****

# Quality assurance protocol for dried blood spots

# Standard operating procedure 3.1

Subtitle: Laboratory protocol for HIV ELISA testing using Abbott Murex 1.2.O test kit

Protocol ID: 3.1

Date: March 2012

Created by: Pieter Smit, Thomas van der Vlis

Email address: [Pieter.smit@lshmt.ac.uk](mailto:Pieter.smit@lshmt.ac.uk)

**Purpose of protocol:**

HIV ELISA Murex test kit 1.2.O from Abbott, an enzyme immune assay for the detection of antibodies to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and 2 (HIV-2) in human serum or plasma, is used for the detection of HIV-1 & 2 on DBS samples.

This protocol describes in detail the testing procedures when using DBS samples. The procedure is divided into two stages. The first stage explains the creation of a masterplate which needs to be incubated overnight. The second stage describes the ELISA test kit protocol using the masterplate as sample material. Test result interpretation is quantitative.

HIV testing should be performed after DBS card quality evaluation (protocol ID 2.0). Printed 96 wells templates are needed to register test results. If bar-coded stickers on the DBS cards are used the printed template might be exchanged for an excel using a bar-code scanner device. The template to be printed can be found at the global health diagnostic website.

C:\Users\Wim\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.IE5\7X5SF0Y0\MC900293214[1].wmf

HEALTH AND SAFETY INFORMATION

CAUTION:

**You are working with potentially infective materials. Read the manufactures manual for safety regulation.**

**Procedure (1):**

**Materials needed:**

- Specimen DBS cards + empty DBS

- Punch machine or Punchers

- Disposable gloves

- Disposable tips

- Pipette’s (multi-pipette)

- PBS buffer (96\*200=19.2ml for 1 plate)\*

- 96 flat wells plate + cover

- Printed 96wells template

- Plate shaker

*\* see page 4 (bottom) for detailed PBS-buffer requirements.*

**Operations:**

* Collect (sample) DBS cards
* Set up 96 flat wells plate (number the plate for reference)
* Leave well A1 to E1 empty for controls
* Punch blank DBS in F1 for negative control
* Punch samples into wells starting at G1 followed by H1 etc
* For each punch, write down card-number on printed template
* For each punch, write down what punch (number) you used in small square (optional)
* Add **200µl of PBS buffer** to each well (except A1 to E1)
* Tap the plate from the side (3x), the spots will turn
* Shake plate for ~30sec on a shaker
* Give the 96 wells plate a number or reference code. Write this number on the template as well
* Cover wells and place in fridge overnight to elute (4 degrees Celsius)

This is your master plate.

Do not store the plate longer than 2 days (at 4 degrees Celsius)

**Procedure (2):**

Murex HIV-1.2.O test (following within 2 days after creating master plate).

**Materials needed:**

- Timer

- Disposable gloves

- Disposable tips

- Pipette’s (multi channel)

**Pre-test**

- Prepare reagents 30 min in advance

- Bring reagents to room temp.

- Bring master plate to room temp

- Bring incubator to 37°C

- Turn on plate reader

- Prepare conjugate diluents\*

- Prepare substrate solution\*

- Prepare wash fluid\*

- Prepare stop solution\*

*\*see page 3-4 of this SOP*

**Operations:**

* Add **50µl of sample diluents** to **control wells** A1-E1
* Add **25µl of sample diluents** to each well that corresponds with a filled well of the masterplate (including G1 and H1)
* Using a multichannel pipette, mix DBS elution (from masterplate) 4-5 times and transfer **75µl** to the test plate and mix well
* Add **50µl** of controls to designated wells (A1-C1 = neg. control; D1=pos. contr-1; E1=pos. contr-2)
* Cover plate and incubate for **30 min at 37**°**C**
* Wash with washer program (washing requirements as stated in manufacturers manual); after washing hit the plate on filter paper (up-side down) and pat dry. Don’t let the wells dry out during the procedure
* Immediately after washing **add 50µl of conjugate** to each well
* Cover plate and incubate **30 min at 37**°**C**
* Wash and pat dry
* Immediately after washing **add 100µl substrate solution**
* Cover plate and incubate for **30 min at 37**°**C**
* Make sure there are no bubbles on the surface (you can pop them with pipette tip)
* **Add 50µl stop solution** to each well
* Tap the plate a few times to mix the stop solution
* Put plate in plate reader, read plate and save file.

***HIV ELISA preparing solutions***

Conjugaat diluents

Tap the bottle gently on the bench to remove any material adhering to the rubber stopper. Pour the whole contents of a bottle of conjugaat diluent into a bottle of conjugaat. Recap the latter and mix by gentle inversion. Allow to rehydrate for at least 30min. with occasional swirling. The color will be red

After reconstituting the conjugaat may be stored at 2-8°C for up to 8 weeks.

Substrate solution

To prepare substrate solution, add a volume of colorless substrate diluents to an equal volume of pink substrate concentrate in either a clean glass or plastic vessel. It is important that this order of addition is followed and that any pipettes and glassware used are clean.

Alternatively the substrate solution may be made by pouring the entire content of the bottle of substrate diluents into the bottle of substrate concentrate. (only stable for 2 days)

Color should be pink, if purple before use it should be discarded. Keep away from sunlight

It is stable refrigerated for up to 2 days, but must be discarded if crystals have formed.

**For a full 96wells plate you need to mix 6ml substrate diluents with 6ml substrate concentrate**.

Washfluid

Add one volume of wash fluid concentrate to 19volumes of distilled or deionised water.

125ml + (19\*125ml)= 2500ml wash fluid

62.5ml wash fluid concentrate to 1187.5ml deinosed water = 1250ml

Washfluid can be stored at 18-30c in a closed vessel under which it will retain activity for 4 weeks. It may develop a yellow color, but this will have no effect.

For one plate you will approximately need 600ml of washing fluid. There is more than enough wash solution.

PBS-buffer

Needed: PBS buffer with 0.05% tween20

Example:

80ml PBS

40µl tween

Use ‘positive displacement’ pipette tips to add the tween20 to the PBS. This is a very thick soap, and normal tips will not work.

Stop solution

0.5M to 2M sulphuric acid

Add between 3ml (for 0.5M) and 11ml (for 2.0M) of analytic grade concentrated sulphuric acid (18.0M) to about 80 ml of distilled or deionised water and then make up to 100ml with more water.

6ml sulphuric acid mixed with 80ml deionised water and then topped to 100ml with water. Total of 100ml stop solution. (using 5,6 ml sulphuric acid 95-97% to 100ml forms 1M) about 1 molair conc stop solution.

Stop solution can be stored for long periods of time.

**Test interpretation:**

*CALCULATION OF RESULTS*

Each plate must be considered separately when calculating and interpreting results of the assay. Approved software may be used for calculation and interpretation of results.

Negative Control

Calculate the mean absorbance of the Negative Controls.

Example:

Well 1 = 0.084, Well 2 = 0.086, Well 3 = 0.070

Total = 0.240

Mean Negative Control = 0.240/3

= 0.080

If one of the Negative Control Wells has an absorbance more than 0.15 OD. above the mean of all three, discard that value and calculate the new Negative Control mean from two remaining replicates.

Cut-off value

Calculate the Cut-off value by adding 0.2 to the mean of the Negative Control replicates (see above).

Mean Negative Control = 0.080

Cut-off value = 0.080 + 0.200 = 0.280

*QUALITY CONTROL*

Results of an assay are valid if the following criteria for the controls are met:

Negative Control

The mean absorbance must be less than 0.3.

Positive Controls

The absorbance of each of the Positive Controls should be more than 0.8 above the mean absorbance of the Negative Control. Assays which do not meet these criteria should be repeated. In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

*INTERPRETATION OF RESULTS*

Non-reactive Results  
Samples giving an absorbance less than the Cut-off value are considered negative in the assay.

Reactive Results

Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay (see Limitations of the Procedure). Such samples should be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate retests are considered repeatedly reactive in Murex HIV-1.2.O and are presumed to contain antibodies to HIV-1 or HIV-2. Such samples should be further investigated and the presence of antibodies against HIV confirmed by other tests. Samples that are non-reactive i.e. with an absorbance less than that of the cut-off value should be considered non-reactive for HIV antibodies.

No sample addition

Absorbance values significantly higher than the Negative Control may be obtained in wells where the sample has been omitted but all the reagents have been added.

## Limitations of Procedure

1. Alterations in the physical appearance of test kit materials may indicate instability or deterioration.
2. Assay which detect antigen only are not replaced by this kit.
3. All highly sensitive assays have the potential for non-specific reactions.
4. This protocol is established and validated within our laboratory setting. A test validation within your laboratory setting is highly recommended.