

ЛАБОРАТОРНА ДІАГНОСТИКА

1(35)/2006

**EVALUATION OF SENSITIVITY OF DIA-HIV ENZYME
IMMUNOASSAY FOR THE DETECTION OF ANTIBODIES TO HUMAN
IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 (HIV-1 AND HIV-2)
MANUFACTURED BY JSC “DIAPROPH MED”**

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The scientific production company “Diaproph Med” represents here the results of sensitivity evaluation of the test-system „DIA-HIV 1/2” intended for the detection of antibodies to human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2). The study was based on the agreement between the JSC “Diaproph Med” and the Testing Laboratory of the Paul Ehrlich Institute (PEI).

The objectives of this test-system evaluation were as follows: 1) to evaluate the seroconversion sensitivity of the „DIA-HIV 1/2” compared to CE-marked anti-HIV ½ assays on 23 commercially available seroconversion panels; 2) to analyze whether the seroconversion sensitivity of „DIA-HIV 1/2” may meet the current state-of-the-art level according to Common Technical Specifications (CTS) of the Directive 98/79/EC; 3) to assess the sensitivity in hospitalized HIV-1 positive patients; 4) to determine the sensitivity of „DIA-HIV 1/2” against a range of available HIV-1 subtypes (panel WWR PBR303) including 3 in-house subtype O specimens; 5) to evaluate the „DIA-HIV 1/2” sensitivity in pre-selected HIV-2 positive specimens.

Materials and methods

Description of the „DIA-HIV 1/2” test-kit

Name of the product	DIA-HIV ½
Catalog No:	T-0106

Manufacturer:	JSC “Diaproph Med”, 35, Svetlitsky str., Kyiv, 04123, Ukraine
Sample volume:	30 мкл
Quantity of determinations/a kit	2 plates with 6 strips, each strip includes 16 wells
Lot No:	280305
Expiration date:	05/08/2006
Storage temperature:	2-8 °C
Principle of the method:	One-step sandwich ELISA variant
HIV-1 antigens:	Recombinant gp120, gp41, p24 i p17
HIV-2 antigens:	Recombinant gp36
КОН'югат:	Mixture of HIV-1 (env, gag) and HIV-2 (env) recombinant proteins
Substrate:	Tetramethylbenzidine (TMB)
Control samples:	Positive control (2 wells) Negative control (3 wells)
Cut-off value :	Mean value of negative controls is + 0.100

ELISA realization

Test-kits „DIA-HIV ½” of the same lot (№ 280305) were given by the JSC “Diaproph Med” for evaluation in the Testing Laboratory of the Paul-Ehrlich Institute (D-63225, Langen, Germany). The experiments were carried out according to the manufacturer’s instruction put into test package. The plates were washed using (Tecan M8/4R, type Columbus Plus, Tecan A-5082, Austria). The optical density (OD) values were evaluated by a Tecan reader (SLT Spectra Rainbow, Tecan A-5082, Austria).

OD/CO ratios ≥ 1.0 , are thought to be positive. OD/CO values between 0.9 and 0.99 are located in the grey zone („indeterminate” results). OD/CO values below 0.9 are interpreted as negative.

Description of panels used to evaluate the „DIA-HIV ½” test-kit sensitivity

32 commercially available seroconversional panels were used to evaluate the „DIA-HIV ½” seroconversional sensitivity. The panels were products of Zeptomatrix, Inc. (375 West Street, W. Bridgewater, MA 02379, USA) and Bioclinical Partners, Inc. (25 Kenwood Circle, Suite 14A, MA 02038, USA).

Results

The „DIA-HIV ½” seroconversional sensitivity was assessed using 15 „DIA-HIV ½” seroconversional panels (see Table 1).

Table 1. Study of seroconversional sensitivity of „DIA-HIV ½” test-kit using different seroconversional panels for detection of antibodies to HIV.

Panel name	Detected HIV-infected sera/total sera quantity of the panel	Day of anti-HIV antibody detection
BBI-PRB 927	2/5	28 th
BBI-PRB 932	4/9	27 th
BBI-PRB 939	9/9	89 th
BCP 6240	10/13	30 th
BCP 6243	9/10	33 th
BCP 6244	14/15	33 th
BCP 6247	10/10	30 th
BCP 6248	7/7	21 th
BCP 65389	7/8	21 th
BCP 65522	3/7	10 th
BCP 66575	10/11	32 th
BCP 67512	22/22	97 th
BCP 67996	12/12	92 th
BCP 68106	8/14	24 th
BCP 73695	7/9	124 th

The „DIA-HIV ½” seroconversional sensitivity was compared with this parameter of other 22 HIV-screening test-kits investigated at the Testing Laboratory of the PET (see Table 2).

Table 2: Detection of anti-HIV seroconversion panels by DIA-HIV 1/2 in comparison to 22 other HIV screening assays

Assay no.	PRB 929 3/7 ¹	PRB 930 1/4 ¹	PRB 954 6/7 ¹	PRB 956 4/5 ¹	BCP 6245 6/11 ¹	BCP 6246 13/21 ¹	BCP 9010 9/11 ¹	BCP 9021 14/17 ¹	BCP 9023 20/22 ¹	BCP 9030 14/16 ¹	No. Panels tested	False negative ²	Score ³	Rank
A	0	0	0	0	0	1	0	0	0	0	10	1	0,10	1
B	1	1	0	0	1	0	0	0	0	0	10	3	0,30	2
C	1	0	0	0	0	1	0	0	1	0	10	3	0,30	3
D	1	0	0	0	0	1	0	0	1	0	10	3	0,30	4
E	1	0	0	1	0	1	1	1	1	0	10	6	0,60	5
F	1	1	0	1	0	1	0	1	1	1	10	7	0,70	6
G	1	1	0	1	0	1	1	1	1	0	10	7	0,70	7
H	1	1	1	1	1	2	2	0	1	1	10	11	1,10	8
I	1	2	1	1	1	2	1	0	2	2	10	13	1,30	9
J	2	2	1	1	2	3	1	2	2	1	10	17	1,70	10
K	1	2	1	1	0	2	3	3	3	2	10	18	1,80	11
L	2	2	1	1	2	2	1	2	3	2	10	18	1,80	12
M	2	2	1	1	2	3	1	2	3	2	10	19	1,90	13
N	2	2	1	2	2	3	1	2	3	1	10	19	1,90	14
O	2	2	1	1	2	3	2	2	3	2	10	20	2,00	15
P	2	2	1	2	2	3	1	2	3	2	10	20	2,00	16
Q	2	2	1	2	3	3	1	2	3	2	10	21	2,10	17
R	2	2	1	2	2	2	3	2	3	2	10	21	2,10	18
S	3	2	1	1	2	3	2	2	3	2	10	21	2,10	19
DIA-HIV 1/2	3	2	1	1	2	3	2	3	3	2	10	22	2,20	20
T	2	2	2	2	2	3	1	3	3	2	10	22	2,20	21
U	3	2	n.d.	2	2	3	1	2	3	2	9	20	2,22	22
V	3	2	2	2	2	3	2	3	3	2	10	24	2,40	23

Notes:

¹ = 1st positive bleed by one of the tests out of total number of panel members

2 = number of missed donations (false negatives) compared to the most sensitive assay

3 = number of panels tested divided by the number of missed positive (false negatives)

n.d. = not done

Table 3: Day delay of DIA-HIV 1/2 in detection of anti-HIV-1 in seroconversion panels in comparison to 22 other HIV screening assays

Assay no.	PRB 929 3/7 ¹	PRB 930 1/4 ¹	PRB 954 6/7 ¹	PRB 956 4/5 ¹	BCP 6245 6/11 ¹	BCP 6246 13/21 ¹	BCP 9010 9/11 ¹	BCP 9021 14/17 ¹	BCP 9023 20/22 ¹	BCP 9030 14/16 ¹	No. panels tested	Total day delay	Average day delay	Rank
A	0	0	0	0	0	4	0	0	0	0	10	4	0,4	1
B	4	3	0	0	3	0	0	0	0	0	10	10	1,0	2
C	4	0	0	0	0	4	0	0	5	0	10	13	1,3	3
D	4	0	0	0	0	4	0	0	5	0	10	13	1,3	4
E	4	0	5	3	0	4	4	3	5	0	10	28	2,8	5
F	4	3	5	3	0	4	0	3	5	3	10	30	3,0	6
G	4	3	5	3	0	4	4	3	5	0	10	31	3,1	7
H	4	3	5	3	3	7	8	0	5	3	10	41	4,1	8
I	4	7	5	3	3	7	4	7	7	8	10	55	5,5	9
J	7	7	5	3	6	11	4	7	7	3	10	60	6,0	10
M	7	7	5	3	6	11	4	7	>7*	3	10	62	6,2	11
K	7	7	5	3	6	7	4	7	>7*	8	10	63	6,3	12
L	4	7	5	3	0	7	>8*	10	>7*	8	10	63	6,3	13
O	7	7	5	3	6	11	4	7	>7*	8	10	67	6,7	14
N	7	7	5	>3*	6	11	4	7	>7*	8	10	69	6,9	15
P	7	7	5	>3*	6	7	>8*	7	>7*	8	10	71	7,1	16
Q	7	7	5	>3*	10	11	4	7	7	8	10	71	7,1	17
R	7	7	5	3	6	11	8	7	>7*	8	10	71	7,1	18
S	7	7	5	>3*	6	11	4	10	>7*	8	10	72	7,2	19
T	11	7	5	3	6	11	8	7	>7*	8	10	75	7,5	20
U	11	7	n.d.	>3*	6	11	4	7	>7*	8	9	68	7,6	21
DIA-HIV 1/2	11	7	5	3	6	11	8	10	>7*	8	10	78	7,8	22
V	11	7	>5*	>3*	6	11	8	10	>7*	8	10	82	8,2	23

Notes:

¹ = 1st positive bleed by one of the tests out of total number of panel members

* kits failing to detect seroconversion over all successive bleeds of a particular panel were assigned two additional days

n.d. = not done

It is clear the results presented in the Tables 1, 2 and 3 suggest the „DIA-HIV ½” test-system was evaluated using 32 standard commercial sera according to the Common Technical Specifications described by the Directive 98/79/EC. The data obtained demonstrate the seroconversional sensitivity of „DIA-HIV ½” comparing with other EC-marked ELISA-based test-kits to meet the EC requirements for such devices.

The test-system „DIA-HIV ½” being produced by the JSC “Diaproph Med” scored 19th among all ELISA-based HIV-tests evaluated by the Testing Laboratory.

Hospitalized patients

109 fresh serum specimens from the routine HIV ambulance of the hospital in Frankfurt (Universitätsklinikum Frankfurt, Johann Wolfgang Goethe Universität, Frankfurt am Main) were analyzed. All samples were tested also by several other anti-HIV screening devices and confirmed by an HIV-1 immunoblot. All samples were correctly positive by „DIA-HIV ½”.

Anti-HIV antibody detection in HIV-1 subtype specific specimens

A commercially available HIV subtype panel, i.e. World Wide HIV Performance Panel (WWRB302, Zeptometrix, Inc.), was tested using a „DIA-HIV ½” kit. The panel comprises HIV samples from different geographical origin with the following HIV subtypes: A, B, C, D and G 4 members each; 3 E-type sera, subtype F one member. In addition, there were 3 HIV-2 sera. All HIV subtypes HIV-2 sera were correctly identified as positive (see Table 4).

Table 4. Anti-HIV antibody determination in patients' sera infected with HIV-1 different subtypes and HIV-2

NN	Member of the panel WWRB302	DIA-HIV ½", OD/cut-off values
1	WWRB302-01 Spain O	1,54
2	WWRB302-02 Ghana A	14,60
3	WWRB302-03 Ghana G	27,92
4	WWRB302-04 Ghana G	13,54
5	WWRB302-05 Ghana A	21,95
6	WWRB302-06 Ghana G	22,24
7	WWRB302-07 Ivory Coast HIV-2	8,42
8	WWRB302-08 Ivory Coast G	17,15
9	WWRB302-09 Ivory Coast A	13,78
10	WWRB302-10 Ivory Coast NEG	0,61
11	WWRB302-11 Mozambique HIV-2	4,32
12	WWRB302-12 Mozambique C	18,85
13	WWRB302-13 Uganda A	14,62
14	WWRB302-14 Uganda D	23,71
15	WWRB302-15 Uganda D	22,65
16	WWRB302-16 Uganda D	28,98
17	WWRB302-17 Uganda D	27,70
18	WWRB302-18 Uganda C	26,64
19	WWRB302-19 Zimbabwe C	20,81
20	WWRB302-20 Zimbabwe C	22,59
21	WWRB302-21 China B	23,65
22	WWRB302-22 Thailand E	22,22
23	WWRB302-23 Thailand E	18,06
24	WWRB302-24 Thailand E	14,79
25	WWRB302-25 India HIV-2	9,42
26	WWRB302-26 U.S.A. B	20,03
27	WWRB302-27 U.S.A. B/D	25,38
28	WWRB302-28 Argentina F	23,26
29	WWRB302-29 Argentina B	14,04
30	WWRB302-30 Argentina NEG	0,15

Detection of subtype O sera

Three different in-house available subtype O sera were tested with „DIA-HIV 1/2”. All three specimens were correctly recognized as positive.

Sensitivity in HIV-2 sera

Together with the 3 immunoblot-confirmed HIV-2 samples of the WWWRB302, 19 anti-HIV positive sera were tested with „DIA-HIV 1/2”. All those samples were determined correctly as positive.

Summary and Conclusion

The sensitivity of the “DIA-HIV 1/2” assay was evaluated in terms of seroconversion sensitivity, sensitivity on fresh HIV-1 positive samples from the routine practice and on HIV-2 samples. With respect to the seroconversion sensitivity „DIA-HIV 1/2” was compared with 23 CE-marked HIV screening immunoassays. „DIA-HIV 1/2” scored 19th among all HIV screening test-kits. Taking into consideration also our previous studies, „DIA-HIV 1/2” was shown to be superior to five CE-marked HIV assays. The sensitivity of „DIA-HIV 1/2” was further assessed by results obtained from (i) 109 HIV-1 positive sera from the routine practice in a hospital; (ii) 29 different HIV-1 subtypes including (iii) 5 subtype O samples and (iv) 19 HIV-2 samples. The test-system „DIA-HIV 1/2” detected all specimens correctly according to their pedigreed status.

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