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

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Key words

- HIV
- Qualitative ELISA
- Thermo Scientific Multiskan FC
- Microplate Photometer
- Thermo Scientific SkanIt Software

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 (H_2O_2) 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 (H_2SO_4). The absorbance is measured immediately at 450 nm. The color intensity is directly related to the concentration of HIV antibodies in a patient sample.

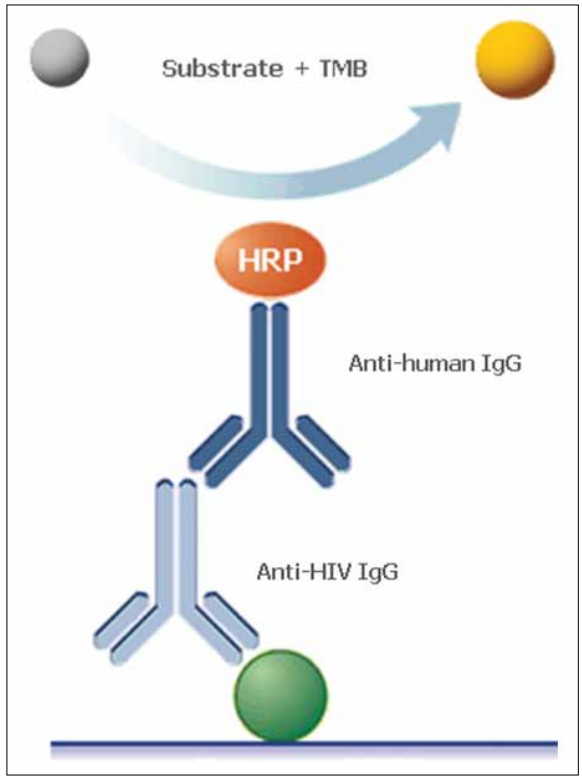

Figure 1. Anilabsystems HIV EIA assay visualization

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 information checked and/or changed according to the instrument configuration.

Thermo Scientific SkanIt Software for Multiskan FC

A new protocol can be created by selecting ***New session*** from the tool bar or from the Home view.

Layout

Create the layout according to the setup on the assay plate. For example, as in Figure 2.

Protocol

Add a Photometric measurement step with the measurement mode ***Normal*** and the ***450 nm*** filter.

Results

SkanIt® Software includes calculations for quantitative and qualitative classification and quality control. The quality control of the kit includes four rules (Table 1).

Control rule	Criteria
Reagent blank	≤ 0.10
Negative control	≤ 0.15 x)
Positive control 1	0.70 ≤ Apc1 < 2.00 x)
Positive control 2	> 0.50 x)

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).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank_Assay Assay	Un_0001 Assay	Un_0009 Assay	Un_0017 Assay	Un_0025 Assay	Un_0033 Assay	Un_0041 Assay	Un_0049 Assay	Un_0057 Assay	Un_0065 Assay	Un_0073 Assay	Un_0081 Assay
B	Blank_Assay Assay	Un_0002 Assay	Un_0010 Assay	Un_0018 Assay	Un_0026 Assay	Un_0034 Assay	Un_0042 Assay	Un_0050 Assay	Un_0058 Assay	Un_0066 Assay	Un_0074 Assay	Un_0082 Assay
C	NEG_0001 Assay 1	Un_0003 Assay	Un_0011 Assay	Un_0019 Assay	Un_0027 Assay	Un_0035 Assay	Un_0043 Assay	Un_0051 Assay	Un_0059 Assay	Un_0067 Assay	Un_0075 Assay	Un_0083 Assay
D	POS2_0001 Assay 1	Un_0004 Assay	Un_0012 Assay	Un_0020 Assay	Un_0028 Assay	Un_0036 Assay	Un_0044 Assay	Un_0052 Assay	Un_0060 Assay	Un_0068 Assay	Un_0076 Assay	Un_0084 Assay
E	POS2_0001 Assay 1	Un_0005 Assay	Un_0013 Assay	Un_0021 Assay	Un_0029 Assay	Un_0037 Assay	Un_0045 Assay	Un_0053 Assay	Un_0061 Assay	Un_0069 Assay	Un_0077 Assay	Un_0085 Assay
F	POS1_0001 Assay 1	Un_0006 Assay	Un_0014 Assay	Un_0022 Assay	Un_0030 Assay	Un_0038 Assay	Un_0046 Assay	Un_0054 Assay	Un_0062 Assay	Un_0070 Assay	Un_0078 Assay	Un_0086 Assay
G	POS1_0001 Assay 1	Un_0007 Assay	Un_0015 Assay	Un_0023 Assay	Un_0031 Assay	Un_0039 Assay	Un_0047 Assay	Un_0055 Assay	Un_0063 Assay	Un_0071 Assay	Un_0079 Assay	Un_0087 Assay
H	POS1_0001 Assay 1	Un_0008 Assay	Un_0016 Assay	Un_0024 Assay	Un_0032 Assay	Un_0040 Assay	Un_0048 Assay	Un_0056 Assay	Un_0064 Assay	Un_0072 Assay	Un_0080 Assay	Un_0088 Assay

Figure 2. Example of a layout created with SkanIt Software

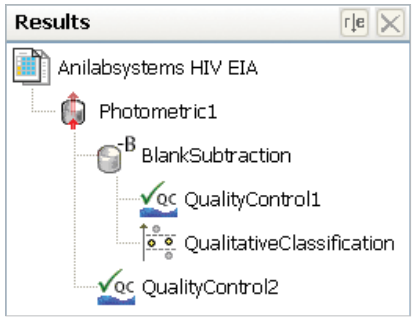

Figure 3. SkanIt Software result tree

Add a Quality Control step under the Photometric (raw data) step. Add the following rule:

1. Samples: Blank_assay ≤ 0.100

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.

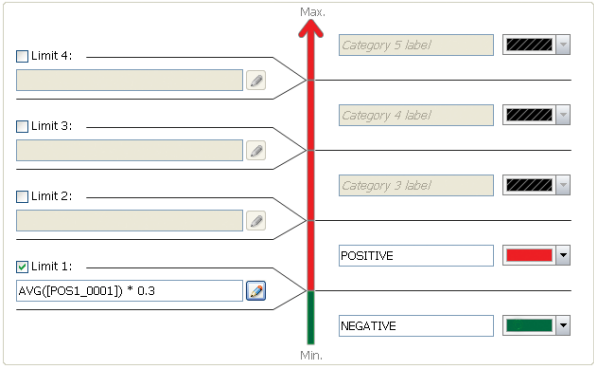

Figure 4. SkanIt Software qualitative classification

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).

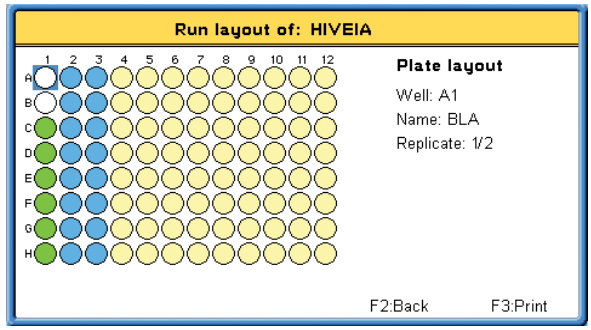

Figure 5. Internal software layout example

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.

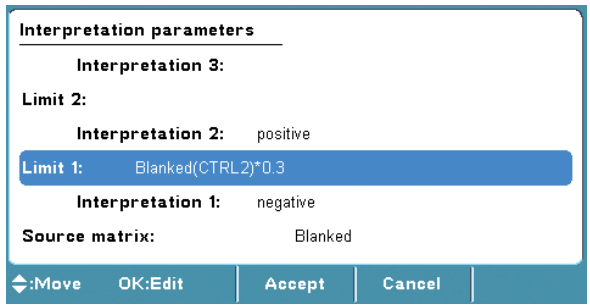

Figure 6. Qualitative classification in the internal software

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: CTRL1/blanked, operator: < , constant: 0.150

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).

Well	Sample	Category	Value
A02	Un_0001 1/2	POSITIVE	1.803
A03	Un_0001 2/2	POSITIVE	1.819
B02	Un_0002 1/2	POSITIVE	0.995
B03	Un_0002 2/2	POSITIVE	0.958
C02	Un_0003 1/2	POSITIVE	1.420
C03	Un_0003 2/2	POSITIVE	1.428
D02	Un_0004 1/2	NEGATIVE	0.309
D03	Un_0004 2/2	NEGATIVE	0.311
E02	Un_0005 1/2	POSITIVE	1.154
E03	Un_0005 2/2	POSITIVE	1.206
F02	Un_0006 1/2	NEGATIVE	0.564
F03	Un_0006 2/2	NEGATIVE	0.605
G02	Un_0007 1/2	NEGATIVE	0.461
G03	Un_0007 2/2	NEGATIVE	0.427
H02	Un_0008 1/2	POSITIVE	0.755
H03	Un_0008 2/2	POSITIVE	0.738

Figure 7. Example of a qualitative result set from the Anilabsystems HIV EIA assay with SkanIt Software

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

Conclusion

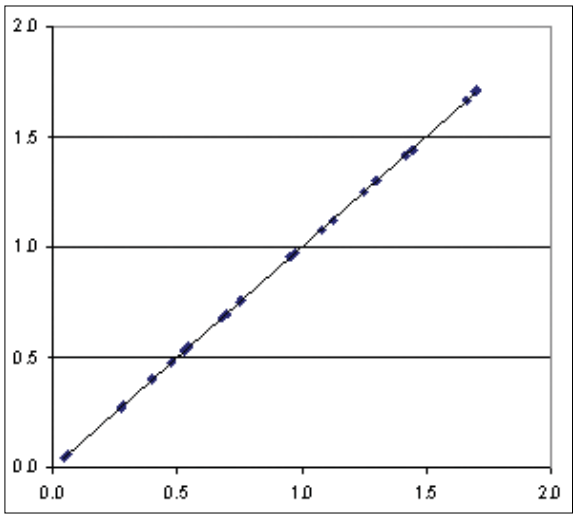

Figure 8. Correlation between the Multiskan FC system and the reference photometer (y = 1.0016x - 0.0005; the calculated correlation factor was over 0.999)

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

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