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Field Evaluation of Alternative Testing Strategies for the Detection of HIV Infection in Beijing[1]

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Objective To identify a cost-efficient alternative antibody testing strategy for screening and confirmation of HIV infection by rapid simple tests (RSTs) and enzyme-linked immunosorbent assays (ELISAs). **Methods** Four RSTs (RST1, RST2, RST3, and RST4) and five ELISAs (ELISA1, ELISA2, ELISA3, ELISA4, and ELISA5) were evaluated in two phases by using banked and serum specimens prospectively collected at regional hospitals and voluntary counseling and testing (VCT) centers in Beijing. A total of 200 banked serum specimens were included in the first phase, including 62 HIV-positive, 127 HIV-negative and 11 indeterminate specimens. All specimens were tested by four RSTs and five ELISAs respectively. The second phase involved prospective testing of 389 routine specimens, including 92 HIV-positive, 287 HIV-negative, and 10 indeterminate specimens. All the specimens were tested by two RSTs (RST2 and RST4) and three ELISAs (ELISA1, ELISA3, and ELISA4), which were selected for their respective excellent sensitivity and/or specificity. Western blot (WB) was used as a gold standard for confirming the reactivity of all the specimens. **Results** Sensitivity, specificity, and efficacy were calculated for each assay in two phases. In the first phase, four assays (ELISA4, RST2, RST3, and RST4) had a specificity of 100%. For the determination of efficacy, ELISA4, RST2, and RST4 were selected in the second phase. ELISA1 and ELISA3 which have a sensitivity of 95.9% and 93.2% respectively also entered this phase. In the second phase, all the five assays (ELISA1, ELISA3, ELISA4, RST2, and RST4) had a sensitivity and specifity of over 90%. ELISA1 had a sensitivity of 99% and ELISA4 a specificity of 99%. **Conclusion** The sensitivity ELISA1 and the specificit of ELISA4 are comparable to ELISA/WB standard strategy. Application of this alternative testing strategy provides a cost-effective method for determining HIV prevalence in Beijing.

Key words: Human immunodeficiency virus type-1; Alternative testing strategy; Rapid simple test; Enzyme-linked immunosorbent assay

INTRODUCTION

Rapid adavances have been made in diagnostic technology since the first HIV antibody test became commercially available in 1985[1]. The challenge today is to identify the most appropriate testing strategy for the reliability of test results under a particular set of circumstances[2]. New developments in preventive interventions such as VCT and the prevention of mother-to-child transmission have created increased demand for efficient HIV testing to expand counseling and testing among high-risk populations[3-4]. The standard laboratory strategy for HIV testing in China is to use ELISA as a screening test, followed by a confirmatory high specificity test. Although Western blot (WB) is the most widely used serological confirmatory test, it is expensive and time-consuming and also has some technical disadvantages[5]. Alternative strategies for confirmation of HIV antibody-positive samples that could replace conventional WB testing have been suggested and evaluated at abroad[6-13].

The aim of this study was to evaluate the use of alternative testing strategies for diagnosis of HIV infection in China.

MATERIALS AND METHODS

All tests were performed at Division of STD/AIDS Prevention, Beijing Centers for Disease

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Control and Prevention. The study was performed in two phases. The first phase was a retrospective study, designed to determine the sensitivity and specificity of various assays. A total of 200 retrospectively selected serum specimens with known serological status were tested, including 62 HIV-positive, 127 HIV-negative and 11 indeterminate specimens.

Four RSTs (RST1, RST2, RST3, and RST4) and five ELISAs (ELISA1, ELISA2, ELISA3, ELISA4, and ELISA5) were included in the first phase: Vironnostika HIV Uniform II Plus O (BioMerieux bv, Boxtel, the Netherlands), Murex HIV-1+2 (Murex Biotech Limited, Dartford, UK), King Hawk anti-HIV-1+2 ELISA kit (King Hawk Co. Ltd, China), Wantai anti-HIV-1+2 ELISA kit (Wantai Co. Ltd, China), Livzon anti-HIV-1+2 ELISA kit (Lizhu Co. Ltd, China), KHB rapid HIV test (Kehua Co. Ltd, China), MiraWell rapid HIV test (MediMira Laboratories Inc., Canada), were used to determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan), Livzon SFD HIV-1+2 (Lizhu Co. Ltd, China). The inclusion criteria for assay were availability of published information on its performance, the ability of the test to detect HIV-1 (group M and O) and HIV-2, as well as IgG and IgM antibodies. Each assay was performed as recommended by the manufacturer.

The sensitivity and specificity of the nine assays were calculated by using the WB results as the "gold standard"[7]. Eleven WB-indeterminate serum speciemns were regarded as ELISA/RST-positive in the evaluation. On the basis of the performance of the assays, two RSTs and three ELISAs were used in the second phase.

In the second phase of the study, five of the nine assays were evaluated prospectively on 389 serum samples consecutively collected from regional hospitals and VCT centers. All samples reactive to any of the five assays were tested by WB. For HIV-1 WB, the Genelabs Diagnostics HIV blot 2.2 (Genelabs Diagnostics, Science Park, Singapore) was used and interpreted according to the WHO criteria requiring positive reaction to at least two *env* bands[8].

RESULTS

Retrospective Evaluation

A total of 200 banked serum specimens with known serological status were tested by four RSTs (RST1, RST2, RST3, and RST4) and five ELISAs (ELISA1, ELISA2, ELISA3, ELISA4, and ELISA5). Sensitivity (the number of WB-positive indeterminate sampme and serum samples determined by the ELISA/RST was divided by the total number of WB-positive/indeterminate serum samples), specificity (the number of WB-negative and serum negative determined by the ELISA/RST was divided by the total number of WB-negative serum samples), and efficacy (the number of WB-positive indeterminate samples and serum positive samples deterimed by the ELISA/RST + the number of WB-negative and serum negative samples determined by the ELISA/RST was divided by the total number of testing serum samples) were calculated for each assay[9] (Table 1). Four assays (ELISA4, RST2, RST3, and RST4) had a specificity of 100%.

TABLE 1

Sensitivity, Specificity, and Efficacy of Nine Assays in Phase 1

Index/Tests	ELISA1	ELISA2	ELISA3	ELISA4	ELISA5	RST1	RST2	RST3	RST4
No. of True Positive	70	63	68	60	63	59	60	58	60
No. of True Negative	119	124	120	127	114	126	127	127	127
Sensitivity (%)	95.9	86.3	93.2	82.2	86.3	80.8	82.2	79.5	82.2
Specificity (%)	93.7	97.6	94.5	100	89.8	99.2	100	100	100
Efficacy (%)	94.5	93.5	94	93.5	88.5	92.5	93.5	92.5	93.5

ELISA4, RST2, and RST4 were used in the second phase. ELISA1 and ELISA3 had a sensitivity of 95.9% and 93.2%, respectively, and also entered the second phase.

Prospective Evaluation

Three hundred and eight-nine prospectively collected serum sampleas were tested by three ELISAs (ELISA1, ELISA3, and ELISA4) and two RSTs (RST2 and RST4), of which 261 concordantly negative serum samples were considered to be true negative, and 128 reactive serum samples determined by any of the five assays were tested using WB. Optical density (OD) ratios of the ELISA, defined as the OD value divided by the calculated cut-off value were used to categorize reactive samples into highly reactive (OD ratio >6.0) and weakly reactive (OD ratio $\leqslant$ 6.0)[10]. Among the 128 serum

samples, 92 WB-positive were concordantly positive determined by thel five assays, with the exception of RST4 showing 3 hemolytic and indeterminate samples. Both ELISA1 and ELISA4 had highly positive reactivity to the 92 serum samples (data not shown). The rest 36 serum samples were discordantly reactive determined by 5 assays (Table 2).

TABLE 2

Comparison of the Discordant Reactivity in the 36 Serum Samples Determined by Five Assays and WB

ID	OD Ratio or Reactivity						ID	OD Ratio or Reactivity					
	ELISA1	ELISA4	ELISA3	RST2	RST4	WB		ELISA1	ELISA4	ELISA3	RST2	RST4	WB
70	–[a]	4.9	13	–	–	Neg[d]	1609	1.4	–	9.3	+	+	Neg
315	–	–	2.9	–	+	Neg	1639	5.2	–	–	–	ND[e]	Neg
1244	–	3.3	1.8	–	–	Neg	1661	1.4	–	–	–	–	Neg
1264	–	–	1.4	–	–	Neg	1694	1.1	–	–	–	ND[e]	Neg
1265	1.4	–	2.5	–	+	Neg	1700	1.5	3.7	–	–	–	Neg
1267	–	–	–	+[b]	–	Neg	1973	2.7	–	–	–	–	Neg
1269	–	–	–	+	–	Neg	1996	–	–	1.3	–	–	Neg
1271	–	–	1.9	–	–	Neg	2066	–	–	–	–	–	Neg
1278	1.6	–	2.6	+	–	Neg	1248	1.1	–	–	–	+	InD[c]
1297	–	–	–	InD[c]	+	Neg	1595	1.8	–	6.8	–	–	InD
1317	–	–	22	+	+	Neg	1687	1.8	–	1.6	–	+	InD
1327	–	–	–	+	–	Neg	1698	6.3	–	–	–	–	InD
1358	–	–	2.2	–	–	Neg	1705	3.1	–	–	–	–	InD
1465	–	–	2.6	–	+	Neg	1857	1.2	–	–	–	–	InD
1498	–	–	5.7	+	+	Neg	1994	4.7	–	–	–	+	InD
1502	–	–	1.3	–	–	Neg	2001	–	1.2	9.9	–	–	InD
1542	–	–	–	+	+	Neg	2010	1.6	6.4	1.2	–	+	InD
1584	1.4	–	9.3	+	+	Neg	2116	1.8	2.1	—	–	+	InD

Note. [a] "–", negative; [b] " + ", positive. [c] InD, indeterminate. [d] Neg, negative. [e] ND, not determined because of hemolytic specimens.

The sensitivity, specificity, and efficacy were calculated for any of the five assays. Ten WB-indeterminate serum samples were regarded as ELISA/RST-positive just as in the first phase. All the five assays had a sensitivitiy and specificity of >90% (Table 3). Among these five assays, ELISA1 and ELISA4 had a sensitivity of 99% and a specificity of 99%, respectively. Combination of ELISA1 and ELISA4 provided an alternative strategy for diagnosis of HIV infection in China.

TABLE 3

Sensitivity, Specificity, and Efficacy of Five Assays in Phase 2

Index/Tests	ELISA1	ELISA3	ELISA4	RST2	RST4
No. of True Positive	101	96	94	92	93
No. of True Negative	278	273	284	278	276
Sensitivity (%)	99.0	94.1	92.2	90.2	91.2
Specificity (%)	96.9	95.1	99.0	96.9	96.2
Efficacy (%)	97.4	94.9	97.2	95.1	94.9

DISCUSSION

In this study, we conducted a two-phase evaluation, which was designed to select appropriate combination of HIV antibody assays that would provide cost effective determination of HIV status and could be implemented in small rural laboratories. The results of our study indicate that combination of ELISA1 and ELISA4 replacing conventional ELISA/WB testing could diagnose HIV-infection accurately. Serum samples reacting concordantly and being highly positive (OD ratio >6.0) determined by

ELISA1 and ELISA4 could be considered as true positive (92 serum samples, 92/389=24%). A true negative serum sample could be defined as having concordantly negative reactivity determined by both ELISA1 and ELISA4 [15 serum samples, Table 2. (261+15)/389=71%]. Serum samples yielding discordant reactivity determined by two ELISAs or weakly reactivity (OD ratio $\leqslant$6.0) determined by any of the two ELISAs could be considered as indeterminate serum samples and should be further tested by WB [21 serum samples, Table 2. 21/389=5%] [10].

The HIV pandemic continues to evolve in most areas of China. Use of WB for confirmation of screening test-positive samples in laboratories implies a high cost for HIV serologic testing[11-12]. An alternative approach of ELISA/ELISA combination substituting ELISA/WB testing algorithm, has at least three advantages[13-14]. First, this strategy is more cost-effective than ELISA/WB testing algorithm. Second, this strategy reduces the possibility that inadvertent sample-handling errors may be undetectable. Third, it has a shorter turnaround time than ELISA/WB testing algorithm, because most of the concordantly and highly positive (24%) and negative (71%) specimens will be reported immediately[15].

In summary, the sensitivity and specificity of ELISA1 and ELISA4 are comparable to ELISA/WB standard strategy, and offer an efficient and economic alternative to the ELISA/WB testing algorithm for the serological diagnosis of HIV infection.

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