

Specifications for WHO Procurement of Diagnostics

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Specifications for WHO Procurement

- Assays are evaluated (as part of Prequalification process) & upon meeting certain performance criteria may be eligible to submit a RFP to WHO for the coming year. If price is felt to be appropriate, a LTA is signed with a yearly review.
- Specifications
 - Operational
 - Technical
 - Commercial



Generic Operational Aspects

In addition to strong laboratory performance, operational characteristics are also important factors

- **Shelf life**

- Kits supplied by the manufacturer should have a long maximum shelf life, also the manufacturer must agree to delivering test kits with at least 80% shelf life left upon delivery i.e. 10 months is shelf life from manufacture is 12 months.

- **Storage temperature**

- High temperatures & fluctuations in temperature during shipment and delivery may reduce the actual shelf life of the products. Ambient temperatures in many countries exceeds the minimum/maximum storage temperature for many kits. Use of a temperature indicator is advised.

- **Kit size**

- Availability of different kit sizes is advantageous to accommodate the different turn-around volumes in various settings. Thus eliminating wastage.



Operational Specifications

- Ability to detect all HIV subtypes
 - Antibody, antigen, mutants, HIV-2
- Specimen throughput (EIA vs. rapid)
 - Over 40 per day per facility could be considered high through-put
 - Specimen type
- Environmental
 - Air conditioning for CD4 enumeration, closed windows (sealed), dust free
 - Electricity, Water
 - Refrigerator
- Safety
 - Avoidance of spills, waste disposal
- Language of package insert



HIV Technical Aspects

● Assay Performance

- WHO evaluation to ensure minimum criteria have been met:
 - **Sensitivity = 100% for EIAs & $\geq 99\%$ for Rapids**
 - Determined from assay performance of the WHO reference panel of HIV positive specimens at WHO CC as part of PQ
 - **Specificity $\geq 98\%$ for both EIAs & Rapids**
 - Determined from performance of the WHO reference panel of HIV negative specimens at WHO CC as part of PQ
 - **Inter-Reader Variability $\leq 5.0\%$**
 - Only a consideration for Rapids when the results are read visually
 - **Seroconversion Panels**
 - Seroconversion sensitivity index must be comparative with assays of same test format



Hepatitis C Technical Aspects

● Assay Performance

- WHO evaluation to ensure minimum criteria have been met:
 - **Sensitivity = 100% for EIAs & $\geq 98.0\%$ for Rapids**
 - Determined from assay performance of the WHO reference panel of HCV positive specimens at WHO CC as part of PQ
 - **Specificity $\geq 98.0\%$ for both EIAs & $\geq 97\%$ for Rapids**
 - Determined from performance of the WHO reference panel of HCV negative specimens at WHO CC as part of PQ
 - **Inter-Reader Variability $\leq 5.0\%$**
 - Only a consideration for Rapids when the results are read visually
 - **Seroconversion Panels**
 - Seroconversion sensitivity index must be comparative with assays of same test format

Hepatitis B Technical Aspects

● Assay Performance

- WHO evaluation to ensure minimum criteria have been met:
 - Sensitivity = **100% for EIAs & Rapids**
 - Determined from assay performance of the WHO reference panel of HBsAg positive specimens at WHO CC as part of PQ
 - Specificity **≥98.0% for both EIAs & Rapids**
 - Determined from performance of the WHO reference panel of HBsAg negative specimens at WHO CC as part of PQ
 - Inter-Reader Variability **≤5.0%**
 - Only a consideration for Rapids when the results are read visually
 - Seroconversion Panels
 - Seroconversion sensitivity index must be comparative with assays of same test format

Example of WHO Specifications

| Assay Name | Test per Kit | Sensitivity ¹ | Specificity ¹ | Shelf-life / Storage Temp (°C) | HIV Type ² | Specimen Type | Equipment Requirements ³ | Cost/Test |
|--|------------------------|--------------------------|--------------------------|--------------------------------|-----------------------|-----------------------------|-------------------------------------|----------------------------------|
| Capillus™ HIV-1/HIV-2 (Trinity Biotech) | 100 | 100% | 100% | 9 months / 2-8 | HIV 1+2 | Serum / plasma, whole blood | G | €1.10 |
| DoubleCheckGold HIV 1&2 Whole Blood (Orgenics) | 20 100 | 100% | 99.3% | 14.4 months / 2-30 | HIV 1+2 | Serum / plasma, whole blood | D, G | US\$1.44 |
| First Response™ HIV 1-2-0 Card (PMC) | 30 | 100% | 98.8% | 16 months / 2-8 | HIV 1+2 | Serum / plasma, whole blood | D, G | US\$0.73 |
| Genie II HIV-1/HIV-2 (BioRad) | 40 | 100% | 99.7% | 9 months / 2-8 | HIV 1 HIV 2 | Serum / plasma | D, G | €2.00 |
| HIV Tridot (J Mitra) | 10 50 100 200 | 99.6% | 99.7% | 12 months / 4-8 | HIV 1 HIV 2 | Serum / plasma | G | €1.05 €0.95 €0.86 €0.76 |

1 Results as obtained in WHO test kit evaluation, final sensitivity & final specificity values shown

2 Assays denoted as HIV 1 HIV 2 are capable of discrimination between HIV-1 and HIV-2, those denoted HIV 1+2 are not capable of discrimination

3 Equipment Requirements A: ELISA reader, B: ELISA washer, C: Consumables, D: Pipette, E: Power supply, F: For large volume testing more than 40 samples daily, G: For small volume testing 1 to 40 samples daily 4 Price does not include freight and administration charges



Viral load Technologies

Nucleic Acid Based

- Additional marker to assess efficacy of treatment

| Assay Name | AMPLICOR™ HIV-1 Monitor® Test v1.5 (Roche) | COBAS® AMPLICOR™ HIV-1 Monitor® Test v1.5 (Roche) | COBAS® TaqMan | AMPLICOR™ HIV-1 DNA Test v1.0 (Roche) |
|---|--|--|--|--|
| Type of assay | RT-PCR, quantitative | RT-PCR, quantitative | Real-Time RT-PCR, DNA PCR (HIV-1 Qual test), quantitative | PCR, qualitative |
| Dynamic Range (copies/ml) | 50 – 100,000 (UltraSensitive) 400 – 750,000 (Standard) | 50 – 100,000 (UltraSensitive) 500 – 1,000,000 (Standard) | 40 – 10 Million copies/ml (95% confidence) | N/A |
| Specimen Type | Plasma, Serum | Plasma, Serum | Plasma, Serum | Whole Blood, DBS |
| Specimen volume | 200 ml (UltraSensitive) 500 ml (Standard) | 500 ml (US, Manual Extraction) 750 ml (US, Automated Extraction) 200 ml (Std, Manual Extraction) 350 ml (Std, Automated Extraction) | 500 ml (Manual Extraction) 1 ml (Automated Extraction) | 200 – 500 ml |
| HIV-1 subtypes amplified and area of genome amplified | Group M, subtypes A-H Gag | Group M, subtypes A-H Gag | Group M, subtypes A-H Gag | Group M, subtypes A-H Gag |
| Time for result | 7-8 hours | 6-8 hours | 5-6 hours | 7-8 hours |
| Cost/test | \$ 17 – 35/test \$ 35 - 90/ test | \$ 17 – 35/test \$ 35 – 90/test | \$ 17 – 35/test \$ 35 – 90/test | \$ 10 – 15 / test \$ 15 – 30 / test |
| Number of samples/run | 9-21 | 9-21 | 21 – 84 (COBAS TaqMan 48 or 96) | 9-21 |
| Equipment required | Thermocycler, ELISA Reader / Washer, Microcentrifuge Not supplied by Roche Total approx \$25,000.- | COBAS AMPLICOR (PCR) COBAS Ampliprep-automated extraction (Optional) | COBAS TaqMan 48 or 96 COBAS AmpliPrep -automated extraction (Optional) | Thermocycler, ELISA Reader / Washer, Microcentrifuge Not supplied by Roche |
| Equipment Cost (\$US) | | COBAS AMPLICOR: \$35,000 - 45,000 AmpliPrep: \$80,000 -100,000 | COBAS TaqMan 48 or 96: \$45,000 - \$60,000 / \$120,000 AmpliPrep: \$80 - 100,000 | Total approx \$25,000.- |



Viral load Technologies

Nucleic Acid Based

| Assay Name | bioMérieux NucliSens EasyQ HIV-1 | Siemens Versant HIV-1 RNA 3.0 Assay | Abbott RealTime HIV -1 |
|---|--|--|--|
| Type of assay | Real Time-NASBA | bDNA | Real Time-PCR |
| Dynamic Range (copies/ml) | 50 - 3,000,000 | 75 - 500,000 | 40 - 10,000,000 |
| Specimen Type | Plasma, Serum, Dried Blood Spots | Plasma | Plasma |
| Specimen volume | 10 - 2,000 µl | 1,000 - 2,000 µl | 200 - 500 - 1,000 µl |
| HIV-1 subtypes amplified and area of genome amplified | All Gag | Group M (subtypes A-G) Pol | Group M (subtypes A-G) and Group O Pol |
| Time for result | 2.5-3 hours | 22 hours | 5 hours |
| Cost/test | \$38-76 | \$87 | 21 - 95 (+3 controls) |
| Number of samples/run | 8-48 | 12-168 | \$20-70 |
| Equipment required | NucliSens miniMAG system or Nuclisens easyMAG system NucliSens EasyQ Analyser Strip centrifuge | Bayer System 340 (bDNA Analyzer, Data Management Software, and computer system) Heatblock Waterbath Vacuum system Centrifuge | M2000sp \$100,000 Or magnetic racks, plate cooler \$500 |
| Equipment Cost (\$US) | \$63,000 | \$10 000 + Bayer System Analyzer | M2000rt: \$ 50,250 |



Viral load Technologies

Non Nucleic Acid Based

| | | |
|--|--|---|
| Assay Name | Cavidi ExaVir™ Load kit version 3 | Perkin Elmer HIV-1 p24 Ultra- ELISA ELAST ELISA amplification system |
| Type of assay | Enzyme immunoassay for quantitation of Reverse Transcriptase activity | Enzyme immunoassay for quantitation of p24 antigen |
| Dynamic Range (copies/ml) | 750 - over 50,000 copies/ml | 400 copies/ml |
| Specimen Type | Plasma | Plasma, Serum or Cell Culture Supernatant |
| Specimen volume | 1000 µl | 100 µl |
| HIV-1 subtypes amplified and area of genome amplified | All, plus HIV-2 Reverse Transcriptase (RT) activity | p24 antigen HIV-1 |
| Time for result | 24 hours | 6 hours |
| Cost/test | \$13-15 | 10 |
| Number of samples/run | 30 | 96 |
| Equipment required | Incubator (33°C), Freezer, ELISA Reader, Computer | Incubator, ELISA Reader, Refrigerator |
| Equipment Cost (\$US) | \$9,000 - \$10,000 (start up pack includes other necessary equipment and 3 kits) | \$7,000 – \$9,000 |



CD4 Enumeration Technologies

Flow Cytometry

- To monitor HIV infection & particularly efficacy of treatment

| Parameter | Double-platform ^a | Single-platform | |
|-------------------------------------|--|---|---|
| | | Volumetric | Bead-based |
| Instruments, Manufacturers | Flow cytometer Partec GmbH (Munster, Germany) Becton Dickinson (CA, USA) Coulter Corporation (FL, USA) | Flow cytometer Partec GmbH (Munster, Germany) Guava Technologies (CA,USA) | Flow cytometer Becton Dickinson (CA, USA) Coulter Corporation (FL, USA) |
| Cost of instrument (US\$) | 20-95 000 | 20-70 000 | 20-95 000 |
| Cost of reagents/test (US\$) | 2-11 | 2-10 | 3-25 |
| Specimen (EDTA) | Whole blood | Whole blood | Whole blood |
| Results | Absolute CD4 count Absolute CD8 count CD4% and CD8% among lymphocytes CD4/CD8 ratio B and NK cells are possible | Absolute CD4 count Absolute CD8 count CD4% and CD8% among lymphocytes CD4/CD8 ratio B and NK cells are possible | Absolute CD4 count Absolute CD8 count CD4% and CD8% among lymphocytes CD4/CD8 ratio B and NK cells are possible |
| Throughput (samples/day) | Up to 250 | Up to 250 | Up to 250 |
| Advantages | One tube assay possible without QC problems EQA available | No need for extra beads or haematology analyser Protocols for aged samples available EQA available | No need for haematology analyser Protocols for aged samples available EQA available |
| Disadvantages | Requires the use of a haematology analyser More prone to clerical errors Fresh samples needed in order to obtain absolute counts | Internal QC for pipetting requires two tubes assay Limited data available | Internal QC for pipetting requires two tubes assay Beads are expensive and require careful handling |



CD4 Enumeration Technologies

Dedicated Assays

| | FACSCount Becton Dickinson (CA, USA) | CyFlow Counter Partec GmbH (Munster, Germany) | CyFlow SL Partec GmbH (Munster, Germany) | PointCare Beckman Coulter Inc. (CA, USA) |
|--------------------------------------|---|---|--|---|
| Instrumentation | Dedicated CD4 counter | Dedicated CD4 counter | Dedicated CD4 and CD4% counter | PointCare System |
| Assay principle | Flow cytometry | Flow cytometry | Flow cytometry | Flow cytometric detection of CD4+ T-cells |
| Detection system | Fluorochrome labelled anti-CD3, CD4 and CD8 MAb | Fluorochrome labeled, anti-CD4, (CD45, CD3, CD8) MAb | Fluorochrome labeled, anti-CD4 /anti CD45 MABs, (CD3/CD4),(CD3CD8),(CD4/ CD8 | Anti-CD4 MAb conjugated with colloidal gold particles |
| Specimen (EDTA) | Whole blood | Whole blood | Whole blood | Whole blood |
| Results | Absolute CD4 and CD8 count, CD4/CD8 ratio, CD4% and CD8% among T cells | Absolute CD4 count | Absolute CD4 count | Absolute and % CD4 count, WBC absolute count, Absolute and % lymphocyte count |
| Cost of instrument (US\$) | 32 000 | 21 050 | 26 050 | 16 500 |
| Cost of reagents /test (US\$) | 5-20 | 2 | 2-3 | 5.5-7.8 |
| Advantages | Automated, fewer steps, less human error, low biohazard risk Absolute CD4 and CD8 counts Quick results EQA available | Reagents available at low cost Equipment includes reagents for 1000 CD4 tests, Quick results EQA available | Reagents available at low cost Equipment includes reagents for 200 CD4 % tests, Quick results EQA available | Simple , no manual gating No manual sample preparation, |
| Disadvantages | Reagent prices vary widely CD4% among lymphocytes not reported, can be calculated from haematology parameters | CD4% among lymphocytes not reported Limited data available | Limited data available | One sample processed at a time 17 min. No EQA available No published data available yet |



CD4 Enumeration Technologies

Dedicated Assays and Manual Assays

| | DEDICATED ASSAYS ctn | MANUAL ASSAYS | |
|--------------------------------------|---|--|---|
| | | Cyto-Spheres | Dynabeads |
| Manufacturer | Sysmex Corp. Japan | Coulter Corporation (FL, USA) | INVITROGEN sarl |
| Instrumentation | pochH-100i or KX-21N for CD4 counting | Haemocytometer Light microscope | Magnet Haemocytometer Light or fluorescence microscope |
| Assay principle | Immunomagnetic isolation of CD4 cells (Dynabeads) and counting by impedance measurement | Direct observation of bead-rosetted cells | Direct observation of immunocaptured cells |
| Detection system | Impedance measurement | Latex beads conjugated | Magnetic beads conjugated to anti-CD4 and CD8 MAb |
| Specimen (EDTA) | Whole blood | Whole blood | Whole blood |
| Results | Absolute CD4 counts, CD4 percentage (haematology parameters (CBC + WBC 3-PD)) | Absolute CD4 count | Absolute CD4 count , Absolute CD8 count, CD4/CD8 ratio |
| Cost of instrument (US\$) | | 2000 | |
| Cost of reagents /test (US\$) | | 08-Apr | |
| Advantages | Simple measurement on haematology analyser | Simple and rapid | Simple and rapid Absolute CD4 and CD8 counts |
| Disadvantages | Manual sample preparation | 10 samples processed at a time, Subjectivity in visual counting, CD4% among lymphocytes or CD8 counts not reported, No EQA available | 6 samples processed at a time Subjectivity in visual counting CD4% among lymphocytes not reported No EQA available |

Generic Commercial Aspects

- Re-branding
 - Two examples on current WHO BPS list
- Commercial viability of the manufacturer
 - Will they stay producing the product in the same manner, if not the PQ becomes invalid & process begins all over again
- Production capacity
 - Enough assays to serve our customers?
- Patent & embargo issues

