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# Quality assurance protocol for dried blood spots

# Standard operating procedure 3.2

Subtitle: Laboratory protocol for HIV ELISA using Vironostika HIV Uni-Form II Ag/Ab test kit

Protocol ID: 3.2

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**Purpose of protocol:**

The Vironostika HIV Uni-Form II Ag/Ab procedure is based on a one step sandwich principle. The Microelisa wells are coated with an optimal mixture of HIV-1 gp160, HIV-1 ANT70 (Group O), HIV-2 gp36 and monoclonal anti-p24 (HIV-1) antibody. These components and their concentrations have been carefully selected for maximum assay sensitivity.

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 Vironoska 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 template 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.

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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 3 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):**

**Materials needed:**

- Timer

- Disposable gloves

- Disposable tips

- Pipette’s (multi channel)

**Pre-test**

- Bring reagents to room temp (30 min.)

- Bring master plate to room temp

- Bring incubator to 37°C

- Turn on plate reader

- Prepare wash fluid\*

- Prepare stop solution\*

*\*see page 3 of this SOP*

**Operations:**

1. Use only a required number of ELISA strips to minimize wastage.
2. Using a multichannel pipette, dispense **75μl** of specimen diluent into all wells except control wells. In control wells dispense 1**00μ**l of diluent.
3. Using a multichannel to transfer **75μl** of DBS eluates into corresponding wells. Transfer the DBS eluates in column-wise sequence.
4. Pipette **50μl** Negative, Antibody Positive and Antigen Positive controls in respective wells. Seal plate with sealers provided in the kit.
5. Tap the plate gently to mix and incubate at **37oC for 1hr**.
6. Wash six times with a soaking interval of 1min between each washing step.
7. Tap the plate upside down on a filtrate paper to remove any trace of liquid in the wells.
8. During washing, prepare **TMB substrate** by mixing equal parts of **TMB solution** and **Urea peroxidase** solution. For 1 plate mix 6 ml of each of the two.
9. Dispense **100μl** of TMB substrate into each well. Seal the plate.
10. Incubate the plate at room temperature in **darkness for 30 minutes**.
11. Stop reaction by adding **100μl** of 1mol H2SO4.
12. Blank ELISA reader on air and read at **450nm** within 15 minutes after stopping reaction.

**Test interpretation:**

## Assay validation and scoring of test results

Each assay will be validated and scored for reactivity by computer using an in-built program **OR** manually according to instructions in the kit insert.

## Test Validity

The test is valid if

1. The Negative control (NCX) <0.250
2. Positive control 1 (PC1) – NCx ≥0.600
3. PC2 - NCx ≥0.600
4. PC3 - NCx ≥0.400

**Interpretation of results**

## Cut-off value

NCx + 0.100

**Final Result**

X< NCx + 0.100 = Negative

X>NCx + 0.100) = Positive

## 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.

## Performance Characteristics

5205 serum and EDTA plasma samples were tested and the ELISA showed a specificity of 99.96%.

## Reference

1. See kit insert.
2. HIV testing strategy SOP

**Preparation of buffers:**

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.