

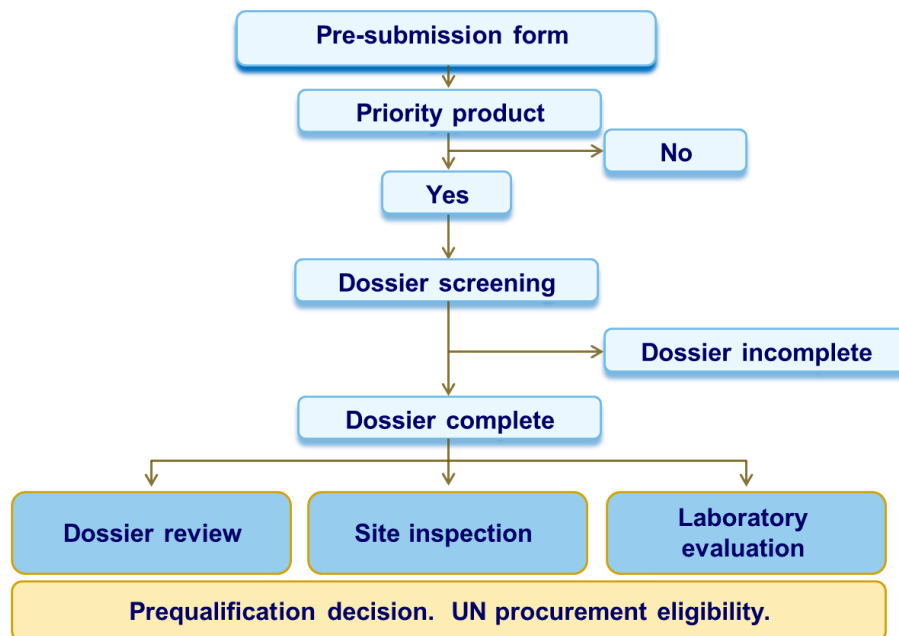
## ANNEX 8: Ensuring the quality of HIV testing services

Acknowledgement: Sands, A<sup>1</sup>

### 8.1 WHO Prequalification of In Vitro Diagnostics Programme

The WHO Prequalification of in Vitro Diagnostics Programme is conducted according to the process outlined in Fig. 8.1A.

**Fig. 8.1A. Overview of the WHO prequalification assessment procedure**



Source: WHO, 2014 (1)

The final WHO prequalification assessment outcome depends on:

1. Results of the dossier assessment and acceptance of the corrective action plan, if required.
2. Results of the inspection(s) and acceptance of the corrective action plan, if required.
3. Meeting the minimum acceptance criteria on the laboratory evaluation.

Applications for prequalification should be submitted in accordance with guidance in Instructions for the completion of the pre-submission form ([http://www.who.int/diagnostics\\_laboratory/evaluations/Application/en/](http://www.who.int/diagnostics_laboratory/evaluations/Application/en/)).

#### Prioritization for WHO prequalification assessment

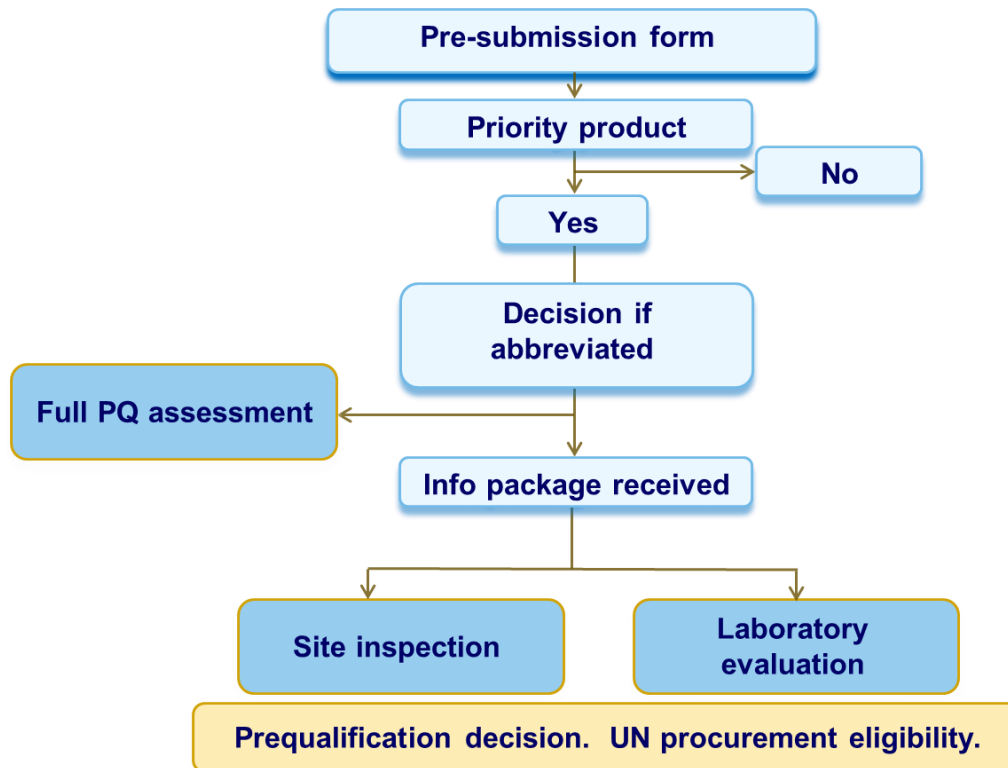
WHO determines whether an application will be prioritized for prequalification assessment based on (1) WHO prioritization criteria and (2) programmatic suitability. At this point in the process, it is necessary to determine if a product is made by the original manufacturer (not re-branded) and to determine which regulatory version of the product has been submitted.

Products that have already undergone a stringent regulatory assessment by certain national authorities may be eligible for an abbreviated WHO prequalification assessment. If so, submission of a product dossier for WHO review is not

<sup>1</sup> World Health Organization, Essential Medicines and Health Products, Geneva

required. However, abbreviated WHO prequalification assessment still requires the conduct of the laboratory evaluation and a shortened inspection that leverages the findings of previous inspections.

**Fig. 8.2A. Overview of the WHO prequalification abbreviated assessment procedure**



Source: WHO, 2014 (1)

### Product dossier review

A product dossier contains documentation and data to demonstrate that the IVD conforms to the essential principles of safety and performance of medical devices. Guidance issued from the International Medical Device Regulators Forum (IMDRF) (formerly the Global Harmonization Task Force) is considered the international best practice related to regulation of medical devices, including IVDs. Other standards and guidance issued by the International Organization for Standardization (ISO) and Clinical and Laboratory Standards Institute (CLSI) can provide specific information on specialized areas such as stability testing for IVDs. The product dossier requested by WHO should be submitted according to the Instructions for compilation of a product dossier ([http://www.who.int/diagnostics\\_laboratory/evaluations/PQDxInfo/en/](http://www.who.int/diagnostics_laboratory/evaluations/PQDxInfo/en/))

The WHO prequalification assessment reviews the performance and use of IVDs specifically from the perspective of WHO Member States, that is, stability, risk assessment and instructions for use. National regulatory authorities (NRAs) undertaking stringent review may not review these aspects in the same way. WHO rates any non-conformities, and the manufacturer is expected to file a corrective action plan that outlines how and when requirements will be met.

## Inspection of the site of manufacture

The WHO inspection schedule is divided into stages:

**Table 8.1A. Stages of the WHO inspection process for site(s) of manufacture**

| Stage                | Content  |
|----------------------|--|
| <b>Stage 1</b>       | Evaluation of readiness for inspection through desktop review of quality documentation or a brief on-site inspection.  |
| <b>Stage 2</b>       | Initial full on-site inspection to determine implementation of the quality management system, facility and warehousing, competence of staff, critical suppliers including outsourced activities, internal audit and management commitment or review. Also, with dossier assessor attends the inspection to confirm aspects of the dossier. |
| <b>Follow-up</b>     | Confirmation of implementation of the corrective action plan submitted in response to Stage 2 inspection; may or may not require another on-site inspection.   |
| <b>Surveillance</b>  | Risk-based; at least annual reporting required.  |
| <b>Re-inspection</b> | Risk-based; conducted after 3–5 years holding WHO prequalification.  |

All sites inspected must meet prequalification requirements and must demonstrate:

- (1) a fully implemented quality management system (design, development and manufacturing including quality control, storage and distribution)
- (2) risk management meeting ISO 14971 requirements
- (3) product stability
- (4) routine manufacturing
- (5) sufficient capacity to ensure reliable delivery.

## Independent laboratory evaluation of performance and operational characteristics

The WHO laboratory evaluations are comparative in nature; each assay under assessment is tested on the same worldwide-sourced clinical specimen reference panel and commercially acquired panels for seroconversion and low antibody and antigen titers. These evaluations aim to assess technical and performance characteristics such as sensitivity and specificity, as well as seroconversion and low titer sensitivity relative to those of other assays of similar format.

Manufacturers send one or two production lots to WHO Collaborating Centres or other laboratories designated by WHO to conduct the evaluation testing. WHO issues a technical report of the performance and operational characteristics and determines if the assay meets WHO prequalification requirements. Specific minimum acceptance criteria are applied to each assay format (Table 8.2A).

**Table 8.1A. Minimum acceptable performance for serology assays for WHO prequalification**

| Characteristic                  | RDT  | EIA  |
|---------------------------------|------|------|
| <b>Sensitivity</b>              | ≥99% | 100% |
| <b>Specificity</b>              | ≥98% | ≥98% |
| <b>Inter-reader variability</b> | ≤5%  | N/A  |
| <b>Invalid rate</b>             | ≤5%  | ≤5%  |

EIA: enzyme immunoassay; RDT: rapid diagnostic test.

Source: List of diagnostics eligible to tender for procurement by WHO in 2014 (including WHO prequalified diagnostics) [http://www.who.int/diagnostics\\_laboratory/procurement/purchase/en/](http://www.who.int/diagnostics_laboratory/procurement/purchase/en/)

In addition to performance, certain operational characteristics that make assays suitable for resource-limited settings are evaluated (Table 8.3A).

**Table 8.2A. WHO prequalification operational characteristics and examples for RDTs and EIAs**

| Operational characteristic   | RDT                                 | EIA   |
|--|-------------------------------------|---|
| <b>Items required but not provided with test kits (consumables, equipment)</b> | Lancets, alcohol swabs, cotton wool | Deionized water, reagent troughs, washer, incubator, reader |
| <b>Time to first result</b>  | 3 minutes, 15 minutes               | 2 hours   |
| <b>Throughput per operator per hour</b>  | 20                                  | 90  |
| <b>Stability/shelf life for storage</b>  | 2 to 30 °C                          | 2 to 8 °C   |
| <b>In-use stability, including at room temperature</b>                         | Use immediately                     | Up to 4 weeks   |
| <b>Specific specimen preparation steps</b>                                     | N/A                                 | 1 step  |
| <b>Number of steps required to perform the test procedure</b>                  | 1 step, 3 steps                     | 4 steps   |

EIA: enzyme immunoassay; RDT: rapid diagnostic test.

Source: WHO 2015 (2) [http://www.who.int/diagnostics\\_laboratory..](http://www.who.int/diagnostics_laboratory..)

### Final prequalification decision

When the prequalification decision has been made, WHO issues a public report, and the product is added to the list of WHO prequalified products. It is, therefore, eligible for WHO and UN procurement. In the post-prequalification stage, the manufacturer is obliged to conduct post-market activities to continue to assure the quality, safety and performance of a WHO-prequalified IVD. The manufacturer is also obligated to notify WHO of any changes to the product or the quality management system, so that these may be evaluated to determine any implication for their listing as WHO-prequalified.

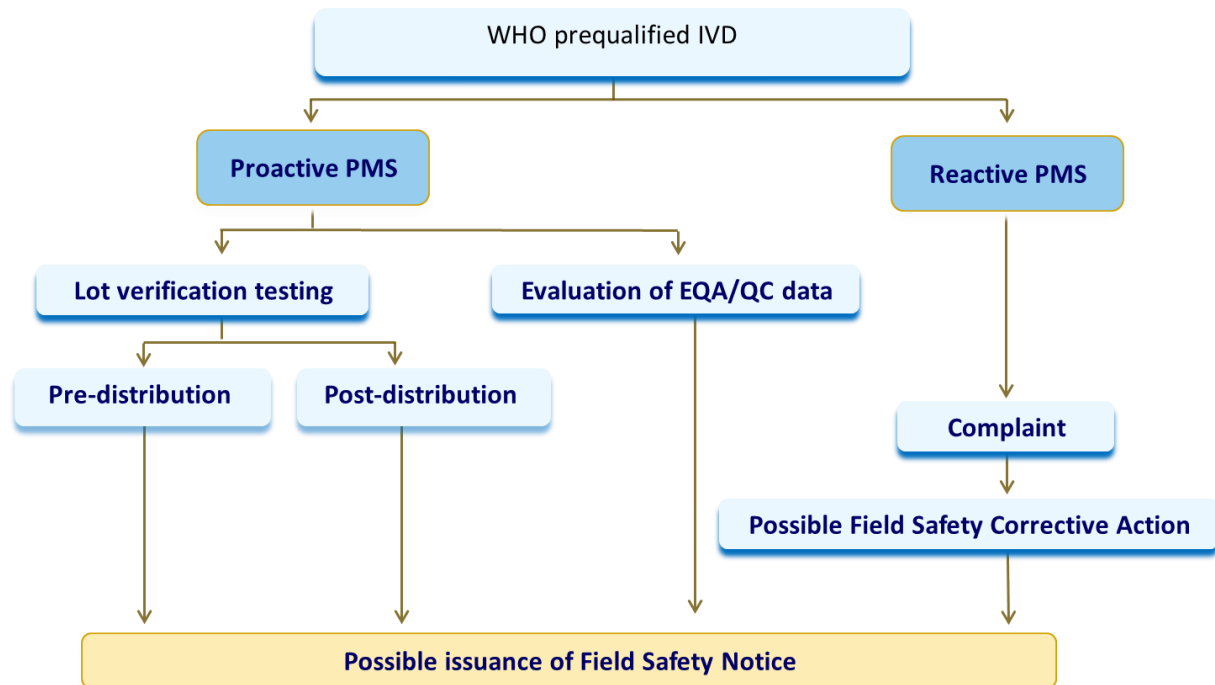
### Other regulatory agencies for in vitro diagnostics

The International Medical Device Regulators Forum (IMDRF) is working toward convergence of national regulatory standards for medical devices, including IVDs. Hence, regulatory reviews undertaken by different national authorities will become more harmonized, with increased efficiencies both for the reviewers and for the manufacturers. The predecessor of IMDRF, the Global Harmonization Task Force (GHTF), was comprised of representatives from both the regulatory authorities and from industry, of Australia, Canada, the European Community, Japan, and the USA. The IMDRF is comprised of only regulatory authorities, with representation from Australia (Therapeutic Goods Administration), Brazil (National Health Surveillance Agency), Canada (Health Canada), China (China Food and Drug Administration), the European Union (European Commission Directorate General Health and Consumer), Japan (Ministry of Health, Labour and Welfare), the Russian Federation (Roszdravnadzor) and the USA (Food and Drug Administration).

## 8.2. Post-market surveillance for in vitro diagnostics

The purpose of post-market surveillance is to protect individual health and public health through continued surveillance of IVDs once they are placed on the market. Post-market activities ensure that manufacturers are aware of any event that may affect the quality, safety or performance of their assay. Manufacturers must then evaluate and assess any residual risks and, as appropriate, take risk mitigation measures. Fig. 8.3A shows the components of post-market surveillance.

**Fig. 8.3A. Proactive and reactive measures for post-market surveillance of in vitro diagnostics**



EQA: external quality assessment; QC: quality control; IVD: in vitro diagnostic; PMS: post-market surveillance.

Source: WHO post-market surveillance of in vitro diagnostics, ([http://www.who.int/diagnostics\\_laboratory/postmarket/en/](http://www.who.int/diagnostics_laboratory/postmarket/en/))

A centralized collection of post-market data on WHO-prequalified IVDs enables coordinated action in WHO Member States and ensures traceability of information. These post-market data include results from pre-distribution and post-distribution lot verification testing as well as complaints and evaluation data. Regulators and users submit these post-market data to WHO in the form of lot testing reports and IVD complaint forms. National regulatory authorities, procurers and implementing partners, such as nongovernmental organizations, are notified of certain reports of adverse events through vigilance information exchange.

Other actions that WHO might take on post-market information include:

- post-market surveillance information exchange with national regulatory authorities
- post-market surveillance information exchange with manufacturers
- publishing post-market surveillance information on WHO's website
- additional surveillance of the IVD concerned
- removal of the product from the list of WHO-prequalified IVDs, if needed
- inspection of the manufacturing site to ensure that corrective or preventive action as a result of any complaint has been implemented.

WHO's Guidance for post-market surveillance of in vitro diagnostics provides further information. ([http://www.who.int/diagnostics\\_laboratory/postmarket/en/](http://www.who.int/diagnostics_laboratory/postmarket/en/))

### 8.3 Principles for preparation of quality control specimens for HIV RDTs

Quality control (QC) refers to a material or mechanism which, when used with or as part of an assay, monitors the analytical performance of that assay. It may monitor the entire assay or only one aspect of it.

**Test kit controls** supplied by the manufacturer (as positive and negative controls), or **internal quality controls** that are integrated within the assay (or test device) are typical forms of QC. However, increasingly internal QC for RDTs comprises of a control line that appears when any type of liquid is added, and therefore does not assure that adequate specimen has been added. Certain regulatory agencies have a requirement that test kit controls are supplied with RDTs supplied within their jurisdiction. However, these test kits controls often have different storage requirements and stability compared to the components of the assay, and thus are usually supplied separately.

In light of the above-mentioned issues with achieving optimal QC for RDTs, an **external quality control specimen** (a low antibody titer biological specimen) can be used to challenge the performance of the assay. In practice, these types of specimens are difficult to acquire as a naturally occurring specimen, and, so it is acceptable to use a biological specimen that has been diluted in a suitable matrix to mimic a low-reactive specimen (See Fig. 8.4A).

External QC specimens are made by taking a strongly reactive HIV-positive specimen and diluting in basematrix (defibrillated plasma) or normal human serum. The diluent should be safe and free of other analytes such as anti-HCV and HsBAg. It is preferable to make large volumes of external QC specimens and then store in small aliquots, until required.

#### Acceptance criteria

- The determination of acceptable QC results is made when the results of the external QC specimen are compared with the expected reference result (band/line intensity<sup>2</sup>).

#### Trend monitoring

- It is critical that QC results are recorded, analyzed and any trends identified that might require occurrence management. For more information on occurrence management see Chapter 8 on the Consolidated Guidelines of HIV testing services (<http://www.who.int/hiv/pub/guidelines/hiv-testing-services>).

Any test kit controls should be run according to the manufacturer's instructions for use, and **external QC specimens should be run:**

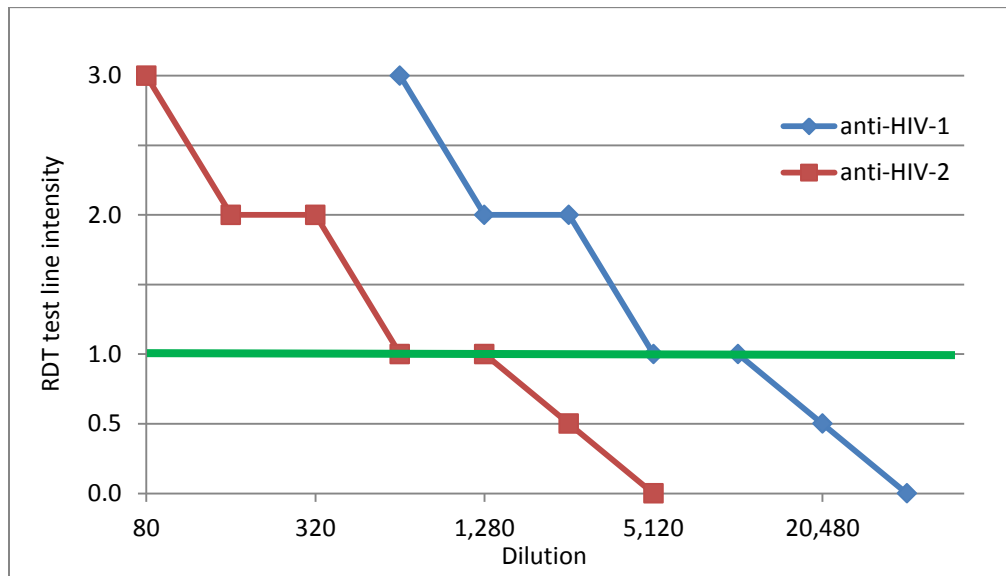
- once weekly, preferably at the beginning of the week
- for any new operator (including trained staff members who have not conducted testing for some time)
- for each new lot of test kits
- for each new shipment of test kits
- when any environmental conditions (for example, temperature and humidity) fall outside the range recommended by the manufacturer.

WHO is developing specific guidance on the preparation of quality control specimens for RDTs.

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<sup>2</sup> As the external QC specimen should be a low-reactive specimen, it is suggested to record the band/line intensity for the QC specimen.

**Fig. 8.4A. Dilution sensitivity of two biological specimens (anti-HIV-1 and anti-HIV-2) on an anti-HIV-1/2 RDT**



Source: WHO, 2015 post market surveillance, ([http://www.who.int/diagnostics\\_laboratory/postmarket/en](http://www.who.int/diagnostics_laboratory/postmarket/en)).

## 8.4 Generic job aid for an HIV RDT

Wonderful HIV-1/2 RDT<sup>3</sup> is an immunochromatographic rapid diagnostic test for the discriminatory detection of HIV-1 and HIV-2 antibodies in human serum or plasma and capillary or venous whole blood specimens.

### Key information

Shelf life: 18 months

Storage conditions: 2–30 °C

Volume of specimen needed:

25 µL (serum/plasma);

50 µL (whole blood)

Time to test one specimen:

16 minutes

### Equipment required but not supplied

- personal protection equipment, such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venous blood collection: venepuncture apparatus and appropriate blood collection tubes.

### Test procedure

- 1) Remove the test device from the protective foil pouch.
- 2) Label the test device with a specimen ID number.
- 3a) For serum or plasma specimens:
  - a. Using the pipette provided in the test kit, apply 1 drop (25 µl) of specimen to the specimen well (S).
  - b. Read results at 15 minutes. Do not read after 30 minutes.
- 3b) For venous whole blood (venepuncture) specimens:
  - a. Using the dropper provided within the test kit, apply 2 drops (50 µl) of specimen to the specimen well (S).
  - b. Then add 2 drops (50 µl) of buffer to the same specimen well (S).
  - c. Read results at 15 minutes. Do not read after 30 minutes.
- 3c) For capillary whole blood (finger-stick) specimens:
  - a. Clean the fingertip with an alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first drop of blood with cotton wool.
  - b. Using the capillary tube, take the specimen until the fill line. Apply 50 µl of the specimen to the specimen well (S).
  - c. Then add 2 drops (50 µl) of buffer to the same specimen well (S).
  - d. Read results at 15 minutes. Do not read after 30 minutes.
- 4) Interpret results as follows:

**Reactive for HIV-1 antibodies:** Two coloured lines appear, one in the control region “C” and one in the test region “T1”.

**Reactive for HIV-2 antibodies:** Two coloured lines appear, one in the control region “C” and one in the test region “T2”.

**Reactive for HIV-1/2 antibodies:** Three coloured lines appear, one in the control region “C” and one each in test regions “T1” and “T2”.

**Non-reactive for HIV-1 & HIV-2:** One coloured line appears in the control region “C”. No coloured lines appear in either of the test regions “T1” or “T2”.

**Invalid:** No control line appears in the control region “C”, irrespective of whether coloured lines appear in either of the test regions “T1” or “T2”; OR high background precludes reading of the test and control lines; OR the specimen is not seen migrate along the test strip.

<sup>3</sup> This is a fictional product.



## 8.5 Principles for preparation of external quality assessment schemes

External quality assessment is an inter-laboratory comparison to determine if the HIV testing service can provide the correct HIV status. EQA assures that assays are performed accurately, results are reproducible, and errors are detected and corrected to avoid misdiagnosis (or misclassification).

EQA may take the form of an **observational visit**, usually by the site supervisor, that observes testing practices. The supervisor should be trained and competent in the principles of quality management and testing practices. These visits should occur at least annually but preferably every three to six months.

EQA also takes the form of **participation in an external quality assessment scheme (EQAS)**, also known as proficiency testing programmes, whereby a panel of blinded specimens is sent to a testing site and is tested according to the usual procedures. The final HIV status assigned (and test results) is reported back to the EQAS provider for analysis.

EQAS panels can be serum/plasma specimens that are clinically collected, however large volumes of specimens are necessary which is impractical for most programmes. An alternative is to use the dried tube specimen (DTS) methodology which allows for sera (mixed with green dye so the pellet can be visualized) to be allowed to dry in the bottom of the tube. (3) This approach provides a practical means to prepare specimens for distribution to outlying laboratories and testing services.

For additional detailed information on how to prepare DTS panels, refer to Parekh BS, Anyanwu J, Patel H, Downer M, Kalou M, Gichimu C, Keipkerich BS, Clement N, Omondi M, Mayer O, Ou CY, Nkengasong JN. *Dried tube specimens: a simple and cost-effective method for preparation of HIV proficiency testing panels and quality control materials for use in resource-limited settings*. J Virol Methods. 2010 Feb;163(2):295-300. doi: 10.1016/j.jviromet.2009.10.013. Epub 2009 Oct 28.

It is crucial that any set of activities for EQA includes the provision for follow-up of any unacceptable EQA results with corrective actions.

National reference laboratories should participate in international EQAS **at least twice per year**. At the same time, national laboratories should aim to conduct national EQAS for all testing services using locally derived and prepared DTS panels.

WHO is preparing specific guidance on this topic.

EQA providers that ship worldwide, including to resource-limited settings:

- College of American Pathologists, USA  
<http://www.cap.org/web/home/lab/proficiency-testing....>
- National Institute of Communicable Diseases, South Africa  
<http://www.nicd.ac.za/>
- National Serology Reference Laboratory, Australia  
<http://www.nrl.gov.au/Our+Services/NRL+Quality+Assurance>
- Réseaux Africain de Recherche sur le Sida, Laboratoire de Virologie Bactériologie, Senegal  
<http://www.rarslbv.org/>
- Royal Australian College of Pathologists  
<http://www.rcpaqap.com.au/serology/>
- UK National External Quality Assessment Scheme  
<http://www.ukneqas.org.uk/content...>
- Zimbabwe National Quality Assurance Programme  
<http://www.zinqap.org.zw/>

## References

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2. *IVD Assays: Laboratory performance and other operational characteristics rapid diagnostic tests (Combined detection of HIV-1/2 antibodies and discriminatory detection of HIV-1 and HIV-2 antibodies: Report 18*. Geneva: World Health Organization; 2015.
3. Parekh B, Kalou M, Alemnji G, Ou C, Gershby-Damet G, Nkengasong J. Scaling up HIV rapid testing in developing countries: comprehensive approach for implementing quality assurance. *Am J Clin Pathol*. 2010;134(4):573-84.