial:** Laboratories in a regional/provincial referral hospital that may be part of a regional or provincial health bureau.
4. **Level IV-National/Multicountry Reference Laboratory:** The national/multicountry public health reference laboratory for one or more countries.

The tiered levels of a laboratory system and the testing performed at each level may vary depending on the population served (e.g., infants, adults), level of service available, physical infrastructure, electricity, water, road conditions, and the availability of trained technical personnel in-country.

Level I Laboratories

Level I laboratories would consist of health post or health center laboratories that would primarily serve outpatients. Essential infrastructure, such as clean water, refrigeration and electricity, may or may not be available. These laboratories would serve as peripheral branches of Level II laboratories, which would be the center or hub. Health posts may collect and/or refer specimens to health center laboratories. Diploma level staff at Level I laboratories would be very limited, with usually no more than one trained laboratory assistant or nurse providing services. Basic quality assurance (QA), QC and record keeping functions must be performed at this level. QA activities would focus on adequate specimen collection and basic reagent use. The laboratory would offer diagnostic and monitoring services for HIV/AIDS, TB and malaria. If essential infrastructure were lacking, then the on-site test menu would be restricted to manual tests. In many instances, Level I laboratories would perform specimen collections and simplified techniques such as the collection of dried blood spot (DBS) and rapid/dipstick tests. Sites with reliable power and water would perform certain automated chemistry tests required for antiretroviral therapy (ART) monitoring. Same day performance and delivery of results must be available while the patient is present in order to provide immediate counseling, treatment and regimen modification.

When required testing exceeds the scope of services available from Level I facilities, the “parent” Level II laboratories would provide a range of consultant services, including the receipt of referral specimens and patients. In many cases, such as with DBS or a TB culture, the specimen may bypass a Level II laboratory and go directly to the nearest performing laboratory.

Level II Laboratories

Level II laboratories would consist of district hospitals or primary hospital laboratories that perform tests beyond the capabilities of Level I facilities. Health posts may refer specimens to Health Center Laboratories under Level I. Serving inpatients, these laboratories would have dedicated laboratory space, formally trained personnel, UPS systems and a consistent source of reagent grade water. The laboratory would be staffed by a minimum of three formally trained technologists or technicians. One staff member who has managerial skills would serve as the senior or supervisory technologist.

The Level II laboratories would have more extensive test menus for diagnoses and treatment. Consolidating testing at the district level for certain tests provides necessary volumes for automated equipment platforms. The Level II laboratories would coordinate the services of Level I laboratories in the district as well as serve as reagent and supply reservoir/back-up repositories for these laboratories. In addition, Level II laboratories would provide the following consultant services and support for Level I laboratories:

- Managerial oversight of an outreach program of peripheral primary laboratories (Refer to Annex J, Reference No. 1)
- Referral laboratory services with a more extensive test menu
- On-site quality assessment visits
- Assistance with resolving technical problems
- Data management support with a strong paper-based laboratory information system (should be part of a national system of data collection by the Ministry of Health [MOH])
- Development and implementation of QA activities (including, but not limited to QC, QI and EQA/PT)
- Periodic review of QC
- Information and training for adequate specimen collection
- Collection of data for assessment of quality indicators
- Approval and annual review of SOPs and policies to ensure alignment with current practices
- Assistance with development of SOPs and safety procedures
- Staff development/training, performance management, competency assessment, and retraining
- Coordination of courier/transport services
- Assistance with results reporting and record retention
- Equipment maintenance and service support including review of maintenance logs
- Follow-up on laboratory incident and accident reports
- Assessment of safety management practices

Level III Laboratories

Level III laboratories would consist of laboratories in tertiary referral facilities such as regional or provincial hospitals. These laboratories would perform a complete menu of testing for HIV/AIDS, TB and malaria as well as testing for many other diseases. Level III laboratories would complete the more sophisticated tests at a higher throughput level that Level II laboratories were not able to perform. These facilities must have dedicated laboratory space that would include a separate microbiology space and UPS systems. While a Biosafety Level III designated area would be desirable, the minimum requirement in areas where specimens are handled would be Biosafety Level II. Reagent grade water would also be required. Formally trained, diploma level technologists who are able to meet workload demands would staff Level III laboratories. One technologist who has managerial skills would serve as the laboratory supervisor. Level III laboratories would act as laboratory resource groups for the facilities in their regions.

In addition, Level III laboratories would provide the following services:

- A more comprehensive test menu than that provided at Level II laboratories

- Coordinate laboratory services and information management with other Level III laboratories
- Perform assessments of laboratories in the region; evaluate the QA data from laboratories in the region
- Coordinate surveillance data collection from lower levels in an effort to obtain country-wide statistics
- May collect and submit inter-laboratory comparisons and EQA data for the region to the national reference laboratory
- Develop training programs and coordination of continuing education
- Assure adequate requisition and reporting mechanisms as well as record retention procedures
- Standardize units, methodologies and reference ranges based on national reference laboratory recommendations
- Determine the amount of patient history/clinical presentation required for tests referred to other levels
- Provide logistical and management support to their service areas

Level IV Laboratories (National/Multicountry Reference Laboratories)

Level IV national/multicountry reference laboratories are recommended to strengthen laboratory capacity for diseases of public health concern. Ideally, they would provide linkages with research laboratories, academic institutions and other public health laboratories, forming integrated laboratory networks that can provide assistance in clinical trials, evaluation of new technologies and surveillance. Senior program employees, laboratory management and senior laboratory technologists/scientists would staff these laboratories. Level IV laboratories would possess the infrastructure, equipment, information systems, and logistical capabilities of sophisticated reference laboratories (i.e., Biosafety Level III specifications). In some countries lacking a unique national/multicountry reference laboratory, Level III laboratories may serve as national reference laboratories.

Level IV National/Multicountry Reference Laboratories would:

- Perform molecular and esoteric testing beyond the technical capabilities of Level III laboratories (e.g., nucleic acid assays, HIV drug resistance studies, TB drug susceptibility studies)
- Develop and/or adopt laboratory standards (i.e., ISO 15189) and processes for laboratory accreditation
- Develop monitoring and evaluation activities for laboratories
- Serve as the national coordinator for HIV, TB and malaria laboratory programs
- Maintain national database of equipment and maintenance in country(s)
- Participate in international EQA programs and develop/oversee national EQA programs
- Provide input on national laboratory policy development
- Determine what information needs to be supplied with the test result to better interpret the results
- Provide courier and logistics management support for the regions

- Develop and implement testing algorithms and automatic performance of additional tests (reflex) for laboratory utilization
- Establish standards for quality management and assist with policy and procedure development
- Provide assistance with reference range validations and development of national reference ranges specific to equipment/methods used
- Coordinate the collection of surveillance data to obtain and monitor country-wide statistics
- Introduce and implement new technologies, appropriate for each level, to reflect current best practices
- Select and evaluate diagnostic tests
- Define sensitivity and specificity requirements in order to select methods that would be evaluated with a method validation plan

Group Work and Recommendations

Breakout Session 1: Review and agree on the laboratory tests and services needed at each level of a tiered, integrated laboratory network.

The participants were divided into five breakout groups. The major task was to review the *Draft Working Resource Document and WHO Technical Consultation Report* to agree on the different levels of laboratories and to establish the tests required at each level of the laboratory network. The basic questions for consensus discussion included:

- 1) What are the definitions of each level of a tiered laboratory network?
- 2) What tests are required at each level?
- 3) What are the infrastructure requirements for testing at each level?

Question 1: The groups agreed that four levels are recommended for laboratory networks in resource-limited countries: Level I (Primary), Level II (District), Level III (Regional/Provincial) and Level IV (National/Multicountry Reference Laboratory). The groups added Level IV, recognizing the need for national coordination of laboratory services, administrative policy support, QA, test development, EQA and surveillance support. The descriptions of each level in a tiered laboratory network are included earlier in this section.

Question 2:

Level I laboratory tests and services

- HIV rapid tests
- Hemoglobin
- Whole blood glucose by glucometer
- Rapid syphilis test
- AFB smear by light microscopy
- Wet mounts
- Urine pregnancy rapid test

- Malaria rapid test
- Malaria smear
- Urine dipstick
- DBS collection
- ALT and creatinine (could be considered at sites with adequate infrastructure)

Level II laboratory tests and services

- All tests and services performed at Level I
- CD4 counts (absolute required; % is preferable)
- HIV serology by EIA (could be considered at Level II if volume and technical capabilities support it)
- Complete blood count (CBC) with differential
- Chemistries
 - Liver function tests (ALT, bilirubin)
 - Serum electrolytes
 - Renal function tests (creatinine, urea nitrogen)
 - Lipid profile
 - Serum amylase
 - Glucose
- Whole blood lactate
- Syphilis serology test
- Cryptococcal antigen
- India ink
- Gram's stain
- Urine dipstick with microscopy
- Type and crossmatch
- CSF/body fluid cell counts
- Hepatitis B
- Hepatitis C (guided by local prevalence)

Note: Microbiology culture and susceptibilities may be offered at higher volume sites.

Level III laboratory tests and services

- All tests and services performed at Levels I and II
- Microbiology culture, identification and susceptibilities
- Blood cultures
- Complete chemistry panel
- AFB smear (by fluorescent technique)
- AFB culture, identification and susceptibility (first-line drugs)*
- Quantitative nucleic acid test for ARV monitoring (PCR, bDNA)
- Qualitative nucleic acid testing for diagnosis of infants (DNA PCR)

*TB susceptibility based on burden and reference laboratory capacity

Level IV laboratory tests and services

- All tests and services performed at Levels I, II and III
- HIV resistance testing
- AFB susceptibility (for first- and second-line drugs)
- Ultrasensitive p24 antigen
- Quantitative nucleic acid testing for ARV monitoring (HIV RNA)
- Qualitative nucleic acid testing (HIV RNA)
- Variety of diagnostic testing
- Other esoteric reference lab testing as needed

See **Annex C** for the summary of workgroup recommendations for laboratory tests performed at each level of a laboratory network.

Question 3: The workgroups agreed with the infrastructure requirements for each level as described earlier in this section.

III. Acquisition and Standardization of Laboratory Equipment

Laboratory test quality relies on the availability of laboratory equipment, reagents and consumables that meet minimum quality standards. In an effort to enhance quality and promise efficient resource use, equipment should be standardized wherever possible in a tiered laboratory network.

Standardization and procurement policies should be defined by MOH in collaboration with physicians, laboratory staff and policy makers. Standardizing the type of platform for laboratory equipment in chemistry, hematology and CD4 across different laboratory levels offers many benefits, including:

- Cost reduction due to bulk procurement
- Ease of service due to a limited variety of platforms
- Higher manufacturer investment in service and distribution capability in-country
- Ease of staff training due to common user interface on the systems
- Minimal additional training needed when staff members move from laboratory to laboratory
- Better standardization of reference ranges and test results, thus better continuity of care for patients who transfer from health centers to district facilities

Standardization of equipment requires that manufacturers provide truly scalable equipment options that meet the low, medium and high volume testing needs of Level I, II, III and IV laboratories. If this is not possible, standardization of no more than two equipment platforms should be considered. While standardization provides many benefits, each country must make sure, through good contract language (see **Section V**), that it receives the financial and maintenance benefits associated with sole sourcing. In many resource-limited countries, there is currently a diversity of equipment platforms as a result of the lack of standardization, regulations that promote competition, donated equipment, and decentralized procurement systems.

To provide guidance on vendor selection, pre-qualification conditions can be set for major laboratory equipment and commodities. Presently, there is limited guidance on the quality of reagents and equipment, especially for hematology and chemistry machines. Purchasers may need to obtain independent evaluation information on the quality of equipment from a variety of sources (particularly other users). The WHO guidelines on diagnostics are expanding and now exist for certain test domains, including CD4, HIV (rapid and ELISA) and rapid syphilis testing. Geneva's Stop TB Department is currently preparing technical specifications for TB lab equipment and supplies plus guidelines for management. The finalized specifications, which are complimentary to this report, will be available in September 2008 and will be posted on WHO's website. Refer to **Annex J** for a complete list of available reference documents.

The first step in standardization of equipment in a laboratory system is to define the tests performed at each level in order to match test system capacity with expected volumes for each test. A list of potential manufacturers who meet basic requirements must be developed and assessed for domestic or international historical performance. Ideally, a list of pre-qualified vendors would be established based on WHO guidelines. A team of laboratory technicians should provide input, and along with procurement specialists, should develop technical specifications for equipment acquisitions. **Table 1** lists important aspects to consider when selecting laboratory equipment.

Table 1: Key Criteria for Selection of Laboratory Equipment

❖ Infrastructure (power, generator, water, temperature, space)
❖ Environmental conditions
❖ Laboratory workload; staff skills and training
❖ Vendor support, reliability and availability (in-country or region)
❖ Availability, stability and temperature sensitivity of reagents, controls and calibrators
❖ Availability of local service, technical and training support
❖ Simplicity of operation; ease of maintenance and calibration
❖ Track record of performance (domestic and/or international)
❖ Analytical performance/technical quality (sensitivity, specificity, reliability, level of detection)
❖ Test menu (consider scalability for various volumes)
❖ Open or closed test/reagent system
❖ System costs (includes equipment, service, reagents, and supplies); cost per reported test
❖ Specimen types
❖ Throughput
❖ Turnaround time
❖ QC and QA required
❖ Availability of EQA and inter-laboratory comparisons
❖ Data management capability; interface capability
❖ Safety
❖ Availability of back-up methods
❖ Supply chain management capability

A variety of acquisition methods exist for laboratory equipment. The three most common are:

1. Purchase
2. Lease
3. Reagent rental

All three methods entail expenses for equipment, service and reagents/consumables, with invoices and payments being handled in different ways. Purchasers should carefully evaluate the equipment acquisition contracts to identify minimum volume commitments, service requirements, training that is included, user support, warranty provisions and reagent pricing discounts. Negotiation of these elements in the contract is required to guarantee that the best value and the lowest costs are obtained from the vendor. Refer to **Annexes H and I**, which include important considerations when procuring laboratory equipment and negotiating contracts. It's important to note that everything in the contract is negotiable (see **Section V**). The input of the MOH officials would be desirable in the procurement and negotiation for laboratory equipment, service, reagents and supplies.

Key considerations for the most common acquisition methods for laboratory equipment are displayed in **Table 2**.

Table 2: Considerations for Laboratory Equipment Acquisition

PURCHASE	LEASE	REAGENT RENTAL
Can result in discounted price	Requires minimum initial cash outlay	Requires minimum cash outlay
Reagent cost may be less	Risk of obsolescence is less as lease term is usually no more than 5 years	Agreements are easy to set up with the vendor
Equipment expense can be depreciated	Equipment can be upgraded for a new model and can be returned after the lease period	Cost of equipment, reagents and service is spread across each test as you are paying a fixed amount on a per-test basis
Can be used as a trade-in for upgraded models	Difficulties returning equipment may exist in certain countries	Reagent costs are higher as cost of equipment and service is bundled in reagent cost
Requires a significant initial cash outlay	Vendors may be hesitant to lease equipment unless risks are minimized (assured return)	Volume commitments must be accurate as these are the basis for pricing
Risk of obsolescence in 5-7 years	Allows options for buying equipment at the end of the lease such as 1 USD or fair market buy-out	Flexible option with predictable fixed costs per month
Equipment must be disposed of at some point	Allows for trying out the equipment before buying it	Equipment issues regarding import and return are similar as for leasing
	Total cost is higher than purchase due to financing	A buy-out option does not exist
	Reagent pricing must be negotiated separately from the lease based on volumes	Most desirable for changing technologies and high-cost systems due to risk of obsolescence in 5 years

Group Work and Recommendations

Breakout Session 2: To develop a consensus to guide standardization of laboratory equipment at each level of an integrated, tiered laboratory network.

The participants were divided into five breakout groups. The major task was to review the *Draft Working Resource Document* tables and equipment considerations in order to make recommendations on laboratory equipment for each level of the laboratory network. The basic questions for consensus discussion included:

- 1) What are the criteria for the selection of laboratory equipment?
- 2) What laboratory equipment is appropriate for the different tests performed at the different levels of an integrated laboratory network?
- 3) What are the recommendations regarding donated equipment?

Question 1: The groups concurred with the criteria for equipment selection listed in **Table 1**.

Question 2: There was agreement that specifications should be defined for each type of equipment. In addition, examples of equipment that meet those specifications should be listed. The WHO guidelines for equipment/methods should be used where available. The workgroups' suggestions regarding tests and equipment for each level of testing as well as manufacturer options are listed in **Annex E**. This list is not meant to be inclusive of all available equipment or vendor choices, but rather to provide options that meet the specifications listed.

Question 3: The workgroups agreed to use the *WHO Guidelines on the Donation of Medical Equipment*. (Refer to Annex J, Reference No. 2). In addition to the WHO guidelines, the following recommendations were made for donated laboratory equipment:

- Donated equipment should be accepted only if part of the laboratory strategic plan.
- Donors should be involved in national laboratory planning.
- Countries should have clear policies at the central level with a list of acceptable reagents and equipment.
- Donors should send equipment specifications prior to delivery.
- Donated laboratory equipment should have at least 80% useful life remaining at time of donation.
- Donated equipment should follow normal supply management processes to assure adequate reagents and supplies; service, maintenance and training systems must be available.
- Vendors should be pre-qualified where possible within the country by the national laboratory system.
- Equipment retirement procedures need to be developed and followed for donated and other equipment.

IV. Equipment Maintenance and Service Contracts

The purchase and maintenance of laboratory equipment are critical to ensuring quality laboratory testing. With quality testing, analyses will be accessible and timely; the requirements for specimen transport will be minimized; and, ultimately, the needs of the patients and communities will be better served. Laboratory equipment maintenance begins with equipment selection and purchase, and is later influenced by infrastructure, training, preventative maintenance and servicing. Therefore, these are all important considerations.

Selection criteria for equipment were previously listed (**Section IV, Table 1**). An important consideration for laboratory maintenance is selecting platforms that are multipurpose (e.g., ELISA platform that provides multiple assays). Having to maintain a single piece of equipment that performs multiple tests will allow for higher quality laboratory testing than having several pieces of equipment with each performing one test or analysis.

Proper infrastructure is also essential for good laboratory maintenance. Equipment must be housed in a proper environment, with consideration of power sources, water quality, dust, climate and appropriate space.

Various purchase methods were previously discussed (**Section IV, Table 2**). The method of equipment acquisition—purchase, lease or reagent rental—is important since it is associated with varying degrees of maintenance support. Proper staff training (initial and follow-up) should be negotiated as part of the equipment purchase. Training is required, not only for the proper operation of laboratory equipment, but also for good laboratory practices, quality testing, proper specimen collection, reagent forecasting and SOP development.

Two types of preventative maintenance are required: laboratory-initiated and manufacturer/service provider-initiated. As part of the initial training, laboratory personnel should learn to provide daily, weekly, monthly and semi-annual preventative maintenance. The timing of the preventative maintenance varies by equipment so a maintenance schedule should be provided for each piece of equipment. Although many maintenance requirements will also be specific to each piece of equipment, there are some general guidelines:

- Internal controls must be run to ensure proper function of the equipment and reagents.
- Proper specimen collection techniques must be practiced and proper reagents must be used.
- Equipment should be covered when not in use and dust should be wiped off at least once a week.
- Maintenance procedures, maintenance logs for documentation, and supervisory reviews of maintenance performed should be developed and shared by laboratories.

The manufacturers' responsibilities for providing preventative maintenance checks on equipment are included in the warranties or service contracts. Quarterly or bi-annual preventative maintenance visits should generally be expected and negotiated. In addition to preventative maintenance details, service contracts and warranties will describe how manufacturers should respond to service needs. Services provided (e.g., troubleshooting, loaners, upgrades, spare parts, scheduling of maintenance visits, training) and costs incurred (e.g., parts, labor, travel, shipping) need to be negotiated and

defined in the service contracts. **Annexes H and I** include important considerations when procuring laboratory equipment and negotiating contracts.

There are significant maintenance challenges in resource-limited countries, including poor infrastructure, equipment placed in remote locations, perceived inability to negotiate with vendors, inadequate procurement and distribution systems, as well as the lack of trained staff, manufacturer presence, communication, information and integration.

These challenges may be addressed by:

- Addressing infrastructure needs
- Developing partnerships between laboratories and manufacturers
- Cross-training personnel on equipment service
- Encouraging manufacturers to establish local representation
- Insisting that manufacturers have available loaners
- Ensuring that laboratories have back up equipment or techniques
- Providing continuing education for laboratory staff
- Developing an integrated laboratory network

In addition, accountability for laboratory performance is critical. Supervision by management staff is integral to maintaining a quality system of equipment and supplies. Also essential to developing proper maintenance practices within a laboratory is involving all staff members in the process. This entails utilizing the individual or collective power of countries/regions with manufacturers and vendors, setting high goals (but reasonable within the laboratory capacity), and helping to redefine existing norms that will make certain that quality laboratory testing is accessible to patients in their communities.

Group Work and Recommendations

Breakout Session 3: To develop a consensus on the key considerations to guide maintenance and service contracts for equipment at the various levels of an integrated, tiered laboratory network.

The participants were divided into five breakout groups. The major task was to review the questions for consensus and to identify maintenance and service issues. In addition, to help guide decision-making, the groups were to make recommendations on equipment service, maintenance and supply delivery. The following questions were discussed:

- 1) What are the issues that exist in the areas of service contracts, service delivery and reagent/supply delivery?
- 2) What maintenance, processes, and service and supply chain management infrastructure must exist to assure continuous testing for patient care at each level of the laboratory network?

Question 1: The major issues identified in each area by the workgroups were:

Service Contracts

- Inadequate service contracts
- High cost of service contracts
- Insufficient laboratory contracting knowledge/expertise
- No service contract in place after equipment warranty expires
- No penalties in contract for failure to deliver service

Service Delivery

- Lack of well-trained local service representatives (vendors or local engineers)
- Difficulties in obtaining service and spare parts for equipment in a timely manner
- Limited presence of suppliers and service providers in-country
- Insufficient preventative maintenance
- Inadequate number of service engineers to cover entire country
- High turnover of in-country trained engineers

Reagent/Supply Delivery

- Problems with accurate inventory and supply chain management especially for cold chain items
- Lack of standardized forecasting models in use
- Cumbersome purchasing processes
- Lack of central purchasing system in certain countries
- Weak delivery system by suppliers

Question #2: Considerations for maintenance and service infrastructure included:

Service Contracts (after-sale maintenance contracts)

- Service contracts should be negotiated at time of equipment procurement.
- Reagent rental agreements with bundled service and reagents should be considered.
- Contracts could be for a minimum of 3 years, renewable with no cost increase annually.
- Service contracts should include at a minimum:
 - Response time (ideally within 48 hours).
 - Number of preventative maintenance visits (as required by the manufacturer).
 - Training of local service engineers and users.
 - Availability of routine/emergency service.
 - Costs incurred outside of the contract.
- Penalties should exist in the contracts for failure to meet the conditions of the agreements.
- A periodic contract review process to determine compliance should be in place.
- Local service providers should be certified by the manufacturer.
- Contracts should provide for backup support (loaners) within 72 hours and access to spare parts.
- Contracts should include a contingency plan for returning equipment for service if repairs cannot be done in-country.

- In-country support should be guaranteed in the contracts.

Service Delivery

- Qualified service technicians should exist locally for the number of equipment.
- Structured formal training programs should exist to train and qualify local engineers.
- Engineers should install, train, service and help users with problem solving (in order to accomplish this, they should speak local language).
- Regular schedules of preventative maintenance should be established and followed by laboratory users and service providers.
- Laboratory sites should obtain and retain documentation of services performed by service providers.
- Hotlines for real time support should be available.
- Engineers should arrive with proper equipment and spare parts.
- Engineers should have access to loaner equipment to swap out if on-site repairs are not possible.
- Troubleshooting and service tips should be shared with users.
- Local service providers should have good relationships with manufacturers.
- Vendors should provide periodic information on recalls and updates.
- Laboratories should actively monitor equipment including service and maintenance as part of the QA program.
- Laboratories should report any adverse events to the manufacturers, documenting downtime and service problems.

Reagent/Supply Delivery

- Defined forecasting and inventory management systems should be operational in each laboratory.
- Reagent rental and standing orders for reagent delivery should be options.
- Central coordinating bodies should perform regular reviews and verify sustainable supply chain management systems.
- Lot assurance should be provided by suppliers.
- Pack size should meet facility and transportation requirements.
- Cold chain requirements should be met in transport and storage at each site.
- Effective clearance procedures and duty waivers should be available.
- National policy should exist for minimum expiry dates on reagents.
- Feedback from users on reagent/supply delivery systems should be obtained.
- Reliable distributors/agents should exist in-country.
- Replacement policy for unusable or expired products should be defined in contracts.
- Quality assessment of products to be used in-country must be performed.
- Quality should drive procurement more than cost.
- Sole sourcing should occur only if unavoidable.
- Global pricing may be useful to reduce high local costs.
- A centralized, transparent procurement system is desirable.
- Streamlined purchasing and payment processes should be in place to avoid stock-outs.

- Competition among quality suppliers should be encouraged.

ANNEXES

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ANNEX A

Meeting Agenda

DAY 1: Tuesday 22 January 2008		
7:30-8:30: Registration – Maputo Hall		
Session #1 Maputo	Moderators: John Nkengasong and Gaby Vercauteren (WHO Geneva)	
	8:45	Welcome and Meeting Objectives Guy-Michel Gershy-Damet (WHO AFRO)
	9:00	Introductory Remarks Deborah Birx (CDC)
	9:10	Introductory Remarks Chana Rabiner (USAID)
	9:20	Introductory Remarks El Hadj Benzeroug (WHO Mozambique)
9:30	Opening Remarks Paulo Ivo Garrido (MOH, Mozambique)	
9:45-10:15: Tea Break		
Session #2 Maputo	Moderators: Guy-Michel Gershy Damet (WHO AFRO)/ Chana Rabiner (USAID)	
	10:15	Scaling-up HIV/AIDS, TB and Malaria Programs in the Next Five Years Marco Antonio de Avila Vitoria (WHO Geneva)
	10:40	Laboratory Challenges in Scaling-Up HIV/AIDS, TB and Malaria Programs Deborah Birx (CDC)
	11:05	Need for Standardization of Laboratory Commodities Trevor Peter (The Clinton Foundation)
	11:30	Country Experience with Standardization of Laboratory Commodities: The Process and Opportunity Alash'le Abimiku (Institute of Human Virology, Nigeria)
12:00	Laboratory Strategic Planning and Standardization of Lab Commodities John Nkengasong (CDC)	
12:30-14:00: Lunch – Hotel Dining Room		
Session #3 Maputo	Moderator: Thomas Lapnet-Moustapha (WHO AFRO)/Phyllis Kanki (Harvard)	
	14:00-14:20	<i>Objective #1: To review and agree on a list of supplies and tests needed at each level of an integrated tiered laboratory network, and classify them as Crucial (critical for the laboratory to operate), Important (the laboratory should have available), or Needed (good to have but not critical for laboratory operations)</i>
		The WHO-Geneva/PEPFAR Collaboration to Prepare an Operations Manual for HIV Care and Treatment at Primary Health Centers: Defining Lab Services Tedd Ellerbrock (on behalf of WHO Geneva/PEPFAR collaboration)
Session #4 Maputo/Beira/Avenida	Breakouts 1	
	14:20-16:00	Room 1 (Maputo): French Speaking, Table 1 Chair: Yao Guillaume Loukou Rapporteur: Lassana Sangare
		Room 2 (Maputo): English Speaking, Table 2 Chair: Sok Srun Rapporteur: Gonfa Ayana
		Room 2 (Beira): English Speaking, Table 3 Chair: Alash'le Abimiku Rapporteur: Jackson Aumone
		Room 3 (Avenida): English Speaking, Table 4 Chair: Charles Massambo Rapporteur: Kekeletso Kao
		Room 3 (Avenida): English Speaking, Table 5 Chair: Veronica Bekoe Rapporteur: Harold Kaura
16:00-16:30: Tea Break – Outside meeting rooms		
16:30-18:00	Breakouts, cont'd	
18:30-19:30: Welcome Reception, Maputo Hall Dinner on your own		

Day 2: Wednesday 23 January 2008

Session #5 Maputo	Moderators: Marco Antonio de Avila Vitoria (WHO Geneva) and Maurine Murtagh (Clinton Foundation)	
	8:00-9:30	<i>Objective #2: To develop a consensus to guide standardization of laboratory equipment at each level of an integrated tiered laboratory network</i>
		Summary of Breakout Presentations, 10 minutes each
		Report back to Assembly: three rapporteurs/chairs (two English speaking, one French) will present summaries of Objective #1 work
	9:30-10:00	Malaria Diagnostic Requirements at Different Levels of a Laboratory Network: Harmonization and Standardization of Equipment
		Earl Long (CDC)
10:00- 10:30: Tea Break		
Session #6 Maputo	Moderator: Jack Nyamongo (MOH, Kenya) and Jessica Justman (Columbia University)	
	10:30	The Tanzania Experience with Standardization of Laboratory Equipment at Different Levels of a Laboratory System
		Charles Massambo (MOH, Tanzania)
Session #7 Maputo/Beira/Avenida	Breakouts 2	
	14:20-16:00	Room 1 (Maputo): French Speaking, Table 1
		Chair: Faustine Malele Rapporteur: Flabou Bougoudogo
		Room 2 (Maputo): English Speaking, Table 2
		Chair: Kgomotso Makhaolo Rapporteur: Sokhal Buth
		Room 2 (Beira): English Speaking, Table 3
	Chair: Janet Robinson Rapporteur: Ekow Biney	
	Room 3 (Avenida): English Speaking, Table 4	
	Chair: Kyle Bond Rapporteur: Rosemary Audu	
	Room 3 (Avenida): English Speaking, Table 5	
	Chair: Thuso Joseph Senosi Rapporteur: Kunda Geoffrey Musonda	
12:30-14:00: Lunch – Hotel Dining Room		
Session #8 Maputo/Beira/Avenida	Breakouts 2, cont'd—Same Chairs and Rapporteurs	
	14:00-15:30	Breakouts, cont'd
	16:00-16:30: Tea Break – Outside meeting rooms	
	16:30-18:00	Breakouts, cont'd
Dinner on your own		

Day 3: Thursday 24 January 2008

Moderators: Dora Mbanya (Cameroon)/ Karin Weyer (WHO Geneva)		
Session #9 Maputo	8:00-9:30	<i>Objective #3: To develop a consensus on key considerations to guide maintenance and service contracts for equipment at various levels of an integrated tiered laboratory network</i>
		Summary of Breakout Presentations, 10 minutes each
		Report back to Assembly: Rapporteurs/chairs of three groups will present summaries of Objective #2 work
Session #10 Maputo	9:30-10:00	Management of Clinical Laboratory Key Aspects for Maintenance and Service Contracts of Equipment
		Joyelle Dominique (University of Maryland, School of Medicine)
10:00- 10:30: Tea Break		
Breakouts 3		
Session #11 Maputo/Beira/Avenida	10:30-12:30	Room 1 (Maputo): French Speaking, Table 1
		Chair: Ludovic Anani Rapporteur: Dora Mbanya
		Room 2 (Maputo): English Speaking, Table 2
		Chair: Paula Fernandes Rapporteur: Douglas Mangwanya
		Room 2 (Beira): English Speaking, Table 3
	Chair: Catherine Mundy Rapporteur: Fales Zulu Mwamba	
	Room 3 (Avenida): English Speaking, Table 4	
	Chair: Christina Mwangi Rapporteur: Mecky Issac Matee	
	Room 3 (Avenida): English Speaking, Table 5	
	Chair: Neil Constantine Rapporteur: Khye Seng Goh	
12:30-14:00: Lunch – Hotel Dining Room		
Breakouts 3, cont'd—Same Chairs and Rapporteurs		
Session #12 Maputo/Beira/Avenida	14:00-15:30	Breakouts, cont'd
16:00-16:30: Tea Break – Outside meeting rooms		
Moderator: Debbie Burgess (Gates Foundation) and Thomas Lapnet-Moustapha (WHO-AFRO)		
Session #13 Maputo	16:00-18:00	Presentations of Breakout 3
		Chairs/Rapporteurs present summary of Objective #3 work
		Discussion
	18:00-18:15	Closing Remarks
		WHO Representative/USG Representative
Dinner on your own		

ANNEX B
List of Participants

Alash'le G. ABIMIKU

Assistant Professor
University of Maryland School of Medicine
USA
aabimiku@ihv.umaryland.edu

Ludovic ANANI

Director of Diagnostic Research and Blood
Transfusion
Ministry of Health
Benin
ananily2002@yahoo.fr

Rosemary AUDU

Lab Manager, Human Virology
Nigerian Institute of Medical Research
Nigeria
rosemaryaudu@yahoo.com

Jackson AMONE

Assistant Commissioner of Health
Ministry of Health
Uganda
amonekaka@yahoo.com.uk

Gonfa AYANA

Chief, National Laboratory Quality System,
Provisional Department
Ethiopian Health & Nutrition Research Institute
Ethiopia
gonfaayana@yahoo.com

John Henry BAGUMA

National Medical Stores
Uganda
jbaguma@natmedstores.org

John BALL

Executive Vice President
American Society for Clinical Pathology
USA
John.Ball@ascp.org

Isaac BAYA

Quality Assurance Manager
National HIV Reference Laboratory
Kenya
chigsus@yahoo.com

Veronica BEKOE

Deputy Chief Biomedical Scientist
Public Health and Reference Laboratory
Ghana
veronicabekoe@yahoo.co.uk

Michele BEST

American Society for Clinical Pathology
USA
Michele.Best@dimensionshealth.org

Ekow BINEY

Health Laboratory Services
Ghana
phrl@africaonline.com.gh

Deborah BIRX

Director, Global AIDS Program
Centers for Disease Control and Prevention
USA
dbirx@cdc.gov

Ana Judith BLANCO

Clinical Laboratory Advisor
Health Alliance International
Mozambique
ablanco@u.washington.edu

Jacques BONCY

Director
National Public Health Laboratory
Haiti
jbony2001@yahoo.fr

Kyle BOND

Associate Director for Laboratory
Centers for Disease Control and Prevention
Nigeria
bondk@ng.cdc.gov

Flabou BOUGOUDOGO

Director General
National Institute of Public Health Research
Mali
flabou@hotmail.com

Deborah BURGESS

Senior Program Officer
Infectious Diseases Development
Bill and Melinda Gates Foundation
USA
debbie.burgess@gatesfoundation.org

Eileen BURKE

Centers for Disease Control and Prevention
EBurke@ke.cdc.gov

Sokhal BUTH
Deputy Director
National Institute of Public Health
Cambodia
buthsokhal@yahoo.com

Abbey CAILLOUX
Staff Associate
Supply Chain Management System
USA
abbey_cailloux@jsi.com

Antonio CHAMUCO
Procurement Specialist
The World Bank
Mozambique
achamuco@worldbank.org

Benedito CHAUQUE
Logistics Activity Manager
USAID

Rachanee CHEINGSONG
Laboratory Director
CDC – Global AIDS Program
Haiti
cheingsongr@ht.cdc.gov

Neil T. CONSTANTINE
Professor of Pathology
University of Maryland School of Medicine
USA
nconstantine@ihv.umaryland.edu

Siobhan CROWLEY
Pediatric & Family HIV Care
World Health Organization- Geneva
Switzerland
crowleys@who.int

Elias CUAMBE
MISAU Mozambique
Mozambique

Joelle DAVIAUD
Technical Officer Pharmaceutical Quality
Assurance
The Global Fund to Fight AIDS, Tuberculosis
and Malaria
Switzerland
joelle.daviaud@theglobalfund.org

Sindisiwe DLAMINI
Swaziland
hsukati@yahoo.co.uk

Joyelle DOMINIQUE
Laboratory Program Manager Clinical Trials and
AIDS Relief/PEPFAR
University of Maryland
USA
dominiqu@umbi.umd.edu

Marie DOWNER
Senior Laboratory Advisor
CDC - GAP/Cambodia
Cambodia
downerM@kh.cdc.gov

Tedd ELLERBROCK
Team Lead, HIV Treatment & Care
CDC- GAP
USA
tve1@cdc.gov

Barbara FELLING
Senior Technical Advisor
Supply Chain Management System
USA
fellingb@jsi.org

Paula FERNANDES
Senior Program Manager, Global Health
Association of Public Health Laboratories
paula.fernandes@aphl.org

Christine FULLMER
Senior Associate
Supply Chain Management System
USA
cfullmer@pfscm.org

Paulo Ivo GARRIDO
Ministry of Health
Mozambique

John GATABAZI
Director, Microbiology Unit
National Reference Laboratory
Rwanda
jgatabazi2002@yahoo.com

Charlotte GAYDOS

Associate Professor of Medicine
Johns Hopkins University
Clinical Laboratory Standards Group
USA
cgaydos@jhmi.edu

Guy-Michel GERSHY-DAMET

Regional Officer for Laboratory
WHO-Regional Office for Africa
Burkina Faso
gersy-dametg@bf.afro.who.int

Khye Seng GOH

Senior Laboratory Technician
American Society for Microbiology
khyegoh@hotmail.com

Luis Felipe GONZALEZ

ICAP Regional Lab Advisor
Columbia University
lg2284@columbia.edu

Vincent HABIAMERE

Medical Officer, Department of HIV/AIDS
WHO- Geneva
Switzerland
habiyamberev@who.int

Donavine HAKIZMANA

Directeur du laboratoire
L'Institut National de la Sante Publique
Burundi
donahakiza@yahoo.fr

Lee HILBORNE

Deputy Director for Global Health
Rand Corporation
USA
leeh@rand.org

Barbara HOFFMAN

Project Director
American Society for Clinical Pathology
USA
Barbara.Hoffman@ascp.org

Aurore HOUNTO

National Reference Laboratory Director
HIV/AIDS
Ministry of Health
Benin
aurorefel@yahoo.fr

Peter HUGHES

Head of Clinical Laboratories
Medical Research Council Uganda Research
Unit on AIDS
Uganda
peter.hughes@mrcuganda.org

Andre INWOLEY

Jessica JUSTMAN

Program Director UTAP International Center
for AIDS Care
Columbia University
jj2158@columbia.edu

Stacy KANCIJANIC

Grants Administrator
American Society for Clinical Pathology
USA
stacy.kancijanic@ascp.org

Phyllis KANKI

Principal Investigator, Harvard PEPFAR/AIDS
Program in Nigeria
Harvard University
pkanki@hsph.harvard.edu

Kekeletso KAO

Director of Laboratory Services
Ministry of Health and Social Welfare
Lesotho
k_mosisili@yahoo.com

Harold KAURA

General Manager, Technical Operations
Namibia Institute of Pathology
Namibia
harold.kaura@nip.com.na

Yenew KEBEDE

Laboratory Technical Officer
Centers for Disease Control and Prevention
Ethiopia
kebedey@et.cdc.gov

Derrick KHUMALO

Swaziland
derricktbkhumalo@yahoo.com

Natacha KOHEMUM

Cote D'Ivoire
adjec@CI.CDC.GOV

Thomas LAPNET-MOUSTAPHA

Pharmacist, HIV/AIDS Unit
WHO-AFRO

Democratic Republic of Congo

lapnett2@afro.who.int

Stefano LAZZARI

Procurement and Supply Management Team
The Global Fund to Fight AIDS, Tuberculosis
and Malaria

Switzerland

stefano.lazzari@theglobalfund.org

Stephen LEE

Program Director, Care and Treatment
Elizabeth Glaser Pediatric AIDS Foundation
slee@pedaids.org

Earl LONG

Research Biologist, Malaria Branch CDC
President's Malaria Initiative

USA

elong@cdc.gov

Yao Guillaume LOUKOU

Professor of Microbiology
National Reference Laboratory

Cote D'Ivoire

gylouk@africaonline.co.ci

Joaquim MACHONE

Head of Laboratory Sector
Ministry of Health

Mozambique

j_machone@yahoo.co.uk

Bereniece MADISON

Laboratory Director
CDC - Zambia

Zambia

MadisonB@cdczm.org

Kgomotso MAKHAOLA

Technical Supervisor
Harvard HIV Reference Lab
National Health Ministry

Botswana

kmakhaola@bhp.org.bw

Ernest MAKOKHA

Acting GAP Lab Director
Centers for Disease Control and Prevention

Kenya

emakokha@ke.cdc.gov

Cleophas MALABA

Director, Chief of Service, Public Health
Laboratories

Ministry of Health

DR Congo

malabac@yahoo.fr

Faustin MALELE

Lab Program Manager
Centers for Disease Control and Prevention

DR Congo

fmalele@cdcdr.org

Saidou MAMADOU

Chief
National Reference Laboratory

Niger

saidou_mamadou@yahoo.fr

Douglas MANGWANYA

Director of Laboratory Services
Ministry of Health

Zimbabwe

dmangwanya@healthnet.org.zw

Jessina MASAMHA

Laboratory Technical Assistant
Centers for Disease Control and Prevention

Mozambique

Charles MASSAMBU

Assistant Director, Diagnostic Services
Ministry of Health and Social Welfare

Tanzania

cmassambu@hotmail.com

Mecky Issac MATEE

Professor of Microbiology
Muhimbili University of Health and Allied
Sciences

Tanzania

mmatee@muchs.ac.tz

Loudoum MBAINADJI

Laboratory Director
National Reference Hospital

Chad

mbainadji@yahoo.fr

Dora MBANYA

Chief, Hematology and Transfusion Services
University Hospital Yaounde, Cameroon
Cameroon
dmbanya1@yahoo.co.uk

Abdou MOHA

HIV/AIDS Country Officer
WHO-Mozambique
Mozambique
mohaa@mz.afro.who.int

Pheona MOHAMED

Laboratory Director
Central Medical Laboratory, Georgetown Public
Hospital
Guyana
phoenar@yahoo.com

Juliette MORGAN

Program Manager CDC Advisor
President's Malaria Initiative
Mozambique
Morganj@mz.cdc.gov

David MOTHABENG

Laboratory Manager
Ministry of Health and Social Welfare
Lesotho
dmothabeng@yahoo.com

Patrick MSIPA

Laboratory Logistics Advisor
USAID | DELIVER PROJECT, JSI
patrick_msipa@jsi.com

Sandra MUCHANGA

NPO/HIV/AIDS-ARV's-MNE
WHO
Mozambique
muchangas@mz.afro.who.int

Catherine MUNDY

Principal Program Associate for Laboratory
Services
Management Sciences for Health
cmundy@msh.org

Anna MURPHY

Consultant
American Society for Clinical Pathology
USA
ammurphy62@hotmail.com

Maurine MURTAGH

Director of Diagnostic Services
The Clinton Foundation HIV/AIDS Initiative
USA
mmurtagh@clintonfoundation.org

Kunda Geoffrey MUSONDA

M.D./Senior Scientist
University Teaching Hospital
Zambia
kundagk@yahoo.com

Jeremie Muwonga MASADI

Chief of Laboratory Division
DRC National HIV/AIDS Program
DR Congo
pmuwonga@hotmail.com

Jubra MUYANGA

JICA
Zambia
jmuyanga@yahoo.com

Fales Zulu MWAMBA

Laboratory Specialist
Ministry of Health
Zambia
medlab@zamnet.zm
falesz@yahoo.co.uk

Christina MWANGI

Laboratory Director
Centers for Disease Control and Prevention
Tanzania
mwangic@tz.cdc.gov

Marasi MWENCHA

Principal Laboratory Advisor
Supply Chain Management System
USA
mmwencha@pfscm.org

Reuben MWENDA

Malawi
remwenda@yahoo.com

Clement NDONGMO

Public Health Laboratory Consultant
National Public Health Laboratory
Haiti
cbndongmo@ht.cdc.gov

Ngozi NGJEUOME
Director, Public Health Department
Federal Ministry of Health
Nigeria
ngonjep@yahoo.com

Do Thi NHAN
Deputy Chief
Care and Treatment, HIV/AIDS Control
Ministry of Health
Vietnam
dothinhan@lifegap.org.vn

Wendy NICODEMUS
Senior Technical Advisor
USAID | DELIVER PROJECT, JSI
USA
wendy_nicodemus@jsi.com

Boris NIKOLIC
Senior Program Officer
Global Health Technologies
The Bill and Melinda Gates Foundation
USA
boris.nikolic@gatesfoundation.org

John NKENGASONG
Branch Chief International Laboratory Branch,
Global AIDS Program
Centers for Disease Control and Prevention
USA
jch5@cdc.gov

Richard Clive Nkunda MWESIGWA
Chief, Molecular Biology Laboratory
National Reference Laboratory
Rwanda
nmrclive@yahoo.com

Karen NURICK
HIV/AIDS Advisor
USAID
knurick@usaid.gov

Trevor PETER
The Clinton Foundation HIV/AIDS Initiative
tpeter@clintonfoundation.org

Chana RABINER
Laboratory Advisor
Office of HIV/AIDS - USAID
USA
crabiner@usaid.gov

Robert REDFIELD
University of Maryland IHV, AIDSRelief
Program
USA
redfield@umbi.umd.edu

Patricia RIZZO-PRICE
Vice President, Global Health Partnerships
Clinical Laboratory Standards Group
USA
pprice@clsi.org

Colin ROACH
GAP Lab Director
Centers for Disease Control and Prevention
Guyana
RoachC@GY.CDC.GOV

Janet ROBINSON
Director, Laboratory Sciences
Family Health International
jrobinson@fhibkk.org

Sameer SAKALLAH
Senior Technical Advisor, SCMS
Crown Agents USA, Inc.
ssakallah@pfscm.org

Jean SAKANDÉ
Director of Laboratories
Direction General de la Pharmacie, Du
Médicament et des Laboratoires
Burkina Faso
jean_sakande@univ-ouaga.bf

Anita SANDS
Technical Officer
WHO-Geneva
Switzerland
sandsa@who.int

Lassana SANGARÉ
Professor of Bacteriology and Virology
University Hospital, Ougadougou
Burkina Faso
lassosangare@yahoo.fr

Jean-Louis SANKALÉ
Senior Research Scientist
Harvard University School of Public Health
USA
jsankale@post.harvard.edu

Souleymane SAWADOGO
Centers for Disease Control and Prevention
Namibia
sawadogos@na.cdc.gov

Leland SCHULLER
Laboratory Officer - Care & Treatment
Elizabeth Glaser Pediatric AIDS Foundation
lschuller@pedaids.org

Thuso Joseph SENOSI
Technical Supervisor
National Health Ministry
Botswana
tsenosi@gov.bw

Haynes SHEPPARD
Consultant
Association of Public Health Laboratories
Haynes.Sheppard@cdph.ca.gov

Yoko SHIMADA
Deputy Director of Diagnostic Services
The Clinton Foundation HIV/AIDS Initiative
USA
yshimada@clintonfoundation.org

Thomas SHINNICK
Chief, TB/Micobacteriology Branch
Centers for Disease Control and Prevention
USA
TShinnick@cdc.gov

Ritu SHRIVASTAVA
Laboratory Director
Centers for Disease Control and Prevention
Vietnam
ShrivastavaR@vn.cdc.gov

Beth SKAGGS
Centers for Disease Control and Prevention
Mozambique
BSkaggs@cdc.gov

Diallo SOULEYMANE
Pharmacist/Biologist
National University Hospital Gabriel Toure
Mali
ntjisdiallo@yahoo.fr

Steven SPECTER
Professor, Molecular Medicine
American Society for Microbiology
USA
sspecter@health.usf.edu

Sok SRUN
Deputy Director, Hospital Services
Ministry of Health
Cambodia
soksrun@camnet.com.kh

Robert STALEY
Procurement Unit Manager
Supply Chain Management System
USA
rstaley@pfscm.org

Matt STREMLAU
Laboratory Technical Working Group
Co-Chair
Office of the Global AIDS Coordinator
USA
StremlauMH@state.gov

Klaus STURBECK
Senior ART Site Development Specialist
CDC - GAP
Mozambique
sturbeckk@mz.cdc.gov

Nguyen Vu TRUNG
Chief, Clinical Laboratories
National Institute of Infectious and Tropical
Diseases
Vietnam
nvutrong@fpt.vn

Willy URASSA
Scientist
WHO-Geneva
Switzerland
urassaw@who.int

Bina VALAYDON
Health Specialist
The World Bank
Mozambique
bvalaydon@worldbank.org

Gaby VERCAUTEREN

Coordinator of Diagnostics and Laboratory
Technology
WHO-Geneva
Switzerland
vercautereng@who.int

Marco Antonio de Avila VITORIA

Medical Officer, Department of HIV/AIDS
WHO-Geneva
Switzerland
vitoriam@who.int

Beryl WEST

Deputy Laboratory Director
Centers for Disease Control and Prevention
Uganda
rqd6@ug.cdc.gov

Larry WESTERMAN

Microbiologist - GAP
CDC - GAP
USA
lwesterman@cdc.gov

Karin WEYER

WHO-Geneva
Switzerland
weyerk@who.int

Jonathan WHANDE

Coordinator, Laboratory Services
Ministry of Health
Zimbabwe
jswhande@yahoo.com

David WHYBREW

Medical and Technical Manager
Crown Agents Health
david.whybrew@crownagents.co.uk

Jason WILLIAMS

Centers for Disease Control and Prevention
South Africa
williamsj@sa.cdc.gov

ANNEX C

SUMMARY OF WORKGROUP RECOMMENDATIONS FOR TESTS PERFORMED AT EACH LEVEL OF A LABORATORY NETWORK*							
Laboratory tests for diagnosis and monitoring ⁽¹⁾	Primary Care Level ⁽²⁾		District Level ⁽³⁾		Regional/Provincial Level ⁽⁴⁾		National/ Multicountry Level ⁽⁵⁾
	Send out ⁽⁶⁾	On-site	Send out ⁽⁶⁾	On-site	Send out ⁽⁶⁾	On-site	On-site
<i>HIV antibody testing</i>							
Lab ELISA	X			X		X	X
Rapid point of care 1		X		X		X	X
Rapid point of care 2		X		X		X	X
Rapid point of care 3		X		X		X	X
<i>HIV virological diagnostic testing</i>							
RNA	X		X			X	X
DNA	X		X			X	X
Ultrasensitive p24 antigen	X		X		X		X
<i>HIV viral load measurement</i>	X		X			X	X
<i>Hematology assays</i>							
Hemoglobinometer such as HemoCue		X		X		X	X
WHO color scale		X					
Full blood count and differential	X			X		X	X
<i>CD4</i>							
Absolute count	X			X		X	X
% desirable if available	X			X		X	X
<i>HIV resistance testing ⁽⁷⁾</i>			X		X		X ⁽⁸⁾
<i>Pregnancy testing</i>							
Urine rapid test		X		X		X	X
<i>Chemistry assays</i>							
Liver function tests		X (if power)		X		X	X
Whole blood glucose (glucometer)		X		X		X	X
Serum glucose				X		X	X
Serum electrolytes	X			X		X	X
Renal function tests		X (if power)		X		X	X
Lipids				X		X	X
Amylase				X		X	X
Lactate				X		X	X

(1) These are generic recommendations and it is recognized that exceptions apply (e.g., some health centers will send out certain tests). When appropriate, these tests may be used for public health surveillance and QA activities.

(2) Primary health care facilities are those providing first point of contact with the health care system.

(3) District level is defined as hospital at the first referral level that is responsible for a defined geographical area containing a defined population and governed by a politico-administrative organization such as a district health management team.

(4) In some smaller countries, there may not be regional laboratories.

(5) Including the national reference laboratory or public health laboratory, which is usually responsible for QA and surveillance activities.

(6) "Send out" refers to not having the testing capability on site, so samples and/or patients are sent to another site for the tests to be performed.

(7) HIV DR testing is only recommended as part of national efforts for surveillance and monitoring as outlined in the WHO HIV DR laboratory strategy. This testing is usually done by sending out samples to an accredited international lab.

(8) May be done at the national level or sent to an accredited lab.

*Adopted from the WHO publication: Summary of WHO Recommendations on Laboratory Investigations for Clinical Care by Level of Health Care Facility.

Note: Test list above is not meant to be all inclusive of the testing performed at each level.

ANNEX C

SUMMARY OF WORKGROUP RECOMMENDATIONS FOR TESTS PERFORMED AT EACH LEVEL OF A LABORATORY NETWORK*							
Laboratory tests for diagnosis and monitoring ⁽¹⁾	Primary Care Level ⁽²⁾		District Level ⁽³⁾		Regional/Provincial Level ⁽⁴⁾		National/ Multicountry Level ⁽⁵⁾
	Send out ⁽⁶⁾	On-site	Send out ⁽⁶⁾	On-site	Send out ⁽⁶⁾	On-site	On-site
<i>Urine analysis</i>							
Urine dipstick		X		X		X	X
Urine dipstick with microscopy				X		X	X
<i>Tuberculosis tests</i>							
<i>Microscopy</i>							
Light		X		X		X	X
Fluorescence		X (if high vol)		X		X	X
<i>Culture and ID</i>							
Solid medium	X		X			X	X
Liquid medium	X		X			X	X
<i>Drug susceptibility test</i>							
First-line			X			X	X
Second-line			X		X		X
<i>Malaria tests</i>							
Rapid test for malaria		X		X		X	X
Microscopy for malaria (thick/thin)		X		X		X	X
<i>Microbiology tests</i>							
Gram's stain				X		X	X
Microbiology culture and ID			X			X	X
Blood culture			X			X	X
Microbiology susceptibilities			X			X	X
Wet mounts/preps		X		X		X	
<i>Syphilis tests</i>							
Syphilis rapid diagnostic test		X		X		X	X
Syphilis serological (RPR, FTA, TPPA/TPHA)				X		X	X
<i>Hepatitis tests</i>							
Hepatitis B by EIA				X		X	X
Hepatitis C by EIA				X (if high prev)		X	X
<i>Cerebrospinal fluid (CSF) tests</i>							
CSF microscopy including cell count, India Ink, Gram's stain and AFB				X		X	X
CSF glucose			X			X	X
Cryptococcal antigen (serum or CSF)				X		X	X

(1) These are generic recommendations and it is recognized that exceptions apply (e.g., some health centers will send out certain tests). When appropriate, these tests may be used for public health surveillance and QA activities.

(2) Primary health care facilities are those providing first point of contact with the health care system.

(3) District level is defined as hospital at the first referral level that is responsible for a defined geographical area containing a defined population and governed by a politico-administrative organization such as a district health management team.

(4) In some smaller countries, there may not be regional laboratories.

(5) Including the national reference laboratory or public health laboratory, which is usually responsible for QA and surveillance activities.

(6) "Send out" refers to not having the testing capability on site, so samples and/or patients are sent to another site for the tests to be performed.

(7) HIV DR testing is only recommended as part of national efforts for surveillance and monitoring as outlined in the WHO HIV DR laboratory strategy. This testing is usually done by sending out samples to an accredited international lab.

(8) May be done at the national level or sent to an accredited lab.

*Adopted from the WHO publication: Summary of WHO Recommendations on Laboratory Investigations for Clinical Care by Level of Health Care Facility.
Note: Test list above is not meant to be all inclusive of the testing performed at each level.

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.

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**.

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.

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.

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.

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.

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.

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.

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).

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.

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.

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.

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.

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.

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 ^c •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.

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.

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.

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).

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.

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/

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

Abbott Laboratories, USA; Website: www.abbott.com

Bionike Inc., USA; Website: www.bionike.com

bioMérieux sa, FRANCE; Website: www.biomerieux.com

Chembio Diagnostic Systems Inc., USA; Website: www.chembio.com

Dade Behring, Inc., USA, Website: www.dadebehring.com

Equipar Diagnostici, ITALY; Website: www.equipar.it

Fujirebio Inc., JAPAN, Website: www.fujirebio.co.jp

Genelabs Diagnostics Pte Ltd., SINGAPORE, Website: www.genelabs.com.sg

Green Cross Life Science Corp, KOREA, Website: www.greencross.com

J. Mitra & Co. Ltd., INDIA, Website: www.jmitra4u.com

Organon Teknika, see bioMérieux

Organics, ISRAEL, Website: www.orgenics.com

Trinity Biotech plc, IRELAND, Website: 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.

MALARIA SMEAR

Refer to Level I for details.

MALARIA RAPID TEST

Refer to Level I for details.

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.

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

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

URINE DIPSTICK - MICROSCOPY

Test:	Urine Dipstick and Microscope
Method:	Manual
Kit/Equipment:	Multi-parameter reagent strips
Company:	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.
-

URINE PREGNANCY RAPID TEST

Refer to Level I for details.

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.

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)

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.

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

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.

MALARIA SMEAR

Refer to Level I for details.

MALARIA RAPID TEST

Refer to Level I for details.

SYPHILIS

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

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

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.

TYPE AND CROSSMATCH

Refer to Level II for details.

URINE PREGNANCY RAPID TEST

Refer to Level I for details.

WET MOUNTS (NaCl and KOH) - DIRECT MICROSCOPY

Refer to Level I for details.

CRYPTOCOCCAL ANTIGEN TEST

Refer to Level II for details.

INDIA INK STAIN

Refer to Level II for details.

ANNEX E

CHART OF LAB TESTS BY LEVEL WITH EXAMPLES OF EQUIPMENT AND VENDORS FOR EACH TEST*

LEVEL I

Test	Examples of Kit/Equipment	Vendor/ Manufacturer
HIV Serology: Rapid Test	HIV Rapid Test Kits	Country-specific; various vendors
Hemoglobin	Hemoglobinometer	HemoCue AB, Sweden
	WHO color scale	WHO
Urine Pregnancy Rapid Test	Beta HCG urine kits	Various vendors
Urine Dipstick	Multi-parameter reagent strips	Various vendors
Whole Blood Glucose	ACCU-CHEK Glucometer	Roche Diagnostics, Switzerland
	Bayer Elite	Siemens USA
AFB Smear	Light microscope	Various vendors
	Ziehl-Neelsen stain, Fluorescent Stain (high vol)	Becton, Dickinson and Company, USA
Malaria Smear	Wright-Giemsa Stain Pack	Various vendors
	Field Stain A and B	Various vendors
Malaria Rapid Test	Malaria Rapid Test kits	Various vendors
Wet Mounts - Direct Microscopy	Light microscope	Various vendors
Rapid Syphilis Test (RST)	RST Kits	Various vendors
Chemistry: ALT and Creatinine	Roche Reflotron Plus	Roche Diagnostics, Switzerland
	VITROS DT60 II	Ortho Clinical Diagnostics
	Humalyzer 2000	Human GmbH, Germany
	BA-88	Mindray Medical International Ltd., China
	Spectrophotometer	Various vendors

*Mention of any products, equipment, or vendors in this document does not indicate endorsement by the World Health Organization, the U.S. Government, the American Society for Clinical Pathology, the Clinton Foundation, the Bill & Melinda Gates Foundation, or the Global Fund. The above list includes examples of products, equipment, and vendors and should not be considered all-inclusive.

ANNEX F: General Supplies for Laboratory Levels I, II and III

LEVEL I

HIV Serology: Rapid Test

1. Positive and negative controls (if not included with kit)
2. Timer with alarm
3. Rapid kit
4. Good source of lighting
5. Phlebotomy (whole blood [WB]), safety and clerical supplies

Hemoglobin

Manual CBC: Hemoglobin

1. Cuvettes
2. Hemoglobinometer
3. Pipettes and tips
4. 4x4 gauze
5. Lint-free tissue
6. Standards and control material
7. Phlebotomy (EDTA WB) and clerical supplies
8. Equipment-specified reagents

HemoCue Hemoglobin

1. Phlebotomy (WB), safety and clerical supplies
2. HemoCue Analyzer
3. Microcuvette
4. Lint-free tissue
5. Alcohol
6. HemoCue cuvette cleaning swabs or cotton-tipped swabs moistened with alcohol or water
7. AC adaptor or batteries (4 AA batteries); extra batteries should be available
8. Hemoglobin controls

Hemoglobin Color Scale

1. Phlebotomy (WB), safety and clerical supplies
2. WHO color scale
3. Chromatography paper

Urine Pregnancy Rapid Test

1. Rapid kit
2. Positive and negative controls (if not included with kit)
3. Collection cups
4. Specimen transport bags
5. Waterproof markers
6. Timer with alarm
7. Appropriate pipettes and tips (if not included with kit)
8. Good source of lighting

Urine Dipstick

1. Reagent strips
2. Positive and negative controls
3. Interpretive color chart
4. Collection cups
5. Specimen transport bags
6. Waterproof markers
7. Pedi UA collection bags
8. Timer with alarm
9. Disposable plastic pipettes
10. 4x4 gauze

Dried Blood Spot (DBS) Collection

1. Phlebotomy (WB-lancet), safety and clerical supplies
2. Appropriate DBS filter paper card
3. Drying stand or rack
4. Individual card packaging: Glassine paper, wrap sheets or gas permeable plastic bag
5. Batch packaging: Gas permeable zip-enclosure plastic bag
6. Desiccant packs
7. Humidity indicator cards
8. Foam mailing pouch or cooler for transporting

Whole Blood Glucose

1. Glucometer
2. Glucometer strips
3. Glucometer calibrator and controls
4. Extra batteries
5. Phlebotomy (WB-lancet), safety and clerical supplies

AFB Smear

1. Disposable wooden applicator sticks or wire loops using sand-alcohol flask/spirit lamp
2. Flask to hold sand-alcohol (if used)
3. Biohazard receptacle with lid and separate disposable bag inserts to burn or autoclave
4. Slides
5. Funnel
6. Filter paper
7. Double basin sink
8. Marking instrument - depends on slide (frosted-lead pencil; unfrosted-diamond stylus)
9. AFB staining (kit or prepared stain)
10. Distilled water
11. Timer with alarm
12. Spirit lamp
13. Methanol and spare wicks for lamp
14. Staining rack
15. Slide warming tray (optional)
16. Drying rack
17. Forceps
18. Phenolic agents or bleach

19. Bottle to store 1:10 bleach solutions for that day's use
20. Sputum collection cups
21. Specimen transport bags
22. 4x4 gauze
23. Waterproof marker
24. AFB positive and negative unstained smears
25. Register of TB suspects
26. Laboratory log book
27. Red ink pen for positives
28. Black/blue pen for negatives
29. Microscopy and clerical supplies

Malaria Smear (thick and thin)

1. Phlebotomy (EDTA WB), clerical and microscope supplies
2. Coplin jars with screw cap lids (4 to 5) or staining rack
3. Diff counter
4. Wooden applicator sticks
5. 4x4 gauze
6. Wright-Giemsa stain or other acceptable malarial stain such as Field Stain A and B
7. Absolute methanol
8. Slides
9. Drying rack
10. Waterproof marker or pencil
11. Slide storage box
12. Water for rinsing
13. Timer with alarm

Malaria Rapid Test

1. Rapid kit
2. QC system developed that includes a source of positive and negative control material
3. Timer with alarm
4. Phlebotomy (EDTA WB), safety and clerical supplies

Wet Mounts – Direct Microscopy

1. Specimen collection devices such as sterile swab transported in tube, collection cups
2. Waterproof marker
3. Specimen transport bags
4. Slides
5. Coverslips
6. Bottle with droppers
7. 5% w/v KOH
8. 0.9% NaCl
9. Spirit lamp (to accelerate clarifying of KOH prep)-optional
10. Forceps
11. Aliquot test tubes to prepare suspensions
12. Petri dish
13. 4x4 gauze
14. Disposable plastic pipettes

15. Microscopy and clerical supplies

Rapid Syphilis Test

1. Rapid kit
2. Positive and negative controls (if not included with kit)
3. Timer with alarm
4. Good source of lighting
5. Phlebotomy (WB-lancet), safety and clerical supplies

Automated ALT and Creatinine

1. Forceps (to remove pedi-tubes from centrifuge)
2. 4x4 gauze
3. Appropriate pipettes and tips
4. Aliquot tubes and caps (to remove cells from serum if delays are encountered or for dilutions if above linearity)
5. Plastic disposable pipettes (to transfer serum)
6. Waterproof markers
7. Equipment-specified calibrators, controls, reagents and consumables
8. Additional print out supplies dependent upon equipment (e.g., paper tape, printer cartridge, paper)
9. Phlebotomy (serum) and clerical supplies
10. Test tube racks
11. Analyzer
12. Diluent (probably saline for this level): if result is above linearity-optional; may report "greater than..." if acceptable

Microscopy

1. Microscope
2. Flat table/bench with chair
3. 10x, 40x, 100x oil immersion objectives
4. Microscope dust cover
5. Immersion oil (tropicalized type)
6. Bottle dropper
7. Spare bulb/fuse
8. Lens cleaner
9. Lens paper
10. Slide storage containers
11. Xylene (to remove oil before placing into storage box)
12. Pictorial reference material or colored atlases

Additional Supplies if Preparing Stains

1. Balance or weight scale (sensitivity of 0.1g)
2. Adhesive labels
3. Amber bottles
4. Distilled water system
5. Filter paper
6. Funnels
7. Boiling flask or Erlenmeyer flask

8. Measuring cylinders (100ml, 500ml and 1000ml)
9. Volumetric flasks
10. Glass cleaner
11. Bottle brush
12. Insulated gloves
13. Heating plate with magnetic stirrers
14. Reagent grade acids, staining crystals, chemicals
15. Parafilm
16. Waterproof markers
17. Weigh boats
18. Disposable plastic pipettes

Phlebotomy

1. Lancet
2. 2x2 gauze
3. 21G needles
4. 22G needles
5. Alcohol prep pad
6. Band-aids or paper tape
7. Safety needle holder and adapter
8. Butterfly safety assembly
9. Disposable tourniquets
10. Phlebotomy chair or stationary chair with arm rests
11. EDTA tubes (plastic)
12. SST tubes (plastic)
13. Red top tubes with clot activator (plastic)
14. Pedi EDTA tubes
15. Pedi red tubes
16. Microtainer EDTA tubes
17. Microtainer amber red tops
18. Betadine preps
19. Sharps container

Microbiological Transport

1. Blood culture tubes
 - a. Aerobic
 - b. Anaerobic
 - c. Pedi
 - d. Myco
2. Urine culture
3. PVA and Formalin
4. Carey Blair
5. Sterile cups
6. Sterile tubes with screw cap lids or plain red top tubes with no clot activator
7. Culturette swabs
8. Sealable plastic transport bags
9. Cool box or thermal cooler

Transport Supplies for Referral Testing

1. Insulated cool boxes to hold ambient temperature and refrigerated specimens
2. Transfer list
3. Transfer log
4. Fax or other system of reporting results from recipient laboratory
5. Appropriate sending containers and preservatives
6. Sealable plastic transport bags
7. Specimen holders or racks

Safety

1. Safety goggles or glasses
2. Gloves (small, medium and large)
3. Fluid-resistant lab coats (to use in the laboratory)
4. Lab coats for patient processing
5. Eye wash station or portable dispenser
6. ABC fire extinguisher
7. Hazardous spill kit
8. Hand soap
9. Paper towels
10. Bleach
11. Disinfectant wipes (optional)
12. Biohazard receptacle with lid and separate disposable bag inserts to burn or autoclave
13. First aid kit

Clerical

1. Binders for SOPs
2. Page protectors for SOPs
3. Log books for QC and patient data management
4. Waterproof markers
5. Pens
6. Paper for all printers (clerical and equipment attached)
7. Printer cartridges for all printers
8. Requisition forms (if separate from request-result forms)
9. Calendar
10. Clock
11. Adhesive labels

Multi-Purpose

1. Disposable plastic pipettes
2. 4x4 gauze
3. Required pipettes (e.g., 10 μ l, 20 μ l, 25 μ l, 50 μ l, 100 μ l, 250 μ l, 500 μ l, 1000 μ l)
4. Small pipette tips
5. Large pipette tips
6. Forceps
7. Wash bottles (to store various reagents such as 1:10 bleach made fresh daily, etc.)
8. Wooden applicator sticks
9. Thermometers for room, refrigerated and frozen temperatures
10. Timers with alarms

11. Paper towels
12. Paper towel stand and holder
13. Appropriate volumetric pipettes for control/calibrator reconstitution
14. Rubber bulbs for volumetric pipettes

Waste Disposal

1. Biohazard bags

LEVEL II

General Laboratory Supplies

Centrifuge Maintenance

1. Reflective strip
2. Lubricant
3. Brushes
4. Timer with alarm (to verify centrifuge's timer)

Pipette Calibration and Maintenance

1. Cleaning and lubricant solutions (refer to manufacturer's acceptable choices)
2. O-rings
3. Inserts
4. Pipette wrench to adjust delivery amounts (pipettes which initially fail calibration)
5. Spare pipettes for Level I to use while current pipette is being calibrated
6. Pipette tips
7. Spectrophotometer calibration kits
 - a. Lint-free tissue
 - b. Spectrophotometer
 - c. Ruler
 - d. Graph paper
8. Analytical balance calibration kit
 - a. Distilled water
 - b. Calculator

Analytical Balance Verification and Maintenance

1. Calibration weights
2. Gloves
3. Forceps
4. Soft bristle brush

HIV Serology

For rapid test, refer to Level I for a list of supplies.

For EIA, refer to Level III for a list of supplies.

Automated CBC with Automated Differential

1. Wooden applicator sticks
2. 4x4 gauze
3. Bleach (for maintenance)
4. Wash bottles (to prepare correct bleach dilution)
5. 100ml graduated cylinder (if wash bottle does not have tick marks)
6. Equipment-specified calibrators, controls and reagents
7. Additional print out supplies dependent upon equipment (e.g., paper tape, printer cartridge, paper)
8. Phlebotomy (EDTA) and clerical supplies
9. Microscope supplies (if peripheral smear review is warranted)
10. Test tube racks
11. Analyzer
12. Calendar (to determine on-board stability expirations)

Manual CBC

WBC Count

1. Hemacytometers
2. Hemacytometer coverslips (standardized thickness)
3. WBC pipette
4. WBC pipette bulb
5. Alcohol pads
6. Lens paper
7. 4x4 gauze
8. Petri dish and cover
9. Timer with alarm
10. WBC diluting fluid (2% acetic acid or 1% HCl or Turks solution)
11. Tally counter
12. Phlebotomy (EDTA WB), clerical and microscope supplies
13. Glass cleaner
14. 500ml beaker to hold cleaner
15. Calculator

Hematocrit

1. Capillary microhematocrit tubes
2. Tube sealant
3. 4x4 gauze
4. Timer with alarm (if not included on centrifuge)
5. Phlebotomy (EDTA WB) and clerical supplies

Differential (Manual)

1. Coplin jars with screw cap lids (4 to 5) or staining rack
2. Diff counter
3. Wooden applicator sticks
4. 4x4 gauze
5. Wright-Giemsa stain
6. Slides
7. Waterproof marker or pencil
8. Slide storage box
9. Water for rinsing
10. Timer with alarm
11. Phlebotomy (EDTA WB), clerical and microscope supplies

CSF/Body Fluid Cell Counts

1. Hemacytometers
2. Hemacytometer coverslips (standardized thickness)
3. Microcapillary tubes (if dilutions are not needed)
4. WBC pipette
5. RBC pipette
6. Pipette bulb
7. Alcohol pads
8. Lens paper

9. 4x4 gauze
10. Petri dish and cover
11. Timer with alarm
12. WBC diluting fluid (2% acetic acid or 1% HCl or Turks solution)
13. RBC diluting fluid (Hayem's or Grower's solution, or 0.85% w/v NaCl)
14. Tally counter
15. Glass cleaner
16. 500ml beaker to hold cleaner
17. Calculator
18. Sterile tubes with screw cap lids
19. Centrifuge (for xanthochromic specimens)
20. WBC and RBC (platelet for differentials) Unopette System if available
21. Slide and differential stain for WBC cell type percentages
22. Sterile disposable pipette
23. Phlebotomy (EDTA WB), clerical and microscope supplies

CD4

1. Pipettes (50 μ l, 100 μ l and 1000 μ l)
2. Pipette tips (100 μ l and 1000 μ l)
3. 12 x 75mm capped polystyrene tubes
4. Vortex mixer
5. Reagent grade distilled water
6. Bleach
7. Phosphate buffer solution (PBS)
8. EDTA (purple top) vacutainer tubes
9. Equipment manufacturer-specific controls
10. Equipment manufacturer-specific reagents
11. Equipment manufacturer-specific sheath fluid
12. Timer with alarm
13. Wash bottles (deionized water and bleach solutions)
14. Phlebotomy (EDTA WB) and clerical supplies
15. Equipment-specified calibrators, controls, reagents and consumables
16. Additional print out supplies dependent upon equipment (e.g., paper tape, printer cartridge, paper)
17. Required pipettes and tips
18. 4x4 gauze
19. Wooden applicator sticks
20. Sharps container
21. Biohazard bags

Automated Chemistry Panel

1. Forceps (to remove pedi tubes from centrifuge)
2. 4x4 gauze
3. Required pipettes and tips
4. Aliquot vials and caps
5. Aliquot tubes and caps (to remove cells from serum if delays are encountered or for dilutions if above linearity)
6. Plastic disposable pipettes (to transfer serum)

7. Waterproof markers
8. Equipment-specified calibrators, controls, reagents, diluents and consumables
9. Additional print out supplies dependent upon equipment (e.g., paper tape, printer cartridge, paper)
10. Phlebotomy (serum) and clerical supplies
11. Test tube racks
12. Analyzer
13. Containers to hold distilled water
14. Calendar (to determine on-board stability expirations)

Whole Blood Lactate

1. Phlebotomy (WB-lancet), safety and clerical supplies
2. Lactate POC device
3. Test strips
4. Control material
5. Additional batteries

AFB Smear

Refer to Level I for a list of supplies.

Cryptococcal Antigen Test

1. Timer with alarm
2. Forceps (to remove pedi-tubes from centrifuge)
3. 4x4 gauze
4. Water bath thermometer
5. Sterile plain red top tubes with no clot activator
6. Sterile disposable pipettes
7. 50 μ l pipette and tips
8. Slide rotator
9. Disposable stirrers
10. Isopropyl alcohol
11. Brush
12. Lint-free tissues
13. Water
14. Phlebotomy (serum) supplies or sterile tubes with screw cap lids (CSF)
15. Clerical supplies
16. For quantitation:
 - a. Second slide
 - b. 12 x 75mm test tubes
 - c. 250 μ l pipette and tips

India Ink Stain

1. India ink reagent droppers
2. Positive and negative saline suspension of *C. albicans* and *C. neoformans*
3. Sterile tubes
4. Sterile saline
5. Sterile disposable pipettes
6. If greater than 1ml of fluid, centrifuge

7. Forceps
8. Slides
9. Coverslips
10. Microscope and clerical supplies

Hepatitis B Surface Antigen and Hepatitis C Antibody Testing

Refer to Level III for a list of supplies.

Gram's Stain

1. Disposable inoculating wires or sterile swabs
2. Biohazard receptacle with lid and separate disposable bag inserts to burn or autoclave
3. Slides
4. Double basin sink
5. Marking instrument—depends on slide (frosted—lead pencil; unfrosted—diamond stylus)
6. Gram's stain (kit or prepared stain)
7. Distilled water
8. Timer with alarm
9. Spirit lamp
10. Methanol and spare wicks for lamp
11. Staining rack
12. Slide warming tray (optional)
13. Drying rack
14. Forceps
15. Phenolic agents or bleach
16. Bottle to store 1:10 bleach solutions for that day's use
17. Appropriate specimen collection devices
18. Specimen transport bags
19. 4x4 gauze
20. Waterproof marker
21. Positive and negative unstained smears using *E. coli* and *S. aureus*
22. Microscope and clerical supplies
23. Media if plating will be performed at this site and sent to tertiary
 - a. Adhesive labels
 - b. Plate sleeves or racks
 - c. Thermometers
 - d. CO₂ canister

Gram's Smear

1. Crystal Violet
2. Iodine
3. Decolorizer
4. Safranin

Malaria Smear

Refer to Level I for a list of supplies.

Malaria Rapid Test

Refer to Level I for a list of supplies.

Rapid Syphilis Test

Refer to Level I for a list of supplies.

TPPA/TPHA

1. Microplate
2. Timer with alarm
3. Forceps (to remove pedi-tubes from centrifuge)
4. 4x4 gauze
5. Required pipettes and tips
6. Kit
7. Phlebotomy (serum) and clerical supplies
8. Test tube racks
9. Tray viewer or good source of lighting

RPR

1. Timer with alarm
2. Forceps (to remove pedi tubes from centrifuge)
3. 4x4 gauze
4. Required pipettes and tips
5. Kit
6. Positive and negative controls (if not included in kit)
7. Phlebotomy (serum) and clerical supplies
8. Test tube racks
9. Rotator cover (old pipette plastic box lid will work if it covers rotating slide)
10. Good source of lighting
11. 0.9% saline for qualitative testing

Type and Crossmatch

1. 4x4 gauze
2. Thermometers for refrigerator, freezer, room temperature, water bath and incubator
3. Circular graph paper for temperature monitoring
4. Ink cartridges
5. Saline (liter boxes)
6. Calibrated disposable pipettes
7. Microscope
8. Slides
9. Coverslips
10. 12 x 75mm test tubes
11. Scissors
12. Aliquot tubes with caps
13. Unit holders
14. Reagents
15. Reagent holders
16. Controls
17. Wash bottles
18. Specimen tube centrifuge
19. Forceps
20. 12 x 75mm tube centrifuge (washing)

21. Unit tags
22. Adapters to pool platelets
23. Unit bags
24. Blood bank results log
25. QC log book
26. Blood bank registry book
27. Unit identification numbers and labels
28. Clerical supplies

Urine Dipstick – Microscopy

1. Reagent strips
2. Positive and negative controls
3. Interpretive color chart
4. Collection cups
5. Specimen transport bags
6. Waterproof markers
7. Pedi UA collection bags
8. Timer with alarm
9. Disposable plastic pipettes
10. Disposable plastic centrifugal aliquot tubes
11. Clerical and microscope supplies
12. Double basin sink
13. Slides
14. Coverslips

Urine Pregnancy Rapid Test

Refer to Level I for a list of supplies.

Wet Mounts – Direct Microscopy

Refer to Level I for a list of supplies.

Microscopy

Refer to Level I for a list of supplies.

Additional Supplies if Preparing Stains

Refer to Level I for a list of supplies.

Phlebotomy

1. Supplies 1-19 same as Level I
2. Disposable plastic pipettes

Microbiological Transport

Refer to Level I for a list of supplies.

Transport/Receiving Supplies for Referral Testing

1. Supplies 1-7 same as Level I
2. Test tube racks and trays
3. Recipient log

Safety

1. Supplies 1-13 same as Level I
2. Chemical safety cabinet

Clerical

Refer to Level I for a list of supplies.

Multi-Purpose

1. Supplies 1-14 same as Level I
2. Glassware cleaner

Waste Disposal

1. Biohazard bags
2. Autoclave or burning receptacle
3. Indicators (to verify acceptable performance of autoclave)

LEVEL III

General Laboratory Supplies

1. Log maintenance book
2. Clerical supplies

pH Meter Maintenance (if preparing media)

1. Lint-free cloth
2. Deionized water
3. Beakers
4. Buffers (3.0 pH, 4.0 pH, 7.0 pH, and 10.0pH)
5. Wash bottle
6. Glassware and pipette washers
 - a. Verification indicator

Centrifuge Maintenance

Refer to Level II for a list of supplies.

Pipette Calibration and Maintenance

Refer to Level II for a list of supplies.

Analytical Balance Verification and Maintenance

Refer to Level II for a list of supplies.

Method Validation

1. Calculator to assess linearity, sensitivity, precision and accuracy

Biosafety Cabinet Class II*

1. Tissue paper to assess inward air flow
2. Disinfectant to wipe interior services (70% ethanol, 0.5% bleach with second wiping using sterile water)
3. UV replacement bulb and UV meter for verification (if UV is installed)
4. HEPA filter replacement
5. Appropriate disposal bag for replaced filters

*Annual verification should be performed by accredited field certifiers.

Thermometer Calibration and Verification

1. Reference thermometer

HIV Serology

For rapid test, refer to Level I for a list of supplies.

HIV Serology by EIA (Automated) [MICHELE TO ADVISE]

1. System-specific:
 - a. Anti-HCV reagent pack
 - b. Hbs Ag reagent pack
 - c. Anti- HBs reagent pack
 - d. HIV 1/2 Antibody reagent pack
 - e. Bulk reagents (e.g., diluents)
2. MLA pipettes
3. Pipette tips
4. Sample wheel, tray or cups
5. Barcode labels
6. Biohazard bags

HIV Serology by EIA [MICHELE TO ADVISE]

1. Precision pipettes to deliver 5µl, 10µl, 100µl, 200µl, 1ml, 5ml and 10ml (accurate within ± 10%) or automated pipettor-dilutor and pipette tips
2. Appropriate containers to prepare diluted specimens and reagents
3. Dry-heat incubator capable of maintaining 37° ± 1°C
4. Microwell plate or strip washer qualified for use with this assay (washer must be capable of dispensing at least 300µl per well and cycling five times)
5. Microwell plate or strip reader qualified for use with assay
6. Household bleach (5% to 8% sodium hypochlorite), which may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite); alternative disinfectants include: 70% ethanol or 0.5% Wescodyne (West Chemical Products, Inc.)
7. Paper towels or absorbent pads for blotting
8. Labeled null strips (for testing partial plates)
9. Clean, polypropylene containers for preparation of working conjugate and chromogen solutions (do not use polystyrene containers)
10. Deionized or distilled water
11. Gloves
12. Laboratory timer
13. EIA reagent reservoirs (optional)

Viral Load

Cavidi ExaVir Load Assay

1. ExaVir Load Reagent Kit (includes ExaVir Load consumables)
2. 1000µl filter pipette tips
3. 200µl filter pipette tips
4. 200µl pipette tips
5. 5ml pipette tips
6. 12.5ml repeater pipette tips
7. 15ml tubes
8. Reagent reservoirs
9. Distilled water
10. Absorbing paper
11. Pasteur pipettes

VERSANT HIV-1 bDNA Assay

1. VERSANT HIV-1 bDNA Reagent Kit
2. 1.5ml tubes
3. 1000µl filter pipette tips
4. 200µl pipette tips
5. Repeater pipette tips
6. 5ml pipette
7. 10ml pipette
8. 15ml tubes
9. 50ml tubes
10. Reagent reservoirs
11. Distilled water
12. Bleach

13. Absorbing paper

Amplicor Monitor vs. 1.5

1. Amplicor Monitor Reagent Kit
2. 96 well plate
3. 1.5ml tubes
4. 1000µl filter pipette tips
5. 200µl pipette tips
6. Repeater pipette tips
7. 5ml pipets
8. 10ml pipets
9. 15ml tubes
10. 50ml tubes
11. Reagent reservoirs
12. Distilled water
13. Bleach
14. Absorbing paper

CBC with Automated Differential

Refer to Level II for a list of supplies.

CD4

Refer to Level II for a list of supplies.

Urine Dipstick - Microscopy

Refer to Level II for a list of supplies.

Chemistry Panel/Whole Blood Lactate

Refer to Level II for a list of supplies.

AFB Smear

Refer to Level I for a list of supplies.

AFB Culture and Susceptibility

1. Acid-alcohol for Ziehl-Neelsen staining
2. Aniline
3. Aqueous methylene blue
4. Basic fuchsin
5. Carbol-fuchsin for Ziehl-Neelsen staining
6. Dry zinc dust
7. Eggs
8. Ethyl alcohol (70%)
9. Glycerol
10. Hydrochloric acid concentrated (reagent grade)
11. Hydrogen peroxide 30% (superoxol)
12. L-asparagine
13. Magnesium sulphate $MgSO_4 \cdot 7H_2O$
14. Magnesium citrate

15. Malachite green
16. Methylated spirit
17. N-naphthylethylene-diamine-dihydrochloride
18. Na₂HPO₄ (anhydrous)
19. p-Nitrobenzoic acid (NPB)
20. Phenol crystals
21. Potassium permanganate KMNO₄
22. Potassium dihydrogen phosphate KH₂PO₄ (anhydrous)
23. Sodium hydroxide (NaOH)
24. Sodium chloride
25. Sodium pyruvate
26. Sodium nitrate
27. Sulfanilamide
28. Sulfuric acid (reagent grade)
29. Tween 80
30. Xylene
31. Prepared AFB media

Microbiology Smear and Culture

Gram's Smear

1. Crystal Violet
2. Iodine
3. Decolorizer
4. Safranin
5. QC Slides

Culture/ID

1. Blood agar
2. MacConkey agar
3. Chocolate agar
4. Hektoen Enteric Agar
5. Thayer Martin Agar
6. Campy CVA Agar
7. Thioglycollate media
8. Anaerobic agar
9. Selenite Broth (Salmonella, Shigella)
10. Trypticase soy agar
11. Trypticase soy broth
12. Triple sugar iron agar
13. Simmons citrate agar slants
14. Oxidase dropper
15. Indole dropper
16. Brain heart infusion with 6.5% NaCl
17. Urea
18. Lysine
19. Arginine
20. Ornithine
21. SIM motility media
22. Bile esculin slants

23. Shigella group antisera
24. Salmonella group antisera
25. Coagulase plasma
26. Desoxycholate solubility reagent
27. Optochin disc
28. Group A disc
29. API strips 20 E
30. API strips non-fermenter
31. Rapid ID strips
32. Gas pak EZ

Susceptibility

1. Kirby Bauer plates
2. Mueller-Hinton agar plain (large)
3. Mueller-Hinton blood agar (large)
4. Antibiotic discs (variety)
5. Etest (certain organisms)
6. Kirby-Bauer dispenser

Other

1. ATCC organisms (all organisms identified)

Blood Cultures

1. Blood culture bottles (aerobic, anaerobic, AFB)
2. Venting units

Malaria Smear

Refer to Level I for a list of supplies.

Malaria Rapid Test

Refer to Level I for a list of supplies.

Syphilis

For rapid test, refer to Level I for a list of supplies.

For TPPA/TPHA/RPR, refer to Level II for a list of supplies.

Hepatitis B and C (Manual)

[MICHELE TO ADVISE]

1. Precision pipettes to deliver 5 μ l, 10 μ l, 100 μ l, 200 μ l, 1ml, 5ml and 10ml (accurate within \pm 10%) or automated pipettor-dilutor and pipette tips
2. Appropriate containers to prepare diluted specimens and reagents
3. Dry-heat incubator capable of maintaining 37 $^{\circ}$ \pm 1 $^{\circ}$ C
4. Microwell plate or strip washer qualified for use with this assay (washer must be capable of dispensing at least 300 μ l per well and cycling five times)
5. Microwell plate or strip reader qualified for use with assay
6. Household bleach (5% to 8% sodium hypochlorite), which may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite); alternative disinfectants include: 70% ethanol or 0.5% Wescodyne (West Chemical Products, Inc.)
7. Paper towels or absorbent pads for blotting
8. Labeled null strips (for testing partial plates)

9. Clean, polypropylene containers for preparation of working conjugate and chromogen solutions (do not use polystyrene containers)
10. Deionized or distilled water
11. Gloves
12. Laboratory timer
13. EIA reagent reservoirs (optional)

Hepatitis B and C (Automated)

[MICHELE TO ADVISE]

1. System-specific:
 - a. Anti-HCV reagent pack
 - b. Hbs Ag reagent pack
 - c. Anti- HBs reagent pack
 - d. HIV 1/2 Antibody reagent pack
 - e. Bulk reagents (e.g., diluents)
2. MLA pipettes
3. Pipette tips
4. Sample wheel, tray or cups
5. Barcode labels
6. Biohazard bags

Type and Crossmatch

Refer to Level II for a list of supplies.

Urine Pregnancy Rapid Test

Refer to Level I for a list of supplies.

Wet Mounts – Direct Microscopy

Refer to Level I for a list of supplies.

Cryptococcal Antigen Test

Refer to Level II for a list of supplies.

India Ink Stain

Refer to Level II for a list of supplies.

LEVEL II				
TEST/OTHER	EQUIPMENT	MODEL #1	MODEL #2	MODEL #3
General Laboratory Equipment - Centrifuge Maintenance	Tachometer (to verify speed)			
HIV Serology	Centrifuge, bench, non-refrigerated, with standard motor-Accepts 13x100mm blood tubes, 240V, 50/60Hz	Beckman Coulter	Heraeus	
HIV Serology	3D bi-directional rotator 240V, 50/60Hz	VWR International Tube Rotator	Drummond	Omega Diagnostics IMMUTREP-ROTATOR OD171 (battery/electric)
HIV Serology	4°C lab refrigerator	Domestic		
HIV Serology	Incubator	NuAire, Inc. Autoflow	Napco	Boekel Scientific
HIV Serology	Automated ELISA plate reader (wavelength range 405- 630 λ)	BioTek EL800	Dynex MRX	Molecular Devices EMax
HIV Serology	Automated ELISA plate washer (8 or 12 strip) with waste container	Thermo Scientific Wellwash	Finstrip	Bio-Tek ELX 50
HIV Serology	EIA incubator	Fisher		
Automated CBC with Automated Differential	Blood tube rocker/rotator	VWR-12620-960	Orbitek-Rocker	Mini Rotator-Vortex
Automated CBC with Automated Differential	4°C lab refrigerator, 240V	Domestic	Isotemp/Fisher	
Manual CBC: Hematocrit	Microhematocrit centrifuge	Hemastat	LW Scientific	Vulcon Technologies Microspin 24
Manual CBC: Hematocrit	Microhematocrit reader	Fisher Scientific StatSpin Card Style Reader #22-269-260	Fisher Scientific Kendall Healthcare Microhematocrit Tube Reader #22-260984	International Equipment Company such as reader model MB
CD4	Blood tube rocker/rotator	VWR-12620-960	Orbitek-Rocker	Mini Rotator-Vortex
CD4	4°C lab refrigerator, 240V (to store controls and calibrators)	Isotemp/Fisher	Domestic	
Automated Chemistry Panel	Centrifuge, bench, non-refrigerated, with standard motor	Fisher 13-100-582	Beckman Coulter	Heraeus
Automated Chemistry Panel	4°C lab refrigerator, 240V (to hold supplies)	Domestic	Isotemp/Fisher	
Automated Chemistry Panel	(-20°C) lab freezer (not self-defrosting) (to store reagents or aliquots of reagents/ calibrators)	Domestic		
Automated Chemistry Panel	Water distiller, complete with wall bracket and tubing	Barnstead International	Thermo Scientific	

LEVEL II				
TEST/OTHER	EQUIPMENT	MODEL #1	MODEL #2	MODEL #3
LEVEL II				
TEST/OTHER	EQUIPMENT	MODEL #1	MODEL #2	MODEL #3

LEVEL II				
TEST/OTHER	EQUIPMENT	MODEL #1	MODEL #2	MODEL #3
AFB Smear	Biosafety Cabinet Class II (if this level will perform any TB activity other than direct specimen smearing, BSC Class II is required)	Baker SterilGARD	NuAire Labguard	Labconco Purifier Logic Class II
Cryptococcal Antigen Test	Centrifuge (1000 x g)			
Cryptococcal Antigen Test	56°C Water bath, 15L, 240V, 50/60Hz	VWR-14231-854	Napco (Levels I-III)	Precision Scientific
Cryptococcal Antigen Test	4°C lab refrigerator, 240V (to store specimens until testing and for kit reagents)	Domestic	Isotemp/Fisher	
Gram's Stain	Incubators (CO ₂ and non-CO ₂)	Fisher		
Gram's Stain	4°C lab refrigerator, 240V (to store media)	Domestic	Isotemp/Fisher	
TPPA/TPHA	Centrifuge, bench, non-refrigerated, with standard motor	Fisher 13-100-582	Beckman Coulter	Heraeus
TPPA/TPHA	4°C lab refrigerator, 240V (to hold supplies)	Domestic	Isotemp/Fisher	
TPPA/TPHA	Blood tube rocker/rotator (optional)	VWR-12620-960	Orbitek-Rocker	Mini Rotator-Vortex
RPR	Centrifuge, bench, non-refrigerated, with standard motor	Fisher 13-100-582	Beckman Coulter	Heraeus
RPR	Blood tube rocker/rotator	VWR-12620-960	Orbitek-Rocker	Mini Rotator-Vortex
Type and Crossmatch	4°C lab refrigerator with continuous temperature monitoring capability	Domestic	Thermo Scientific	
Type and Crossmatch	(-40°C) laboratory freezer refrigerator with continuous temperature monitoring capability	Domestic	Thermo Scientific	
Type and Crossmatch	56°C Water bath, 15L, 240V, 50/60Hz	VWR-14231-854	Napco (Levels I-III)	Precision Scientific
Type and Crossmatch	Platelet rotator	Thermo Scientific		
Type and Crossmatch	Platelet incubator	Thermo Scientific	Fisher	
Type and Crossmatch	Test tube agglutination viewer	BD Diagnostics	Fisher	
Urine Dipstick - Microscopy	Centrifuge			
Microbiological Transport	4°C lab refrigerator, 240V	Domestic	Isotemp/Fisher	

**Mention of any equipment in this document does not indicate endorsement by the World Health Organization, the U.S. Government, the American Society for Clinical Pathology, the Clinton Foundation, the Bill & Melinda Gates Foundation, or the Global Fund. The above list includes examples of supply/equipment options and should not be considered all-inclusive.*

ANNEX H

Vendor Questionnaire: Important Considerations for Service Contract Negotiations

Laboratories should ask vendors the following questions:

QUESTIONS	YES	NO
Equipment Installation		
Are there pre-installation checklists and specifications provided by the vendor to assist staff with equipment familiarity, space and heat requirements?		
Is there a pre-installation site visit to address all physical requirements (e.g., space, power, heat output)?		
Are user manuals available that staff can utilize?		
<ul style="list-style-type: none"> • Are the user manuals translated into local language? (Note: CD-ROMs in laboratories without computer capabilities are not an acceptable resource.) 		
Can the vendor perform all instrument validations for the equipment?		
Can the vendor assist with the development of reference ranges for the laboratory?		
Is there a final installation checklist completed and given to the customer with supporting documentation?		
Is all documentation provided in a notebook for laboratory QA?		
Service and Maintenance		
Is the expected mean time between failures for the equipment and uptime defined in the contract?		
Is the acceptable response time for phone support, including callback time and on-site support defined in the contract?		
<ul style="list-style-type: none"> • Are there penalties/reimbursement in the service contract when service response time is not met? 		
Is there a hierarchy defined in the contract for problem resolution when vendor support is unsatisfactory?		
<ul style="list-style-type: none"> • Are the contract-defined communications channels between the user and the manufacturer stated in the contract? 		
Are all operators' manuals, including troubleshooting and maintenance, provided to the laboratory by the vendor?		
Does the maintenance/service manual include basic troubleshooting suggestions with flags or alerts?		
Is the frequency of vendor-initiated preventative maintenance visits defined in the service contract (usually quarterly or every 6 months)?		
Has the service technician assigned to the area been adequately trained to support the specific equipment?		
Is there a checklist performed by the technician prior to ending a service call to prove that the equipment is functioning at an acceptable level?		
<ul style="list-style-type: none"> • Is a copy of the checklist provided to the customer? 		
Does the contract provide for replacement or loaner equipment in the case of equipment failure?		
Is there support available for replacement parts that are shipped and to be		

installed by staff?		
Is there a mechanism for tracking and receiving replacement parts?		
Are the hours of operation for the hotline included in the contract?		
<ul style="list-style-type: none"> Do the hotline hours match the lab's hours of operation? 		
<ul style="list-style-type: none"> Is support available consistently during these hours? 		
<ul style="list-style-type: none"> If unresolved over the phone, is action taken by the hotline to get on-site assistance and follow-up on the support? 		
Does the vendor maintain a service history according to equipment serial numbers so that all service call details can be retained and chronic issues can be identified?		
Is there a sufficient number of trained technicians to handle the laboratory's territory?		
<ul style="list-style-type: none"> Is back-up coverage provided if the assigned technician cannot arrive within the defined response time? 		
Does the technician provide to the customer written information relating to the service call?		
Is the response time for an unresolved issue on a service call defined in the contract?		
Reagent Acquisition		
Can the vendor provide delivery with adequate lead time to minimize stock-outs?		
<ul style="list-style-type: none"> Is the vendor able to supply a continuous supply of reagents, calibrators and controls? 		
Do the initial supplies sent for training have sufficient expiration dates to continue operation until the standing order is in place or are other arrangements made?		
Is the system an open system?		
<ul style="list-style-type: none"> Have all reagents been validated on the system if open? 		
Can the laboratory negotiate reagent/consumable pricing based on consolidated volumes for all equipment?		
Is there a complete ordering inventory document given by the vendor (catalog numbers of parts and reagents needed for the system)?		
Is it possible to arrange for standing orders for reagents?		
<ul style="list-style-type: none"> Can the vendor help with forecasting reagent needs based on volumes? 		
<ul style="list-style-type: none"> Can standing orders be adjusted quarterly to account for changing volumes? 		
Does the contract describe the costs associated with reagents not used/needed (applied to account or not refundable)?		
Equipment Training		
Does training for computerized equipment meet staff needs (level of computer proficiency)?		
Is training customized for different sites?		
Is training tailored to meet staff competencies in laboratory practice? Does training include:		

<ul style="list-style-type: none"> • Dry run of a call to the hotline to help learn what information must be readily available during call (e.g., serial number, callback number)? 		
<ul style="list-style-type: none"> • Maintenance and troubleshooting procedures including staff performance with limited guidance? 		
<ul style="list-style-type: none"> • Documentation of maintenance performed using vendor supplied logs? 		
<ul style="list-style-type: none"> • Performance of both random and multiple tests for batch analysis? 		
Is the effectiveness of training measured?		
Are all necessary reagents, controls, calibrators, consumables and reagent holders available on-site at the time of validation and training?		
<ul style="list-style-type: none"> • If not, will they be available during training or during a follow-up visit to ensure correct usage? 		
Support After Installation		
After installation, will the vendor representative visit the site? (How often?)		
After installation, will the vendor representative check-in by phone? (How often?)		
After installation, will the vendor respond to calls from the users? (How quickly?)		
Other		
Does the vendor offer external peer comparison programs?		
<ul style="list-style-type: none"> • Are the reports timely enough to be used effectively? 		
<ul style="list-style-type: none"> • Is there an additional charge to use these reports? 		
If the customer is part of the peer comparison program, does the vendor review external QC data submitted by the customer and supply some feedback regarding the data?		
If the customer is interested in participating in the peer comparison program, is it organized and operational during on-site training?		
Does the equipment include a QC data management package?		
<ul style="list-style-type: none"> • To what extent must staff use additional resources to monitor QC on a daily and monthly basis? 		
Are Material Safety Data Sheets (MSDS) provided for all hazardous chemicals?		

ANNEX I-Considerations when Procuring Laboratory Equipment and Negotiating Contracts

When procuring laboratory equipment and reagents at the program level, the following steps should be taken:

1. Assess the laboratory system needs: Evaluate the clinical testing needs of the patient populations at the laboratory sites in order to determine volumes and test menu requirements. If in a laboratory system, evaluate the needs of all laboratories in the system.
2. Research the international market to identify available equipment that may meet your needs; request information on the equipment from all vendors as well as from organizations such as the FDA that certify/approve equipment.
3. Develop the specifications for the equipment purchase (see example below).

Specifications for the purchase of the automated Chemistry System are as follows:

- a. The new system must include user-friendly software including a backup system that is capable of patient data management including test autoverification, QC management and equipment function management.
 - b. The new system will provide an integrated chemistry and immunoassay solution that will provide analytical TAT for STAT testing of less than 20 minutes for critical testing including chemistry panels and cardiac markers.
 - c. The new system will provide throughput of at least 1000 tests per hour.
 - d. The proposal must include the capability to perform all current tests on the test menu of the various laboratories on the primary platform or provide an acceptable alternative solution. Tests provided by third parties must be fully validated and supported by the system vendor. Any tests not fully validated and supported by the vendor should be noted as such.
 - e. The proposal must include a scalable chemistry system (high, moderate, and low testing volumes) that will meet the needs of each hospital/health center in the system.
 - f. A mirror image backup chemistry/immunoassay system will be provided for any equipment placed at the hospital sites.
 - g. All correlations for tests on the system will be done by the vendor for the life of the contract.
 - h. Mean time between failure rate must be described for the equipment and must be standard for the industry.
 - i. Guaranteed response time on urgent and non-urgent service calls must be included.
 - j. The system must interface with LIS Cerner Classic version 3.06.
 - k. The system must be installed with minimum renovations. If any renovations will be required, then they must be stipulated in the proposal.
 - l. Complete technical training on all systems and related middleware will be provided for at least two key operators per system.
 - m. All software updates, other than enhancements, must be installed at no additional charge for the life of the systems.
 - n. Complete operational training will be provided to all laboratory staff who will be operating the equipment.
4. Issue a request to vendors/suppliers for proposal or tender for the equipment.

5. Review proposals to determine if the system meets your written specifications.
6. To determine the best system for your needs, use prioritized criteria in a decision matrix; use this matrix to evaluate all systems and then determine which equipment meet your needs (include local service availability as an important criteria).
7. Ask several vendors (usually the top rated two or three on decision matrix) to provide proposals that describe equipment, reagents, controls/calibrators, consumables and service (all systems regardless of procurement method should include these costs).
8. Ask vendors to provide proposals for different procurement options including direct purchase, lease and reagent rental. Each proposal should include costs for equipment, reagent, consumables and service (after the warranty).
9. Review the proposals to determine the exact costs of equipment, reagents, calibrators, consumables and service for each procurement option. Evaluate service options in the proposals.
10. Make it clear to each vendor/supplier that you are negotiating with other vendors/suppliers and that competition for your business exists.
11. Determine what you are willing or able to pay for the equipment and negotiate that price.
12. Negotiate group discounts on equipment from list price based on number of equipment, total value of contract and your budget.
13. Negotiate discounts on reagent list pricing based on total test volumes projected.
14. Negotiate for calibrators and controls to be included at no cost in the contract.
15. Negotiate payment for any water systems required.
16. Negotiate on-site training for all sites at no expense.
17. Negotiate upgrades to equipment if released during term of a lease or reagent rental.
18. Negotiate additional needs if not already included in the proposal (e.g., manufacturer training for additional lab key operators or engineers; number of available local service engineers for the systems).
19. Assure the contract has an uptime guarantee of at least 98%.
20. The contract must include stipulations for shipment method for reagents including calibrators and controls (standing order, on demand) and any freight charges that may apply. Negotiate free shipping for standing orders at defined frequencies. Negotiate shipment charges.
21. Contract should stipulate expected time for fulfillment of non-standing reagent orders.
22. Contract should stipulate terms for reimbursement if reagents/kits are recalled or withdrawn from market.
23. Contract should include at no cost software upgrades that are needed for existing system operation.
24. Contract should include terms of the warranty provided.
25. Contract should include conditions for termination of the contract (especially on lease or reagent rental agreements).
26. Contract should include payment terms.
27. Reagent rental contracts will specify a reagent commitment that must be carefully evaluated; contract should include defined pricing schedule for reagents and any price increases over the term of the contract.
28. The contract should contain details of the service contract provided after the warranty period; service contracts should be negotiated at time of purchase based on number of equipment purchased; cost of contract should never exceed 10% of equipment cost.
29. Service contracts should include at a minimum the following items:
 - a. Defined number of service visits and cost of any additional calls.

- b. Acceptable response for routine and urgent service calls (e.g., 95% of all urgent calls for such issues as equipment down will be responded to within 24 hours).
- c. At least two preventative maintenance visits per year.
- d. Coverage for costs of freight if parts or equipment must be shipped out.
- e. Coverage for parts, labor and travel.
- f. Stipulation for service and maintenance training for staff/engineers.
- g. Loaner equipment available within defined period.
- h. Access to spare parts may need to be included.
- i. Mechanism for shipping back unreparable equipment at vendor cost.
- j. Penalties and mechanisms for escalation when defined service response rate is not met.
- k. Details of hotline services including hours of operation.
- l. Service documentation provided to user.
- m. Define the term and cost of the contract.
- n. Define all equipment covered under the contract.
- o. Mechanism for contract review.

ANNEX J

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ANNEX K ABBREVIATIONS

AFB	Acid-Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
APC	American Power Conversion
API	Trademark name for Microbiology test strips
ART	Antiretroviral Therapy
ASCP	American Society for Clinical Pathology
bDNA	Branched-Chain DNA
BMP	Basic Metabolic Panel
BSA	N,O-Bis(Trimethylsilyl)acetamide (silylation reagent)
BSC	Biological Safety Cabinet
BSL	Biosafety Level
CBC	Complete Blood Count
CD	Cluster of Differentiation (refers to cell surface proteins)
CD3	Protein present on T- cells
CD4	Protein present on T- 'helper' cells
CD8	Protein present on T- 'cytotoxic' cells
CDC	Centers for Disease Control and Prevention (United States)
CK	Creatine Kinase
CLSI	Clinical and Laboratory Standards Institute
CMV	Cytomegalovirus
CO ₂	Carbon Dioxide
CPK	Creatine Phosphokinase
CSF	Cerebrospinal Fluid
CV	Coefficient of Variation
DBS	Dried Blood Spot
DI	Deionized water
DNA	Deoxyribonucleic Acid
DR	Drug Resistance
DST	Drug Susceptibility Testing
EDTA	Ethylenediaminetetraacetic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
EQA	External Quality Assessment
FBC	Full Blood Count
FTA	Fluorescent Treponema Absorption
GHTF	Global Harmonization Task Force
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GLI	Global Laboratory Initiative
GLP	Good Laboratory Practice
H ₂ O	Dihydrogen Monoxide (water)
HCl	Hydrochloric Acid
HCT	Hematocrit
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HVZ	Herpes Varicella Zoster
Hz	Hertz
ID	Identification

ISE	Ion Selective Electrode
IUATLD	International Union against Tuberculosis and Lung Disease
KOH	Potassium Hydroxide
LFT	Liver Function Test
LIS	Laboratory Information System
mL	Milliliter
MOH	Ministry of Health
MOTT	Mycobacteria Other Than Tuberculosis
MSDS	Material Safety Data Sheet
NaCl	Sodium Chloride
NCCLS	National Committee for Clinical Laboratory Standards
NPB	p-Nitrobenzoic Acid
PBS	Phosphate Buffer Solution
PCP	Pneumocystis carinii pneumonia
PCR	Polymerase Chain Reaction
PEPFAR	President's Emergency Plan for AIDS Relief
PLT	Platelet Count
PNB	Para-Nitro Benzoic Acid
POC	Point-of-Care
PPE	Personal Protective Equipment
PT	Proficiency Test
QA	Quality Assurance
QC	Quality Control
QI	Quality Improvement
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
RST	Rapid Syphilis Test
SCMS	Supply Chain Management System
SD	Standard Deviation
SOP	Standard Operating Procedure
SST	Serum Separator Tube
STAT	Latin word for "immediately"
TAT	Turnaround Time
TB	Tuberculosis
TPHA	Treponema Pallidum Hemagglutination
TPPA	Treponema Pallidum Particle Agglutination Assay
UPS	Uninterruptible Power Supply
USG	United States Government
VDRL	Venereal Disease Research Laboratory
WB	Whole Blood
WBC	White Blood Count
WHO	World Health Organization