Performing a Qualitative HIV ELISA assay with the Thermo Scientific Multiskan FC Microplate Photometer

Abstract
This application note describes how to perform a qualitative ELISA for HIV antibodies with the Thermo Scientific Multiskan FC microplate photometer and an Ani Labsystems HIV Enzyme Immunoassay (EIA) kit.

The assay can easily and reliably be performed with the Multiskan® FC using either the PC (external) or internal software.

Introduction
ELISA is the most commonly used type of test to screen for HIV infection. As a method, it is relatively simple, highly sensitive and suitable for testing large numbers of samples.

The in vitro enzyme immunoassay kit from Ani Labsystems Ltd. Oy detects HIV antibodies in human serum or plasma.

The assay is an indirect solid-phase enzyme immunoassay with horseradish peroxidase as the marker enzyme.

The patient serum is pipetted into the coated microplate well. If present in patient serum, the HIV antibodies combines with HIV peptides attached to the polystyrene surface of wells. Residual of the patient sample is removed by washing, and horseradish peroxidase conjugated anti-human IgG (sheep) is added. The wells are then washed again, and a colorless enzyme substrate (H₂O₂) and chromogen (TMB) are added. The enzyme reaction of the chromogen/substrate produces a colored end product (Figure 1).

The enzyme-chromogen/substrate reaction is terminated with acid (H₂SO₄). The absorbance is measured immediately at 450 nm. The color intensity is directly related to the concentration of HIV antibodies in a patient sample.
Materials and Methods

- A solid-phase enzyme immunoassay for the detection of antibodies to HIV in human serum or plasma. (Ani Labsystems Ltd. Oy, Product no. 61 11 011)
- Thermo Scientific Multiskan FC (Product no. 51119000 or 51119100)
- Photometric filter 450 nm (factory installed in Multiskan FC)
- Thermo Scientific Finnpipette F1 (Product no. 4641070 Single Channel, 4661030 Multichannel)
- Thermo Scientific Wellwash 4 Mk 2 (Product no. 5160770)
- Thermo Scientific iEMS Incubator/Shaker (Product no. 5112200)

The Multiskan FC system can be operated by either keypad (internal software) or PC. Software is provided for both options together with the instrument, and this note describes both methods of operation.

The air blank measurement will automatically be performed during the assay.

The negative control was assayed as a single sample, the positive control 2 in duplicate, and the positive control 1 in triplicate.

Unknown samples were prepared by spiking the sample diluent with the positive controls 1 and 2.

For user convenience, the protocols for this and also other assays can be downloaded from www.thermo.com/readingroom

If downloaded, the layout will need to be modified according to the current layout and the filter.

<table>
<thead>
<tr>
<th>Control rule</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent blank</td>
<td>≤ 0.10</td>
</tr>
<tr>
<td>Negative control</td>
<td>≤ 0.15 x</td>
</tr>
<tr>
<td>Positive control 1</td>
<td>0.70 ≤ Apc1 &lt; 2.00 x</td>
</tr>
<tr>
<td>Positive control 2</td>
<td>&gt; 0.50 x</td>
</tr>
</tbody>
</table>

Table 1. The quality control rules for Anilabsystems HIV EIA. x that appear on the table indicate that the absorbance of the reagent blank should have been subtracted from these values before the comparison. Apc1 stands for mean absorbance of the positive control 1.

As one of these control rules uses raw data and the others use blank subtracted data as the source, two different QC steps must be added to the result tree (Figure 3).
Multiskan FC Internal Software (keypad and display)

A new protocol can be created by selecting File/New or changing the parameters of the current protocol and selecting Save as.

Main view

In the measurement parameters, select 450 nm as filter 1 and the measurement mode Normal.

Processing

Create the layout according to the setup on the assay plate. For example, as in (Figure 5).

![Diagram of assay layout](image)

When performed via the internal software, the negative control is coded as CTRL 1, the positive control 1 as CTRL 2 and the positive control 2 as CTRL 3.

The qualitative classification is added by enabling Interpretation and determining the blanked value of Control 2*0.3 as limit 1.

![Qualitative classification interface](image)

The quality control is enabled by selecting Yes in the Quality Control step.

In the internal software, the check is added by enabling quality control and adding the rules (Table 1):

1. Sample: Blank/raw, operator: <, constant: 0.100
2. Sample: POS1_0001 < 2.00
3. Samples: POS2_0001 ≥ 0.50

The three other rules are added to the Quality Control step under the Blank Subtraction step in the result tree. This means that the rules automatically use blank subtracted data as source data. Add the following rules:

1. Samples: NEG_0001 ≤ 0.15
2. Samples: 0.7 ≤ POS1_0001 < 2.00
3. Samples: POS2_0001 ≥ 0.50

This is how the formulas appear with the default names. It is possible to change the name of the measurement step, calibrators, etc. The rule should be made according to the current assay naming convention. If these rules are not followed, the software gives a warning to alert the user to check the assay results and retest the specimens.

Qualitative classification

The classification is made by adding a Qualitative Classification step to the result tree. The two categories and the limit to separate them from each other are added to the step: Sample: POS1*0.3. The names of the categories can, for example, be Negative and Positive (Figure 4). Save the session.
3. Sample: CTRL2/blanked, operator: > , constant: 0.7

4. Sample: CTRL2/blanked, operator: < , constant: 2.0

5. Sample: CTRL3/blanked, operator: > , constant: 0.5

Note: Only four rules per protocol can be added via the internal software control, and therefore one of the QC criteria must be followed separately.

If these rules are not followed, the software gives a warning and the user should check the assay results and repeat the test.

The absorbance values of the Multiskan FC were compared to a reference instrument.

**Results**

The absorbance of each sample is compared to the absorbance value of control 2*0.3. If the absorbance of the sample is higher than the limit, the sample is considered to be positive for HIV antibodies. If the absorbance of the sample is lower than the limit, the sample is considered to be negative for HIV antibodies.

Both of the softwares calculate and report the interpretation of each sample.

When controlled via a PC, SkanIt Software color coding can be used to show the interpretation results more visually (Figure 7).

The results of the Multiskan FC photometer correlate very well with the reference instrument (Figure 8).

**Conclusion**

The Multiskan FC microplate photometer is an excellent tool for performing qualitative ELISA assays. The system offers the user all software and data processing parameters needed for easy and reliable result reporting. Assay performance can easily be controlled with the quality control features.

Ready-made assay protocols can be downloaded from www.thermo.com/readingroom

**Further Information**

For further information please refer to the following Web pages:
- www.thermo.com/readingroom
- www.thermo.com/mpi
- www.anilabsystems.com/products_idt_HIV.html

**Figure 8.** Correlation between the Multiskan FC system and the reference photometer ($y = 1.0016x - 0.0005$, the calculated correlation factor was over 0.999)

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