Report on HIV test kit Evaluation for Government of Indonesia

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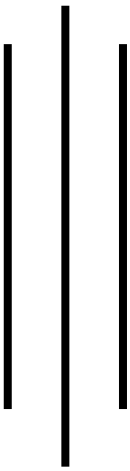

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Introduction

Human Immunodeficiency Virus (HIV) infection is recognised as an important health problem in Indonesia since the first HIV infection case diagnosed in 1987. In the last few years the number of HIV infection cases increased in an alarming rate.

The diagnosis of HIV infection is usually made on the basis of the detection of anti-HIV antibody. Laboratories in Indonesia conduct HIV testing for clinical diagnosis, blood and blood products screening, epidemiological surveillance and research purposes.

Diagnostic technology should be available in an appropriate manner with a good quality system. In order to provide support to all health care activities, laboratory service should get a special attention: good standard, qualified manpower, good infrastructure and equipment and it must be evaluated to maintain high standards in laboratory techniques. The diagnostic reagents used by these laboratories should also have good quality.

An evaluation on the diagnostic reagents has to be done to prove their good quality and their performance in local conditions. This evaluation on diagnostic reagents is better to be conducted prior to marketing of each diagnostic reagent in the country.

Background

The first report of HIV infection in Indonesia was in 1987. In 1998, the official cumulative number of reported HIV-positive cases was 819 (227 AIDS). This number increased to 1678 cases (635 AIDS) as of September 2001. Recent changes in the epidemiology of Indonesian HIV infection led to re-categorization of Indonesia as a “concentrated epidemic” by the World Health Organization (WHO). HIV infection is now above 5% among drug users in selected cities (Jakarta, Bogor, Bali), and among selected groups of female sex workers (Merauke, Bali, West Java, Riau). Jakarta, the largest city in Indonesia, reports the highest number of HIV infections and the second highest rate of infection with the highest per capita rate of infection in the country is in Papua.

In this setting, the need for accurate, reliable and readily accessible HIV antibody testing has focused attention, in part, on the number of different HIV tests used in Indonesia. In country evaluation of Indonesian sold HIV test kits using Indonesian samples has not been available in the past. HIV test kit evaluations performed and published elsewhere include few, if any, samples from Indonesia. Past WHO test kit evaluations include a limited number of Asian samples and are conducted under circumstances different from the testing circumstances in Indonesia. The scattered reports of unreliable HIV test results in Indonesia have brought into question the performance characteristics of selected HIV antibody test kits. Reports of false positive

and false negative test results may, if fact, represent predictable, test variation expected in a low prevalence setting. On the other hand, differences in test performance could be due to serologic variations in the samples tested, reagent transportation or storage problems in a tropical region, or due to problems with actual testing and quality control procedures.

In order to address these issues, the Ministry of Health made the decision to evaluate test kits used in Indonesia using samples collected throughout the country. All available test kits were evaluated because circumstances surrounding HIV testing vary over Indonesia. Cost, test kit availability, technical expertise, number of samples requiring testing, laboratory facilities, reagent transport and storage requirements contribute, in part, to the most appropriate test kit choice in any one site. Large facilities with a high degree of technical expertise, large numbers of samples to test, and the availability of confirmatory testing will appropriately choose to use a sensitive, automated, enzyme-linked immunoassay. In this circumstance, results of a sensitive test can be confirmed with a more expensive but specific assay (i.e. Western blot). Smaller facilities and/or facilities with a small volume of samples, smaller budgets, and where confirmatory testing is not available will appropriately choose one or more than one rapid tests. A realistic balance between sensitivity with specificity will be determined by test kit choice. All facilities need to insure strong quality assurance/quality control programs.

Assay selection

Test kits for the detection of antibodies to HIV, which are registered to the National Agency of Drug and Food Control (NADFC) of Indonesia and acceptable for testing with plasma were evaluated. These test kits were classified into two groups, which were the Enzyme immunoassay (EIA)-based kits and the simple/rapid assays. Five EIA-based test kits and 7 simple/rapid assay-based test kits were evaluated in this study. Two of the EIA-based test kits can detect both antibodies to HIV and the antigen of HIV. One simple/rapid test kit was not evaluated because the package insert specified fresh serum as a requirement for testing.

Study preparation

The study was started with the recruitment of a team, which consisted of members from Directorate of Laboratory Services and Centre for Diseases Control (Indonesian Ministry of Health), National Agency of Drug and Food Control, National Reference Laboratory for HIV testing (Clinical Pathology Department, Medical Faculty University of Indonesia and Dr. Cipto Mangunkusumo Hospital), Central Blood Transfusion Unit (Indonesian Red Cross), provincial laboratory (Surabaya Provincial Laboratory) and private laboratory (Prodia Clinical Laboratory). This team developed a proposal to improve the quality of HIV testing in Indonesia, which was improved and

refined by John Parry from the Central Public Health Laboratory, United Kingdom as World Health Organisation (WHO) temporary consultant. Also consulting for the proposal were Elizabeth Donegan from the University of California San Francisco laboratory partner for the Aksi Stop Aids Program (ASA) of Family Health International funded by USAID, Elizabeth Dax from the Australian National Serology Reference Laboratory and Gaby Vercauteren from the WHO headquarter.

Ministry of Health and WHO appointed the Clinical Pathology Department, Medical Faculty University of Indonesia and Dr. Cipto Mangunkusumo Hospital as the National Reference Laboratory (NRL) for HIV testing and the evaluation centre for HIV diagnostic reagents. Two national consultants from that department were recruited to conduct the evaluation. Dr Donegan was recruited to partner on-site during the evaluation process. The two national consultants were trained at the Australian National Serology Reference Laboratory prior to the evaluation process. During their training, they had refined and developed the Indonesian evaluation protocol using in part protocols proposed by John Parry (WHO consultant) and by Elizabeth Donegan (ASA/FHI partner). In order to compare the results of this HIV test kit evaluation with other published results, particularly those of the WHO, the chart-reporting format of the WHO was adapted for this evaluation as much as possible.

The facilities in the NRL were improved by adding new equipment that are needed for the study, such as - 80ºC freezer, calibrated pipettes, calibrated timers, and vortex mixer donated by FHI.

Laboratory procedures for each of the test kits to be evaluated were written. Distributors for the each of the test kits were invited to calibrate, service and test automated instruments used in the evaluation. Distributors of the rapid tests were invited to inspect the facilities and observe the test kit evaluation for their test kit.

In preparation for the evaluation, the Central Blood Transfusion Unit of the Indonesian Red Cross contacted blood centres throughout Indonesia. HIV screen antibody positive plasma and HIV screen negative plasma frozen and stored at the Central facility at -40°C was transported from storage to the HIV National HIV Reference Laboratory and used for the evaluation. A computerised inventory system and sample labelling system was put in place.

Materials and Methods

Biosafety Standards

Universal precautions for laboratory acquired HIV infections were observed. All participating laboratory staff wears “laboratory only” coats and disposable gloves discarded after single use. Counter tops were clean twice a day with

bleach. All disposable items were either soaked in bleach ≥10% for one or more hours or incinerated. Disposable waste was discarded in either a safety unit (needles, small items) or into a designated disposal plastic bag. Discarded items were then incinerated.

Quality Control

Temperatures of the -80° C freezers used to store the plasma inventory, of the cold room and refrigerator used to store reagents and testing laboratory ambient temperature was monitored with NSBT thermometer. The instrument distributor calibrated the instruments. Pipettes used for the evaluation were calibrated. A quality control panel of plasma samples was made and tested prior to the evaluation (appendix 1). Reagent lots and out-date were checked and recorded.

Specimen acquisition and storage

A total 458 frozen plasma samples were transferred from the Central Blood Transfusion Unit (CBTU) to NRL on dry ice. Two hundred and eighty two of these units had been reported to CBTU as having anti-HIV positive test results (EIA and/or Rapid tests; generally EIA: Abbott or Organon EIA, rapid tests: Abbott Determine and/or Entebe). A hundred and seventy six units had been reported to CBTU as having anti-HIV negative test results with the above tests. After transfer on dry ice, units were thawed, Western blot

(Cambridge Bioscience, USA) tested and dispersed in aliquots prior to the study conduct. Plasma bags were thawed at room temperature, and aliquots made and refrozen on the same day. The following aliquots were made: 30 one ml aliquots, 5 five ml aliquots and the remaining plasma stored in twenty-five ml aliquots. Samples were colour-coded and the inventory stored in a -80°C freezer.

Western blot (WB) testing was performed according to the manufacture's directions and interpreted as recommended by the manufacturer (CDC criteria). Western blots with any two or more of the following bands present: p24, gp41, and gp120/160 were interpreted as WB positive. When any bands were visualised but the pattern did not meet criteria of positivity, the WB was interpreted as indeterminate. If no band was present, the WB was interpreted as negative.

Of the 282 plasma samples referred as anti-HIV positive, 153 were confirmed positive with WB, 90 were WB negative and 39 were WB indeterminate. For this evaluation the WB negative samples were evaluated together with the plasma samples referred as anti-HIV negative. Thirty-nine samples with indeterminate WB results were eliminated for further evaluation.

Panel Selection

All 153 anti-HIV positive/WB positive samples, the 90 anti-HIV negative but WB negative samples as well as the 176 anti-HIV negative samples were used for this evaluation.

Thirty-six blood banks from 15 provinces throughout Indonesia contributed plasma bags to this evaluation (table 1).

Table 1: Origin of plasma samples by province.

Province	Number of plasma samples
Bali	15
Central Java	49
East Java	42
East Kalimantan	12
Jakarta	159
Lampung	5
North Sulawesi	11
North Sumatera	13
Papua	23
South Kalimantan	11
South Sumatera	11
Southeast Sulawesi	13
West Java	32
West Sumatera	13
Yogyakarta	7
Unknown	3
Total	419

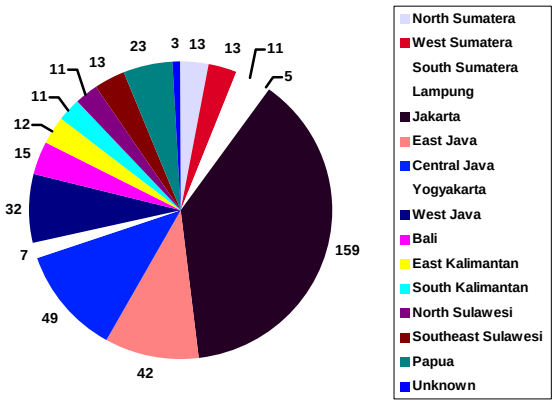

Figure 1: Provincial distribution of the evaluation panel.

Site

All testing were performed at Clinical Pathology Department, Medical Faculty, University of Indonesia, Dr. Cipto Mangukusumo Hospital, Jakarta, which has been appointed as the National Reference Laboratory for HIV testing.

Specimen panel

Specimens from the panel were given sequenced number. Randomisation using random table were made and sample chosen for panels were put in the rack according to the random number. The rack was labelled with the panel name. For testing, one panel was thawed at a time and refrigerated at 2 – 8^{o}C until aliquots were exhausted or 1 month has elapsed at which stage another panel was thawed. During testing, samples should be returned to 4^{o}C immediately after their addition to an assay. The racks of samples, which are to remain at 4^{o}C following thawing should have the date of thaw on the rack. If not used or re-frozen within 1 month, any remaining volume should be discarded. Following completion of testing, samples should be re-frozen at –70^{o}C and this will be indicated with a black marker pen on every tube's side and cap.

Recording of each test kit's general characteristics

Two questionnaires (one for EIAs and one for simple/rapid assays) were used to record information on the test kit's general characteristics (appendices 2 and 3). The information includes test kit's name, manufacturer, principle or assay type, antigen type, solid phase, sample volume, incubation time, and wavelength for EIAs. This information will be obtained from the package inserts.

Equipment preparation

The manufacturer has checked the equipment used for the evaluation and a certificate of validated performance submitted to the evaluator. The equipments were readers, washers, incubators, and micropipettes.

All temperature controlled equipment were monitored on a daily basis and records retained.

Washers were checked for proper performance at the start of each day on which EIAs will be performed.

Testing procedure

All samples were tested singly. Falsely reactive samples were retested in duplicate and the final result was that which occurs 2 of 3 times.

Before starting the assay, package insert was examined and protocol for each assay developed.

All testing will be performed as directed in the manufacturer's package insert. The person or operator, who did the test, were trained prior to the actual testing by either the manufacturer's technical staff or by the evaluators. During training a training record was completed and initialised by the trainer and the trainee. The training record was filed into a designated folder.

Worksheets for each test kit were developed on an Excel spreadsheet. On the day of testing, sample ID was entered into the worksheet with a barcode reader and printed. Then other data such as the lot or batch number, expiry date, date of testing and the operator's ID were written on the worksheet.

The testing results were written on the worksheet with the printout attached to it (If available) by the operator. The validity of the testing has been checked by the operator and verified by the evaluators. Each person checking the validity signed her initial on the worksheet.

If the test was valid, then the operator enters the testing results into the electronic spreadsheet.

For simple / rapid test, result was recorded as directed in the manufacturer's package insert independently by three observers on 3 separate worksheets. When the three observers interpreted the result differently from each other, the consensus was recorded as that interpretation which occurred 2 out of 3 times. In cases where all three interpretations were different, the result was recorded as indeterminate. In these cases the testing device was re-examined to ensure that no clerical errors or sample mix-ups had occurred.

To comply with the quality system, the following data must be provided for each run:

Operator

Run date

Run number

Batch number

Batch expiry date

Verified data entry and calculation

Assay reproducibility

The appropriate Quality Control (QC) sample was prepared (appendix 1) and tested in every run. A “run” for EIA assays was a number tests that are done simultaneously on one plate or batch of 100 tests. The QC sample was tested in at least 7 replicates on each run. The testing of multiple replicates of QC provided data for analysis of the variability of an EIA while also allowing monitoring of the assay run-to-run.

For simple/rapid assays, a “run” was a batch of 20 simultaneous tests. The QC sample was tested singly on each simple/rapid assay run.

The date of thaw of QC sample was recorded on the tubes. Remaining volume in a thawed aliquot was discarded after 1 week.

Data management

Data entry

Data were entered manually into Excel spreadsheet for analysis. The relevant entries from Excel spreadsheet containing the panel's characteristics were copied to this one and built upon.

Data entry was double-checked by a second person by printing an entered copy and comparing it with the original data. The second person, which was checking the data initialled and dated the original data to verify the checking process was completed satisfactorily.

Data analysis

Sensitivity, specificity, positive predictive value and negative predictive value were calculated for each kit. Positive and negative delta values were calculated for each EIA.

Sensitivity is the ability of the assay under evaluation to detect correctly specimens that contain antibody to HIV. Sensitivity analysis was performed on samples whose Presumed Antibody Status (PAS) is positive, based on the Western blot result. The calculation of sensitivity in Excel spreadsheet was by:

- Dividing each Optical Density (OD) by the cut-off (CO) in-order to calculate the OD/CO ratio for each sample
- Assigning a reactive / Positive (P) result if the OD/CO is ≥ 1 and a non-reactive / Negative (N) result if the OD/CO) is < 1.
- Determining the total number of samples that were non-reactive (false negative) and the total number of true positives.
- Calculating the sensitivity with the following formula:

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives + False negatives}}$$

Specificity is a measure of the ability of an assay to determine as non-reactive those samples that do not contain specific antibodies. The calculation of specificity on Excel spreadsheet was by:

- Dividing each Optical Density (OD) by the cut-off (CO) in-order to calculate the OD/CO ratio for each sample
- Assign reactive / Positive (P) results if the OD/CO is ≥ 1 and a non-reactive / Negative (N) result if the OD/CO is < 1.
- Determining the total number of samples that were reactive (false positive), and the total number of true negatives.
- Calculating the specificity with the following formula:

$$\text{Specificity} = \frac{\text{True negatives}}{\text{False positives} + \text{True negatives}}$$

Positive predictive value (PPV) is the probability that when the test is reactive, the specimen does contain antibody to HIV. This was calculated using the following formula:

$$\text{PPV} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

Negative predictive value (NPV) is the probability that when the test is negative, a specimen does not have antibody to HIV. This was calculated using the following formula:

$$\text{NPV} = \frac{\text{True negatives}}{\text{False negatives} + \text{True negatives}}$$

95 % confidence limits of the sensitivity, specificity, PPV and NPV were calculated using the formula:

$$p \pm \sqrt{\frac{p(1-p)}{n}}$$

95 % confidence limits are a means of determining whether observed differences in sensitivity, specificity, PPV or NPV between assays are significant or not.

The delta value is statistic that will define how far the negative or a positive sample population's distribution is removed from the cut-off value. The delta value is a ratio between the distance of the distribution's mean of log[OD/CO] from the cut-off and the standard deviation of the whole distribution around the mean. The calculation a delta value using Excel spreadsheet was by:

- Calculating the OD/CO ratio for each sample
- Calculating the $\log_{10}$ of each OD/CO
- Calculating mean of all the $\log_{10}$ OD/COs
- Calculating the standard deviation of all the $\log_{10}$ OD/COs
- The delta value was then determined by dividing the mean of $\log_{10}$ OD/COs by the Standard Deviation (SD) of $\log_{10}$ OD/COs

The positive delta value was calculated from the results of all samples whose PAS is positive. The negative delta value was calculated from the results of samples whose PAS is negative.

The reproducibility of EIA-based kits was determined by calculating its intra-assay and between assays coefficient of variation (CV) of OD/CO ratio of the QC sample. Calculation of CV was made by dividing the SD of OD/CO ratio by the mean of OD/CO ratio.

The inter-reader variability of simple/rapid assays was expressed as a percentage of specimens which initial test results were differently interpreted by different readers.

Evaluation of the ease of use of test kits

Evaluation of the ease of use of each test kit was done by using 2 questionnaires that had been developed (appendices 4 and 5). A total ease of use score was calculated for each kit.

Result

Table 2: General characteristics of EIA-based HIV test kits.

No	Name of the assay	Manufacturer	Assay type	Antigen type	Coated antigens	Solid phase	Number of test per kit	Volume of sample needed (μL)
1	Abbott HIV 1/2 gO	Abbott	EIA	recombinant protein, synthetic peptide	HIV-1 env group M, HIV-1 env group O and HIV-1 core , HIV-2 env.	beads	100 or 1000	150
2	Enzygnost anti-HIV 1/2 Plus	Behring	EIA	recombinant antigen	gp 41(HIV-1), gp 41 (HIV-1 subtype O), gp 36 (HIV-2)	microwells	2 x 96 or 10 x 96	100
3	Murex HIV-1.2.O	Abbott	EIA	synthetic peptides, recombinant protein and core protein	Immunodomi-nant region HIV-1 (O), env. (HIV-1 and HIV-2), HIV core protein	microwells	96 or 480	50

Table 2: General characteristics of EIA-based HIV test kits (continued).

No	Name of the assay	Manufacturer	Assay type	Antigen type	Coated antigens	Solid phase	Number of test per kit	Volume of sample needed (µL)
4	HIV Uniform II Ag/Ab	Organon Teknika	EIA	peptides	gp 160, HIV-1 ANT70, HIV-2 env peptide, anti HIV-1 p24	microwells	192 or 576 or 2304	50
5	Vidas HIV Duo	BioMérieux	EIA	synthetic peptides and monoclonal antibody	gp 41(HIV-1), gp 36 (HIV-2), peptide specific to HIV-1 group O and anti-p24	solid phase receptacle (SPR)	60	200

Table 3: Performance of EIA-based HIV test kit compared to Western blot results.

No	Kit	N	Sensitivity (%)	95% Confidence limit	Specificity (%)	95% Confidence limit	NPV# (%)	95% Confidence limit	PPV# (%)	95% Confidence limit	Positive Delta value	Negative Delta value	CV# intra batch (%)	CV# between batch (%)
1	Abbott HIV 1/2 gO	419	100.0	99.6-100.0	84.6	81.1-88.0	78.9	75.0-82.8	100.0	99.6-100.0	2.7	3.9	2.4 - 6.1	20.6
2	Enzygnost anti-HIV 1/2 Plus	419	99.4	98.6-100.0	94.0	91.7-96.3	90.5	87.7-93.3	99.6	99.0-100.0	5.3	3.0	2.9 - 7.8	6.8
3	Murex HIV-1.2.O*	419	100.0	99.6-100.0	92.1	89.5-94.7	87.9	84.8-91.0	100.0	99.6-100.0	3.4	3.2	6.0*	NA**
4	HIV Uniform II Ag/Ab	419	98.0	96.7-99.4	94.0	91.7-96.3	90.4	87.5-93.2	98.8	97.8-99.8	3.4	4.2	7.9 - 8.7	12.7
5	Vidas HIV Duo	419	98.0	96.7-99.4	97.7	96.3-99.2	96.2	94.3-98.0	98.9	97.8-99.9	10.2	3.7	3.8 -13.6	16.6

NPV = negative predictive value, PPV = positive predictive value, CV = coefficient of variation
* Only one batch of kits evaluated. ** Not applicable, since only one batch of kits evaluated.

Table 4 : Comparison between study and WHO evaluation results. (# ND = no data)

No	Kit	Sensitivity (%)		95% Confidence limit		Specificity (%)		95% Confidence limit	
		This study	WHO[1,2]	This study	WHO[1,2]	This study	WHO[1,2]	This study	WHO[1,2]
1	Abbott HIV 1/2 gO	100.0	ND#	99.6-100.0	ND#	84.6	ND#	81.1-88.0	ND#
2	Enzygnost anti-HIV 1/2 Plus	99.4	100.0	98.6-100.0	99.6-100.0	94.0	99.7	91.7-96.3	99.1-100.0
3	Murex HIV-1.2.O	100.0	100.0	99.6-100.0	99.6-100.0	92.1	99.4	89.5-94.7	98.6-100.0
4	HIV Uniform II Ag/Ab	98.0	ND#	96.7-99.4	ND#	94.0	ND#	91.7-96.3	ND#
5	Vidas HIV Duo	98.0	ND#	96.7-99.4	ND#	97.7	ND#	96.3-99.2	ND#

Table 5a : Technical aspects of EIA-based HIV test kits.

No	Name of the assay	Manufacturer	Total incuba-tion time (hh:mm)	Wavelength (nm)		Stability of reagent after reconstitution at (….°C)					
				Single	Double	Controls	Antigen	Sample diluent	Conju-gate	Substrate	Wash buffer
1	Abbott HIV 1/2 gO	Abbott	1 : 30	492	None	NA*	NA*	NA*	3 weeks (2-8)	60 minutes (room temp.)	None
2	Enzygnost anti-HIV 1/2 Plus	Behring	1 : 30	450	-	exp. date (2-8)	NA*	NA*	NA*	discard after use	1 weeks (2-8)
3	Murex HIV-1.2.O	Abbott	1 : 30	450	450/620-690	NA*	NA*	NA*	8 weeks (2-8)	2 days (2-8)	1 month (RT**)
4	HIV Uniform II Ag/Ab	Organon Teknika	1 : 30	450	450/620-700	NA*	NA*	NA*	None	8 hours (15-30, dark)	2 weeks (2-8)
5	Vidas HIV Duo	BioMérieux	No data	No data	No data	NA*	NA*	NA*	NA*	NA*	NA*

*NA = not applicable, reagents are ready for use
**RT = room temperature

Table 5b : Additional technical aspects of EIA-based HIV test kits.

No	Name of the assay	Manufacturer	Number of controls per test run		Number of blanks	Number of standard	Incubation temperature (...^{o}C)	Reading time limit (min)	Total time to perform the assay (hh:min)	Number of speci-mens each run (min. - max.)
			Negative	Positive						
1	Abbott HIV 1/2 gO	Abbott	3	2 for anti-HIV1, 2 for anti-HIV2	5, if using the Abbott Comman-der	None	39 - 41	120	03:45	1 - 89
2	Enzygnost anti-HIV 1/2 Plus	Behring	4	2	None	None	37	60	03:15	1 - 90
3	Murex HIV-1.2.O	Abbott	3	1 for anti-HIV1, 1 for anti-HIV2	None	None	37	15	03:30	1 - 91
4	HIV Uniform II Ag/Ab	Organon Teknika	3	1 for anti-HIV1, 1 for anti-HIV2, 1 for HIV-1 Ag	None	None	37	15	02:45	1 - 90
5	Vidas HIV Duo	BioMérieux	1	1	None	2	No data	No data	02:45	1 - 12

Table 5c : Additional information on EIA-based HIV test kits.

No	Name of the assay	Manufacturer	Cut-off (CO) value calculation	Definition of positive results	Definition of grey zone (if any)	Storage at (…^{o}C)
1	Abbott HIV 1/2 gO	Abbott	NCx+0.10	equal to or greater than CO	None	2-8
2	Enzygnost anti-HIV 1/2 Plus	Behring	NCx+0.400	equal to or greater than CO	None	2-8
3	Murex HIV-1.2.O	Abbott	NCx+0.200	equal to or greater than CO	None	2-8
4	HIV Uniform II Ag/Ab	Organon Teknika	NCx+0.100	equal to or greater than CO	None	2-8
5	Vidas HIV Duo	BioMérieux	0.25	equal to or greater than 0.35	between 0.25 and 0.35	2 - 8

Table 5d : Additional equipment needed by EIA-based HIV test kits.

No	Name of the assay	Equipment needed but not provided in the kit												
		Washer	Incubator / water-bath	Spectrophotometer	Refrigerator (storage)	Agitator / rocker	Aspiration device	Automatic pipette	Multichannel pipette	Disposable tips	Dilution tubes / rack, microtiterplate	Plate covers	Absorbent paper	Reagent trough
1	Abbott HIV 1/2 gO	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes, if using multichannel pipette
2	Enzygnost anti-HIV 1/2 Plus	Yes	Yes	Yes	Yes	No	Yes	Yes	No, but better if available	Yes	No	No	Yes	Yes, if using multichannel pipette
3	Murex HIV-1.2.O	Yes	Yes	Yes	Yes	No	Yes	Yes	No, but better if available	Yes	No	No	Yes	Yes, if using multichannel pipette

Table 5d : Additional equipment needed by EIA-based HIV test kits (continued).

No	Name of the assay	Equipment needed but not provided in the kit											
		Washer	Incubator / water-bath	Spectrophotometer	Refrigerator (storage)	Agitator / rocker	Aspiration device	Automatic pipette	Multichannel pipette	Disposable tips	Dilution tubes / rack, microtiterplate	Plate covers	Absorbent paper	Reagent trough
4	HIV Uniform II Ag/Ab	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No, but better if available	Yes	No	No	Yes	Yes, if using multichannel pipette
5	Vidas HIV Duo	No	No	No	No	No	No	Yes	No	Yes	No	No	No	No

Table 6 : Ease of use score of EIA-based HIV test kits.

No	Features	Scoring	Abbott HIV 1/2 gO	Enzygnost anti-HIV 1/2 Plus	Murex HIV-1.2.O	HIV Uni-form II Ag/Ab	Vidas HIV Duo
1	Machine based	y=1,n=0	0	0	0	0	1
2	Dedicated equipment needed	y=0,n=1	0	1	0	1	0
3	Format of strip	≤4=2, 8=1, 16=0	2	0	0	1	2
4	Type of specimen	plasma/serum only =0, both plasma & serum =1	1	No data	1	1	1
5	Any restriction of anticoagulant	y=0,n=1	1	No data	0	1	1
6	Specimen volume	<50 uL=2, 50-100 uL=1, >100 uL=0	0	1	1	1	0
7	Sample preparation step	y=0,n=1	1	1	1	1	1
8	Controls included in kit's cost	y=1,n=0	1	1	1	1	1
9	Controls ready for use	y=1,n=0	1	1	1	1	1
10	Reagent preparation needed	y=0,n=1	0	0	0	0	1
11	Need of additional reagent	y=0,n=1	1	1	0	0	1
12	Incubation period	<2hr=2, 2-3hr=1, >3hr=0	2	2	2	2	2
13	Need of special incubation condition	y=0,n=1	0	1	1	0	0
14	Number of steps (excl. wash)	3=2, 4=1, 5=0	0	0	0	1	2
15	Availability of specimen addition monitoring	y=1,n=0	0	0	1	0	0
16	Storage of reagents	ambient possible=1, 2-8°C=0	0	0	0	0	0
17	Reagent stability after reconstitution (at 2-8°C)	<1wk=0, 1-4wk=1, 6-8wk=2, exp. date=3	1	1	2	1	3
18	Grey Zone	y=0,n=1	1	1	1	1	0
Total score			12	11	12	13	17

Table 7: General characteristics of simple/rapid HIV test kits.

No	Name of the assay	Manufacturer	Assay type	Antigen type	Coated antigens	Solid phase	Number of test per kit	Volume of sample needed (μL)	Final dilution of sample
1	Serodia HIV-1/2	Fujirebio	Passive particle agglutination	Inactivated HIV-1 & HIV-2 antigens	No data	Gelatin Particles	100 or 220	25	1:32 & 1:64
2	Entebe HIV Dipstick	Hepatika Laboratories	Colloidal gold dot blot	Synthetic peptides	gp41(HIV-1), gp36 (HIV-2)	Polystyrene comb	96	100	1:2
3	Immunocomb II HIV 1&2 BiSpot	PBS Orgenics	Indirect solid-phase EIA	Synthetic peptides	gp41& gp120 (HIV-1), gp36 (HIV-2)	Plastic combs	36	50	No data
4	HIV-Spot	Genelabs Diagnostics	Gold dot blot	Recombinant protein (HIV-1) & purified peptide (HIV-2)	gp41 & gp120 (HIV-1), env (HIV-2)	Porous membrane	20 & 100	1 drop	None
5	Hexagon HIV	Human	Immunochro-matography	Synthetic peptides	gp41 & gp 36	No data	40	40	None
6	Determine HIV 1/2	Abbott	Immunochro-matography	Recombinant protein & synthetic peptide	No data	No data	100	50	None
7	HIV 1&2 Antibody Rapid Test	Oncoprobe Biotech	Immunochro-matography	Recombinant protein	gp120, gp41 & gp36	Membrane	25	40	2:3 - 2:5

Table 8: Performance of simple/rapid HIV test kit compared to Western blot results.

No	Kit	N*	Sensitivity (%)	95% Confidence limit	Specificity (%)	95% Confidence limit	NPV# (%)	95% Confidence limit	PPV# (%)	95% Confidence limit	Inter-reader variability (%)
1	Serodia HIV-1/2	415**	98.0	95.8-98.7	93.5	91.1-95.9	89.9	87.0-92.8	98.8	97.7-99.8	3.8
2	Entebe HIV Dipstick	419	97.4	95.0-98.2	98.9	97.9-99.9	98.0	96.7-99.4	98.5	97.3-99.7	8.6
3	Immunocomb II HIV 1&2 BiSpot	419	98.0	96.7-99.4	99.6	99.0-100.0	99.3	98.6-100.0	98.9	97.9-99.9	0.5
4	HIV Spot	416##	94.1	91.8-96.3	100.0	99.6-100.0	100.0	99.6-100.0	96.7	95.0-98.4	3.1
5	Hexagon HIV	419	96.7	95.0-98.4	99.3	98.4-100.0	98.7	97.6-99.8	98.1	96.8-99.4	1.0
6	Determine HIV 1/2	419	98.7	97.6-99.8	88.7	85.7-91.7	83.4	79.9-87.0	99.2	98.3-100.0	6.7
7	HIV 1&2 Antibody Rapid Test	419	97.4	95.9-98.9	97.4	95.8-98.9	95.5	93.6-97.5	98.5	97.3-99.6	2.9

*N = number of specimens.
** 4 specimens gave +/- result.
# NPV = negative predictive value, PPV = positive predictive value.
3 specimens were not adsorbed into the reaction cell.

Table 9: Comparison between study and WHO evaluation results.

No	Kit	Sensitivity (%)		95% Confidence limit		Specificity (%)		95% Confidence limit	
		This study	WHO[1,2]	This study	WHO[1,2]	This study	WHO[1,2]	This study	WHO[1,2]
1	Serodia HIV-1/2	98.0	100.0	95.8-98.7	99.6-100.0	93.5	100.0	91.1-95.9	99.7-100.0
2	Entebe HIV Dipstick	97.4	100.0	95.0-98.2	99.6-100.0	98.9	96.4	97.9-99.9	94.4-98.4
3	Immunocomb II HIV 1&2 BiSpot	98.0	100.0	96.7-99.4	99.6-100.0	99.6	99.7	99.0-100.0	99.1-100.0
4	HIV Spot	94.1	94.5	91.8-96.3	89.7-97.4	100.0	99.0	99.6-100.0	96.4-99.9
5	Hexagon HIV	96.7	ND[#]	95.0-98.4	ND[#]	99.3	ND[#]	98.4-100.0	ND[#]
6	Determine HIV 1/2	98.7	97.9-100.0*	97.6-99.8	ND[#]	88.7	100.0*	85.7-91.7	ND[#]
7	HIV 1&2 Antibody Rapid Test	97.4	ND[#]	95.9-98.9	ND[#]	97.4	ND[#]	95.8-98.9	ND[#]

ND[#] = no data
* From Branson BM[3].

Table 10a: Technical aspects of simple/rapid HIV test kits.

No	Name of the assay	Manufacturer	Stability of reagent after reconstitution at (2-8°C)							Number of controls per run	
			Controls	Antigen	Sample diluent	Conjugate	Substrate	Wash buffer	Others	Negative	Positive
1	Serodia HIV-1/2	Fujirebio	NA*	7 days	NA*	None	None	None		None	6
2	Entebe HIV Dipstick	Hepatika Laboratories	NA*	None	NA*	NA*	None	No data		1	1
3	Immunocomb II HIV 1&2 BiSpot	PBS Orgenics	NA*	None	NA*	NA*	NA*	NA*		1	1
4	HIV-Spot	Genelabs Diagnostics	6 months	None	None	2 months	None	NA*	Liquid buffer : 6 months	1	2
5	Hexagon HIV	Human	None	NA*	None	NA*	NA*	NA*		None	None
6	Determine HIV 1/2	Abbott	None	NA*	None	None	None	None	Chase buffer : NA*	None	None
7	HIV 1&2 Antibody Rapid Test	Oncoprobe Biotech	None	NA*	NA*	None	None	None	None	None	None

NA* = not applicable, reagents are ready for use.

Table 10b: Additional technical aspects of simple/rapid HIV test kits.

No	Name of the assay	Manufacturer	Incubation temperature	Reading	Total time to perform the assay (hh:min)	Number of sera per run (min. - max.)	Definition of positive results	Storage at (…°C)
1	Serodia HIV-1/2	Fujirebio	RT***	Visual	02:30	1 - 24	Agglutination	2 - 10
2	Entebe HIV Dipstick	Hepatika Laboratories	RT***	Visual	00:30	1 - 94	Pink/red dot	2 - 8
3	Immunocomb II HIV 1&2 BiSpot	PBS Orgenics	RT**	Visual	00:40	1 - 34	Blue spots on the upper spot and either middle &/ lower spots	2 - 8
4	HIV-Spot	Genelabs Diagnostics	RT**	Visual	00:10	1 - 20	Two spots	2 - 8 & 25
5	Hexagon HIV	Human	RT**	Visual	00:08	1 - 20	Colour of Test > Reference	2 - 8
6	Determine HIV 1/2	Abbott	RT**	Visual	00:15	1 - 20	Two bars	2 - 30
7	HIV 1&2 Antibody Rapid Test	Oncoprobe Biotech	RT**	Visual	00:16	1 - 20	Two bands	15-30

RT** = room temperature

Table 10c: Additional equipment needed by simple/rapid HIV test kits.

No	Name of the assay	Manufacturer	Equipment needed but not provided in the kit												
			Washer	Incubator / water-bath	Spectrophotometer	Refrigerator (storage)	Agitator / rocker	Aspiration device	Automatic pipette	Multichannel pipette	Disposable tips	Dilution tubes / rack, microtiterplate	Plate covers	Absorbent paper	Reagent trough
1	Serodia HIV-1/2	Fujirebio	No	No	No	Yes	No	No	Yes	No, but better if available	Yes	Yes, microti-ter plate U	No	No	Yes, if using multichan-nel pipette
2	Entebe HIV Dipstick	Hepatika Laboratories	No	No	No	Yes	No	No	No	No	No	No	No	No	No
3	Immunocomb II HIV 1&2 BiSpot	PBS Orgenics	No	No	No	Yes	No	No	Yes	No	Yes	No	No	No	No
4	HIV-Spot	Genelabs Diagnostics	No	No	No	Yes & No	No	No	No	No	No	No	No	No	No
5	Hexagon HIV	Human	No	No	No	Yes	No	No	No	No	No	No	No	No	No
6	Determine HIV 1/2	Abbott	No	No	No	Yes & No	No	No	Yes	No	Yes	No	No	No	No
7	HIV 1&2 Antibody Rapid Test	Oncoprobe Biotech	No	No	No	Yes & No	No	No	No	No	No	No	No	No	No

Table 11: Ease of use score of simple/rapid HIV test kits.

Feature	Scoring	Serodia HIV-1/2	Entebe HIV Dipstick	Immuno-comb II HIV 1&2 BiSpot	HIV-Spot	Hexagon HIV	Determine HIV 1/2	HIV 1&2 Antibody Rapid Test
Type of specimen	(plasma/serum only =0, plasma & serum=1, whole blood, plasma & serum =2,)	1	1	1	1	2	1	1
Use of fresh specimen is compulsory	(y=0,n=1)	1	1	1	1	1	1	1
Specimen volume	(<50 uL=1, >50uL=0)	1	0	1	1	1	1	1
Need of additional reagent	(y=0,n=1)	1	1	1	1	1	1	1
Availability of reading equipment	(y=1,n=0)	0	0	0	0	0	0	0
Need of additional equipment	(y=0,n=1)	0	1	0	1	0	0	1
Number of steps	(1=3, 2=2, 3=1, >3=0)	1	1	0	0	0	3	2
Processing time	(<15min=2, 15-30 min=1, >30 min=0)	0	1	0	2	2	2	1
Availability of controls	(y=1,n=0)	1	1	1	1	0	0	0
Availability of specimen addition monitoring	(y=1,n=0)	0	0	1	1	0	1	1
Reading time range	(<2min=0, 2-5 min 1, >5 min=2)	2	2	2	2	0	2	No data

Table 11: Ease of use score of simple/rapid HIV test kits (continued).

Feature	Scoring	Serodia HIV-1/2	Entebe HIV Dipstick	Immuno-comb II HIV 1&2 BiSpot	HIV-Spot	Hexagon HIV	Determine HIV 1/2	HIV 1&2 Antibody Rapid Test
Reagents are ready for use	(y=1,n=0)	0	0	1	0	1	1	1
Storage of reagents	(ambient possible=1, 2-8C=0)	0	0	0	1	0	1	1
Reagent stability after reconstitution (at 2-8C)	(<1wk=0, 1-4wk=1, 6-8wk=2, >8 wk & exp date=3)	1	1	3	3	3	3	3
Total score		9	10	12	15	11	17	14

Discussion

Several lessons were learned from this test kit evaluation. First, several of the anti-HIV test kits used in Indonesia do not have published performance profiles. None of the evaluated test kits evaluated perform at a level higher than the profile indicated by the WHO. Several of the test kits had inferior performance profiles as compared with published WHO profiles or a performed, in this evaluation, at the lower limit of confidence limits suggested by confidence levels published by the WHO or others.[1, 3]

The results of this HIV antibody test kits evaluation suggests that individual in country decisions to purchase and/or allow the sale of HIV antibody test kits benefit from focused in country test kit evaluations using anti-HIV test kits evaluated under local conditions using test kits sold in country and local samples.

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Appendix 1

Preparation and Use of Quality Control samples

Introduction

Variation in the kit's performance may have an effect on the test results. The use of QC samples in routine Enzyme Immunoassays is to monitor the kit's performance variation, which includes both systematic variation and random variation, but it does not determine the validity of a test run.

Preparation of Quality Control Samples

Aim

Quality control sample prepared is to be used to evaluate the reproducibility of anti-HIV detecting kits, which are based on EIA principles.

Materials

- The source of control material is obtained from fresh-frozen plasma bag with high antibody titre (OD > 2.000) with volume < 80 mL.
- As the diluent, anti-HIV non-reactive, HBsAg negative and anti-HCV non-reactive fresh-frozen plasma will be used
- These plasma bags are obtained from the central blood transfusion unit and kept frozen at -70°C until time of preparation.

Equipment and supplies

- Sterile 500 μL Cryotubes and boxes (100)
- Cryo-labels
- Dispenser or repeater
- Sterile dispenser or repeater tips
- Water-bath
- Sterile 50 mL centrifuge tubes
- Centrifuge with 50 mL capacity rotor
- Sterile 100 mL, 0.5 and 1 L plastic bottles
- EIA-based anti-HIV reagent kits
- Semiautomatic pipette: 20-200 μL and 200-1000 μL
- 8 or 12 channel semiautomatic pipette: 20-200 μL
- Microplate washer
- Microplate reader
- Pipette tips: 200 μL and 1000 μL
- Incubator

Establishing antibody levels by titration

All testing will be performed according to the manufacturer's package insert.

Reactive plasma preparation

- The reactive plasma is heat-inactivated at 62°C for 20 minutes.

- After inactivation the reactive plasma mixed well using a rotator or hand mixing and aseptically poured into sterile 50 mL centrifuge tubes. The plasma is centrifuge at 3000 rpm for 10 minutes to separate any precipitation that might develop during freezing.
- The supernatant is collected into a sterile100 mL plastic bottle and kept at 4°C until volume required is determined.
- Aliquot the remainder into 500 uL aliquots and store at –70^{o}C. Aliquots are labelled with a green labels to indicate inactivation.

Preparation of diluent

- The anti-HIV, HBsAg and anti-HCV non-reactive plasma is thawed in water-bath at 37°C for 20 minutes.
- The thawed plasma is mixed well by inversion, aseptically poured into sterile 50 mL centrifuge tubes and then centrifuged at 3000 rpm for 10 minutes to separate any precipitation that develop during freezing.
- The supernatant is collected into sterile 0.5 L plastic bottle and kept at 4°C.

Making and testing a serial dilution of the reactive plasma

- Determine the volume of plasma necessary to produce doubling dilutions according to the volume required by the assays. Alternatively a master doubling dilution series can be prepared and maintained at –70^{o}C.

- Make a two-fold serial dilution of the reactive plasma starting at the dilution of 1:2 until 1: 32768 (15 serial dilution) using the diluent.
- Each titration will be tested singly using each anti-HIV EIA for which an appropriate QC sample is not available.
- Record and plot the OD/CO ratios to view the sigmoidal response curve. If needed, further dilutions can be tested to obtain a more accurate result.

Determination of dilution for the QC sample

- Select the titration that produce OD/CO ratio between 2-3. For the assays that have high cut-off (0.500-0.600), the selection of dilution is the titration that produce OD/CO ratio between the positive control and the cut-off.

Preparation of QC sample

- Calculate the total volume required. This will depend on:
    - The sample volume required by the assay per run
    - How many runs of the assay are performed on average per unit time.
- Calculate the volume of reactive plasma needed. Using the following formula: $x_1.y_1 = x_2.y_2$ (x_1= volume of neat plasma, $y_1 = 1$, x_2= total volume required, y_2 = titration level)
- Add the calculated volume (x_1) of neat reactive plasma to a sterile appropriate-sized tube / bottle.

- Add the required volume of diluent using a pipette, cylinder or volumetric flask.
- Mix them thoroughly either by inversion or magnetic stirrer, depending on the volume being mixed.
- Aliquot the mixture into 500 μL labelled-cryotubes and keep at 4°C until homogeneity testing is completed.

Homogeneity testing for QC samples

- Take 10 aliquots randomly.
- Assign number to each aliquot.
- Test each aliquot in duplicate.
- Calculate the CV of OD/CO ratio.
- Accept the batch if the CV < 20 %.

Storage

- Once a batch of QC samples has been accepted, store them at -70°C.

Usage

- One aliquot will be use for 1 week and during the usage it is stored at 4°C.
- When a new aliquot has to be taken from the freezer the date of thaw should be written on the tube and the last week's aliquot has to be discarded.

Establishment of QC range

When an assay is to be used on an ongoing basis, a range into which the QC sample result should fall needs to be determined.

- 3 kits with different lot or batch numbers have to be used to establish QC range
- Test at least 12 replicates of one QC sample aliquot using one batch of the test kit. Repeat the testing of the same QC sample aliquot using the same batch of test kit with the same number of replicates.
- Using new QC sample aliquots for each of the other 2 batches of the test kits, test the same number of replicates as in the first batch. Repeat as above.
- Calculate the mean and standard deviation of OD/CO ratio from the 72 results.
- If the number of outliers < 10 % of the points, remove the outliers and re-calculate the mean and standard deviation of the OD/CO ratio.
- Use the new range of mean $\pm$ 2 SD as the QC sample range.

Use of QC sample in routine run

- Test one QC sample in duplicate on each run.
- Calculate the mean of OD/CO ratio
- Record each of the results in the QC chart (Shewart chart and Cusum chart)

- If the result was out of range or a trend of systematic error was noticed, do an investigation to determine the probable cause.
- The common causes of variation are:
    - Systematic variation
        - High absorbance
            - Insufficient washing
            - Incorrect wave length
            - Contaminated substrate
            - Incubation time too long or temperature too high
            - Assay background
            - Using a kit batch which reacts higher than the mean of all batches
        - Lower absorbance
            - Problem with blank
            - Expired kit
            - Contaminated conjugate
            - Incubation time too short or temperature too low
            - Incorrect storage of kits
            - Incorrect filter wavelength
            - Kit reagents not at room temperature when tested
            - Using a kit batch, which reacts lower than the mean of all batches.
    - Random variation

- Poor pipette precision
- Poor mixing of sample
- Reader not calibrated
- Washing ineffective or not consistent
- Transcription error
- Sample mix up

Records

Records will include:

- Certificates from the manufacturers on the performance of the incubator, washer, reader, and pipettes.
- Temperature monitoring record of the incubators, refrigerators, freezer and cold room.
- Worksheets.
- Printout of the results.
- Calculations
- Sigmoidal response curve
- Control charts.

Appendix 2

QUESTIONNAIRE FOR GENERAL CHARACTERISTICS OF THE SIMPLE / RAPID ASSAY

1. Name of the assay : ……..
2. Manufacturer : ……..
3. Assay type : ……..
4. Antigen type : ……
5. Solid phase : ……
6. Number of test per kit : ……
7. Lot number 1-3 : ……
8. Expiry date 1 – 3 : ……
9. Shelf life at (..^{o}C) : ……
10. Volume of sample needed (μL) : ……
11. Final dilution of sample : ……
12. Stability of reagent after reconstitution at (….^{o}C)
    - Controls : ……
    - Antigen : ……
    - Sample diluent : ……
    - Conjugate : ……
    - Substrate : ……
    - Wash buffer : ……

13. Number of control per test run
    - Negative : ……
    - Positive : ……
    - Blank : ……

14. Incubation temperature : ……
15. Reading : ……
16. Total time to perform the assay (hh:min): ……
17. Number of sera per run (min. - max.) : ……
18. Definition of positive results : ……

19. Definition of grey zone (if any) : ……

20. Storage at (…^{o}C) : ……

21. Equipment needed but not provided in the kit (tick ✔ which applies)

- ❑ Washer
- ❑ Incubator / water-bath
- ❑ Spectrophotometer
- ❑ Refrigerator (storage)
- ❑ Agitator / rocker
- ❑ Aspiration device
- ❑ Automatic pipette (μL)
- ❑ Multichannel pipette (μL)
- ❑ Disposable tips
- ❑ Dilution tubes / rack, microtiterplate
- ❑ Plate covers
- ❑ Absorbent paper
- ❑ Reagent trough

Completed by……..Date……

Appendix 3

QUESTIONNAIRE FOR GENERAL CHARACTERISTICS OF THE EIA-BASED ASSAY

1. Name of the assay : ……
2. Manufacturer : ……
3. Assay type : ……
4. Antigen type : ……
5. Solid phase : ……
6. Number of test per kit : ……
7. Lot number 1-3 : ……
8. Expiry date 1 – 3 : ……
9. Shelf life at (..^{o}C) : ……
10. Volume of sample needed (μL) : ……
11. Final dilution of sample : ……
12. Total time of incubation (hh:min.) : ……
13. Wavelength (nm) single : ……
    double : ……
14. Stability of sample after reconstitution at (….^{o}C)
    - Control : ……
    - Antigen : ……
    - Sample diluent : ……
    - Conjugate : ……
    - Substrate : ……
    - Wash buffer : ……
15. Number of control per test run
    - Negative : ……
    - Positive : ……
    - Blank : ……
16. Incubation temperature : ……
17. Reading : ……
18. Number of sera per run (min. - max.) : ……
19. Cut-off value : ……

Grey zone (if any) : ……

20. Storage at (…^{o}C) : ……

22. Equipment needed but not provided in the kit (tick ✔ which applies)

- ❑ Washer
- ❑ Incubator / water-bath
- ❑ Spectrophotometer
- ❑ Refrigerator (storage)
- ❑ Agitator / rocker
- ❑ Aspiration device
- ❑ Automatic pipette (μL)
- ❑ Multichannel pipette (μL)
- ❑ Disposable tips
- ❑ Dilution tubes / rack, microtiterplate
- ❑ Plate covers
- ❑ Absorbent paper
- ❑ Reagent trough

Completed by……..Date……

Appendix 4
EVALUATION OF EASE OF USE FOR SIMPLE / RAPID TEST KIT

		Score
1. Type of specimen :	□ plasma / serum only	0
	□ whole blood only	1
	□ both whole blood and plasma & serum	2
2. Use of fresh specimen is compulsory :	□ yes	0
	□ no	1
3. Specimen volume required	□ ≤ 50 μL	1
	□ > 50 μL	0
4. Need of additional reagent (apart from available reagents in kit)	□ yes	0
	□ no	1
5. Availability of reading equipment / instrument	□ yes	1
	□ no	0
6. Need of additional equipment (apart from available equipment in the kit)	□ yes	0
	□ no	1
7. Number of processing / testing steps	□ 1	3
	□ 2	2
	□ 3	1
	□ > 3	0
8. Processing / testing time	□ < 15 minutes	2
	□ 15 – 30 minutes	1
	□ > 30 minutes	0
9. Availability of positive / negative control specimens :	□ yes	1
	□ no	0
10. Availability of specimen addition monitoring system	□ yes	1
	□ no	0
11. Reading time range	□ < 2 minutes	0
	□ 2 – 5 minutes	1
	□ > 5 minutes	2
12. Reagents are ready for use	□ yes	1
	□ no	0
13. Storage of reagents	□ ambient t^o possible	1
	□ 2 – 8^{o}C required	0
14. Stability of reconstituted reagents at 2 – 8^{o}C	□ < 1 week	0
	□ 1 – 4 weeks	1
	□ 6 – 8 weeks	2
	□ expiry date	3

Completed by……..Date…….

Appendix 5
EVALUATION OF EASE OF USE FOR EIA TEST KIT

1. Machine based	□ yes	1		□ no	0
2. Dedicated equipment needed	□ yes	0		□ no	1
3. Format of strip			□ ≤ 4		2
			□ 8		1
			□ 16		0
4. Type of specimen	□ serum or plasma only				0
	□ both serum and plasma				1
5. If plasma, any restriction anticoagulant	□ yes	0		□ no	1
6. Specimen volume required			□ < 50 μL		2
			□ 50 – 100 μL		1
			□ > 100 μL		0
7. Sample preparation step needed	□ yes	0		□ no	1
8. Controls included in the kit's cost	□ yes	1		□ no	0
9. Controls are ready for use	□ yes	1		□ no	0
10. Reagent preparation step needed	□ yes	0		□ no	1
11. Additional reagents needed	□ yes	0		□ no	1
12. Incubation period			□ < 2 hours		2
			□ 2 – 3 hours		1
			□ > 3 hours		0
13. Need of special incubation condition	□ yes	0		□ no	1
14. Number of step (excluding washing step)			□ 3		2
			□ 4		1
			□ 5		0
15. Availability of sample additional monitoring	□ yes	1		□ no	0
16. Storage of reagents		□ ambient t^o possible			1
		□ 2 – 8^{o}C required			0
17. Stability of reconstituted reagents at 2 – 8^{o}C		□ < 1 week			0
		□ 1 – 4 weeks			1
		□ 6 – 8 weeks			2
		□ expiry date			3
18. Reading the result : availability of grey zone		□ yes	0	□ no	1

Completed by……Date ……

Table of content