DONOR INFECTIOUS DISEASE TESTING

Multinational assessment of blood-borne virus testing and transfusion safety on the African continent...

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ABBREVIATIONS

GDP = gross domestic product; GEE(s) = generalized estimating equation(s).

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ABSTRACT

BACKGROUND

Failures of blood screening due to low test quality or poor laboratory technique increase the
risk of transfusion-transmitted infections. For this reason, the World Health Organization has recommended a quality control (QC) system for African blood centers.

STUDY DESIGN AND METHODS

We conducted a cross-sectional research assessment of test performance at 51 blood centers in 17 African countries. A blinded, standardized panel containing 25 samples positive for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) and negative controls was tested by the centers using their operational infectious disease testing consisting of rapid tests, enzyme immunoassays (EIAs), or antigen- antibody EIAs. Nucleic acid testing was not performed.

RESULTS

The overall performances of the 42 assays were the lowest for hepatitis B surface antigen (75.6% sensitivity, 94.5% specificity), then for HCV (80.0% sen- sitivity, 98.1% specificity) and for HIV (81.4% sensitivity, 99.6% specificity). Poor sensitivity was driven by the use of rapid tests, which had sensitivities of 47.4% for HBV, 63.7% for HCV, and 72.4% for HIV. From a blood screening point of view, 321 (5.6%) infected units would have been transfused due to false-negative results. Assuming that those that were missed by rapid tests (84%) would have been detected by EIAs, 270 viral contaminations (92 HIV, 65 HCV, and 113 HBV) would have been avoided.

CONCLUSION

These results support the discontinua- tion of rapid tests and implementation of antigen-antibody EIAs whenever possible in Africa. This successful QC program highlights the need for promoting such periodic external quality assessment studies.

Transfusion-transmitted infections by viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are prevented by testing of blood products throughout the world. Paradoxically, high-income countries, where the prevalence of these pathogens is low, have the most comprehensive screening systems, while other countries, where the prevalence is the highest, have the worst systems.[1] In consequence, the risk of transfusion-transmitted infection has become very low in the former[2,3] while remaining high in the latter, particularly in sub-Saharan Africa.[4] The same sub-Saharan African countries with the highest red blood cell requirements for anemic children and women are those which have not yet achieved robust and regular volunteer donor recruitment and cannot reduce the risk of transfusion-transmitted agents by expensive nucleic acid testing (NAT). Moreover, countries that depend on antibody or antigen screening assays are challenged by the relatively high cost of these tests, the difficulty in maintaining the cold chain, and a shortage of well-trained staff. This is significant, because failures of serologic screening due to low test quality or from poor laboratory expertise may adversely affect this otherwise cost-effective intervention.[5] For this reason, the implementation of a quality control (QC) system has been recommended to the African Blood Centers by the World Health Organization (WHO), but has not yet been broadly implemented. A newly formed network of African blood transfusion specialists has therefore decided to perform a baseline evaluation of serologic testing performance. Building on the experience of a pilot study,[6] the group now reports a QC study involving 51 blood centers belonging to 17 African countries. The aims of this research study were:

1. to allow each participating blood center to benchmark its laboratory procedures and assays;
2. to identify the most frequent reasons for poor quality, to define a consensus strategy based on appropriate assays; and
3. to provide to the health authorities preliminary data on the feasibility of ongoing QC assessment.

MATERIALS AND METHODS

Study design

The study, conducted by the National Institute of Blood Transfusion in Paris, France, was a cross-sectional assessment of test performance using a standardized and blinded-coded panel. This panel was made up of 25 samples including eight negative samples; five anti-HIV– (four HIV-1 and one HIV-2), four anti-HCV–, and five hepatitis B surface antigen (HBsAg)-positive samples (confirmed by neutralization assay); and three mixed samples to mimic coinfections (HCV/HIV, HBV/HCV, and one HIV/HCV; Table 1). All samples (except S3) were obtained after dilutions in a negative sample to obtain a range of the marker concentrations. Each sample was pedigreed in the French Laboratory Reference with the following enzyme immunoassays (EIAs): Vidas HIV DUO Ultra (bioMérieux, Craponne, France), Genscreen HIV-1/2 v2 (Bio-Rad, Marnes-La-Coquette, France), and Genscreen HIV Ag/Ab Ultra (Bio-Rad), for anti-HIV; ETI MAK4 (Dia Sorin, Saluggia, Italy) for HBsAg; and Monolisa HCV Ag/Ab Ultra (Bio-Rad) for anti-HCV. Moreover, positive confirmatory results for HIV and HCV were obtained with WB HIV (HIV Blot 2.2, Abbott, Rungis, France) and recombinant immunoblot assay (RIBA) HCV (Ortho Clinical Diagnostics, Issy, France). The assays were performed according to the manufacturer's instructions. The panel was distributed in a coded fashion and tubes within each panel were numbered uniquely to allow for blinded testing. The sample panels were sent to a coordinator in each participating country under appropriate transport conditions for maintaining frozen samples. The country coordinators were responsible for the retrieval of panels on arrival in the country and redistribution to participating centers. The panels were required to be maintained frozen at a minimum of not more than -20°C before and during reshipping to the participating centers in the country. Sixty panels were distributed to 51 labs of 17 countries (Fig. 1).

Testing of the panels in participating labs

The panel was required to be tested twice in each lab by using routine techniques and test conditions normally applied to donor screening in the lab. The 51 labs used 42 different assays, (Table 2): 10 for HIV (five rapid tests, one Ab EIA, four Ag/Ab combination assays), 15 for HCV (eight rapid tests, five Ab EIA, two Ag/Ab combination assays), and 17 for HBsAg (10 rapid tests, seven EIA). Twenty-two labs tested the panel with more than one (two to four) assay with the objective to use the different assays that they would be able to obtain if one of them was temporarily unavailable. This strategy generated a total of 233 series of results: 89 for HIV (48 with rapid tests, one with Ab EIA, 40 with Ag/Ab combination assays), 72 for HCV (30 with rapid tests, 16 with Ab EIAs, 26 with Ag/Ab combination assays), and 72 for HBV (31 with rapid tests, 41 with EIAs). Among the 60 panels tested, 58 were tested through one assay for HIV Ab screening (22 rapid tests, one EIA, 35 Ag/Ab EIAs), and two panels were tested according to a strategy based on two HIV assays (one with two rapid tests, one with a rapid assay in combination with a Ag/Ab EIA). All panels were tested with only one assay for HCV (25 rapid tests, 14 Ab EIAs, and 21 Ag/Ab EIAs) and for HBsAg (22 rapid tests and 38 Ag EIAs).

Statistical analysis

The results were analyzed in two ways. First, we evaluated the performance of assays by category. Sensitivity was defined as the percentage of correct results among the positive samples and specificity as the percentage of negative results among the negative samples. An assay quality score was established as the percentage of correct results among the results expected as positive or as negative. Overall quality scores according to type of test were also quantitatively analyzed using generalized estimating equations (GEEs) methods in a repeated-measures logistic regression model (SAS 9.1.3 Service Pack 4, SAS Insti- tute, Cary, NC). We also explored country effects using hierarchical cluster analysis. Using the Ward method, the 17 countries were clustered into three groups (high, medium, and low performance), first according to the sensitivity and specificity of each country's test results for each virus separately and second according to the nominal gross domestic product (GDP) per capita of each country. The Ward method ensures that the countries clustered together within each group are more homogeneous. Country groupings were then explored as covariates together with test type in subsequent GEE models.

A final analysis was aimed at evaluating the reliability of screening strategies regarding test type used for each virus and included calculation of the number of false-positive results (which could have led to wrongly discarded blood donations) and false-negative results (which could have led to the infection of transfusion recipients).

For labs using a combination of assays to identify an infected blood unit, viral screening was considered as positive when at least one of both assays provided a positive result.

Table 1: Results of pedigree testing in the reference laboratory of the 25 samples included in the panel*

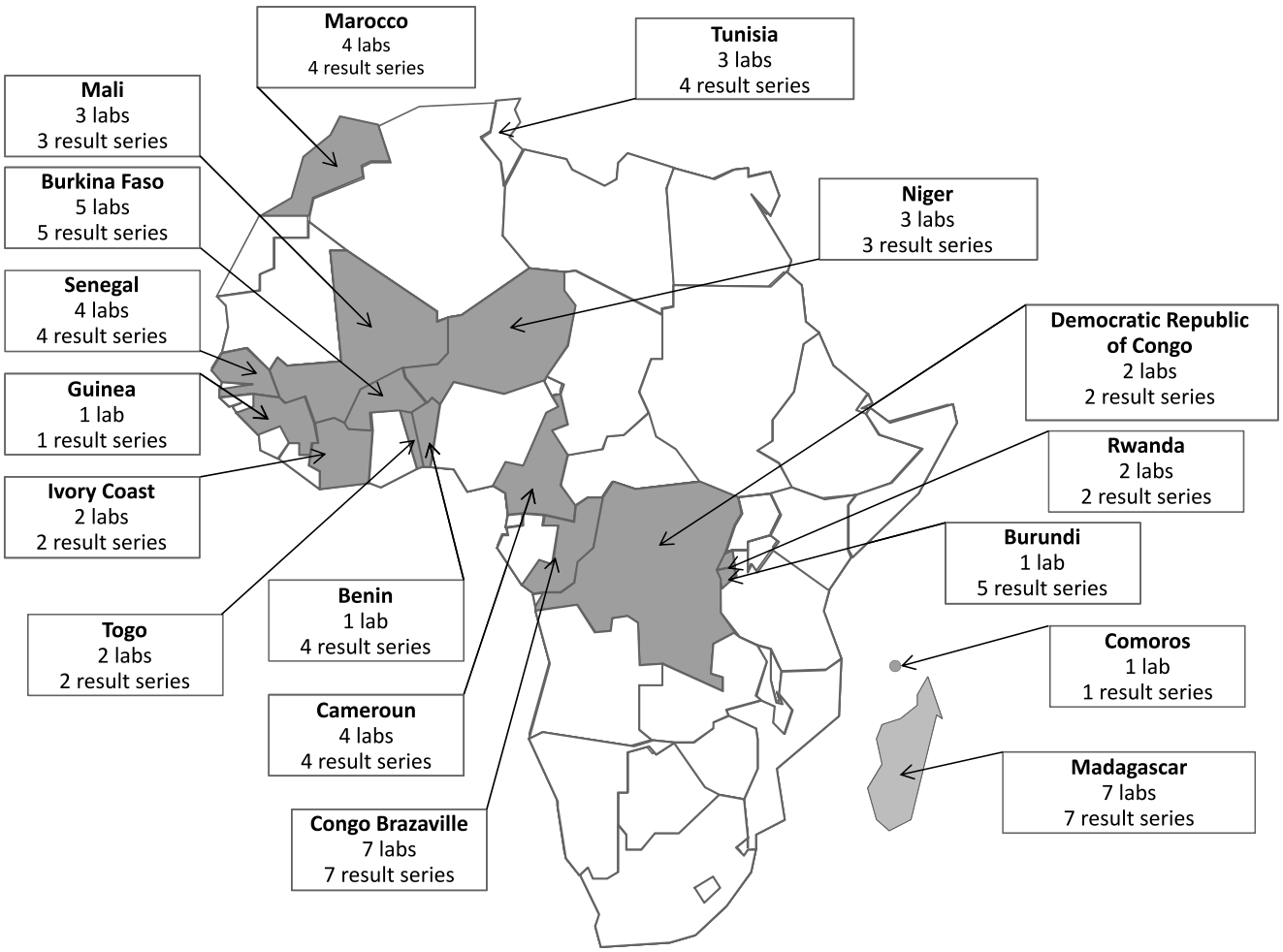
Samples		HIV				HBV	HCV	
		Vidas Duo Ultra (bioMérieux) S/CO	Genscreen HIV-1/2 v2 (Bio-Rad) S/CO	Genscreen HIV Ag/Ab Ultra (Bio-Rad) S/CO	WB HIV-1 (Abbott)†	ETI MAK-4 (Dia Sorin) S/CO‡	Monolisa HCV Ag/ Ab Ultra (Bio-Rad) S/CO	RIBA HCV (Ortho Clinical Diagnostics)†
S2	HIV-1 gt B	53.1	19.7	>11.4	gp160, gp120, p24	0.06	0.11	NT
S10	HIV-1 gt B	58.4	20.3	>11.4	All bands	0.09	0.21	NT
S22	HIV-1 gt B	7.04	2.4	0.68	p24	0.06	0.18	NT
S17	HIV-1 gt B	7.32	2.41	0.65	p24	0.20	0.12	NT
S7	HIV-2	67.2	18.3	6.09	pep HIV-2, p24	0.15	0.30	NT
S5	HCV gt1a	0.36	0.18	0.27	NT	0.12	6.2	Core 4+, NS3 4+, NS4 4+, NS5 4+
S13	HCV gt3a	0.3	0.12	0.27	NT	0.06	3.03	Core 1+, NS3+, NS4 4+/–, NS5+
S24	HCV gt 3a	0.28	0.11	0.23	NT	0.09	2.15	Core 1+, NS3+, NS4 4+/–, NS5+/–
S18	HCV gt 1b	0.32	0.15	0.25	NT	0.00	2.24	Core 1+, NS3+, NS4 4+/–, NS5+/–
S3	HBsAg (>100 ng/ mL) gt D‡	0.36	0.13	0.27	NT	100.6	0.21	NT
S14	HBsAg (10 ng/mL) gt B‡	0.36	0.12	0.29	NT	102	0.20	NT
S21	HBsAg (1 ng/mL) gt B‡	0.32	0.13	0.26	NT	16.1	0.18	NT
S25	HBsAg 0.2 ng/mL) gt B‡	0.36	0.13	0.27	NT	2.90	0.2	NT
S15	HBsAg (1 ng/mL) gt D‡	0.32	0.16	0.27	NT	16.2	0.18	NT
S6	HIV-1 + HCV	58.2	20.3	>11.4	All bands (except p18)	0.00	6.40	Core 4+, NS3 4+, NS4 4+, NS5 4+
S11	HIV-1 + HBsAg	58.6	20.1	>11.4	All bands (except p18)	102	0.18	NT
S20	HCV + HBsAg	0.32	0.13	0.27	NT	99.5	5.96	Core 4+, NS3 4+, NS4 4+, NS5 4+
S1	Negative	0.32	0.13	0.30	NT	0.15	0.09	NT
S4	Negative	0.36	0.13	0.29	NT	0.09	0.11	NT
S8	Negative	0.32	0.12	0.29	NT	0.15	0.09	NT
S9	Negative	0.32	0.11	0.29	NT	0.00	0.14	NT
S12	Negative	0.32	0.10	0.27	NT	0.03	0.18	NT
S19	Negative	0.28	0.11	0.31	NT	0.10	0.16	NT
S23	Negative	0.36	0.41	0.24	NT	0.00	0.10	NT
S16	Negative	0.32	0.28	0.37	NT	0.06	0.11	NT

* Numeric data represent ratios of sample optical density (OD) to the cutoff OD. Result was considered as positive when S/CO >0.9 and for Gencreen HIV Ag/Ab Ultra when >0.5. All samples (except S3) were obtained after dilutions in a negative sample to obtain a range of the marker concentrations.

† Positive bands only.

‡ HBsAg level (in ng/mL) determined against the French reference panel. gt = genotype; NT = not tested.

Figure 1: Francophone African countries that participated in the study.



RESULTS

Overall performance of the assays

Among the 42 assays 26 were used by at least two centers generating 2 to 32 series of results per assay (Table 2).

Overall, 79.1% of the 1539 results expected positive were found positive, and 98.5% of the 4176 expected negative were negative, leading to an overall quality score of 93.3%.

The overall sensitivity was the lowest for HBsAg with 75.6% versus 80.0% for HCV and 81.4% for HIV. The overall specificity was also lowest for HBsAg at 94.5% versus 98.1% for HCV and 99.6% for HIV. The sensitivities of rapid tests were always the lowest regardless of the marker: 47.4% for HBsAg, 63.7% for HCV, and 72.4% for HIV versus 96.8, 96.9, and 93.1%, respectively, with the EIAs.

GEEs were used with the rapid tests as reference category. For HIV, Ag/Ab EIA assays performed significantly better than rapid tests (odds ratio [OR], 3.21; 95% confidence interval [CI], 2.04-5.05) but the single Ab EIA test used here performed worse than rapid tests (OR, 0.51; 95% CI, 0.44-0.60).

For HCV, the Ag/Ab EIA assays were not significantly better than rapid tests (OR, 1.50; 95% CI, 0.58-3.84), but the Ab EIA tests were (OR, 2.37; 95% CI 1.50-3.74). For HBV, the Ag EIAs were significantly better than the rapid tests (OR, 3.29; 95% CI 1.85-5.88).

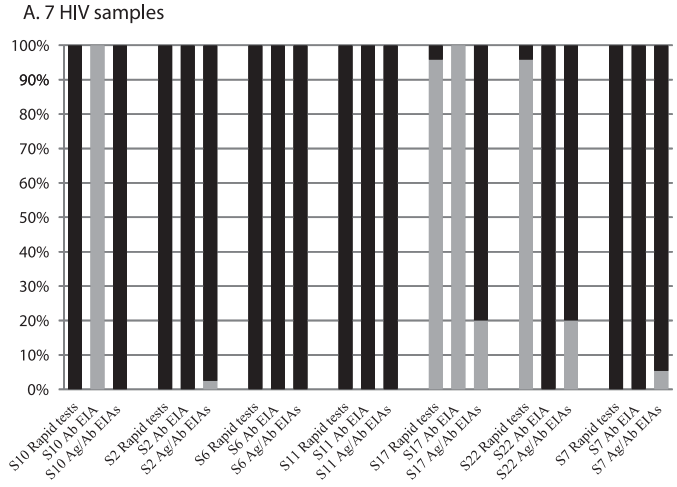
Sixteen (38%) of the 42 assays demonstrated a 100% quality score in at least one lab (Table 2); of these 16, only four assays had perfect scores in all labs that used them. When analyzed by test type, the number of assays achieving a 100% quality score in at least one lab was 4 of the 23 rapid tests (one of five for HIV, three of eight for HCV, 0 of 10 for HBV), six of the 13 Ab or Ag EIAs (zero of one for HIV, four of five for HCV, two of seven for HBV), and six of the six Ag/Ab EIAs (all of four for HIV, all of two for HCV).

TABLE 2. Results of HIV, HCV, and HBsAg testing according to the assays

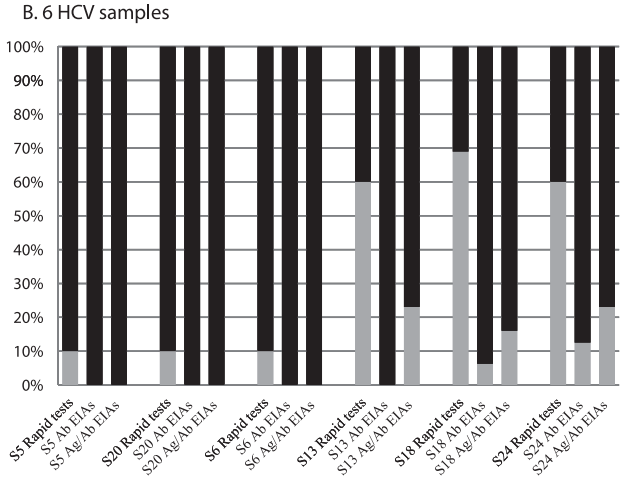
Assay	Supplier	N series of results	Total results expected positive†	Overall sensitivity‡ (%)	Range according to the centers (%)	Total results expected negative	Overall speci city§ (%)	Range according to the centers (%)	Total results expected correct	Assay quality score|| (%)
HIV		89	618	81.4		1595	99.6		2213	94.5
Rapid tests										
Determine HIV-1/2	Abbott	32	223	71.9	71.4-100	571	99.3	88.9-100	794	91.6
Immunocomb II HIV-1&2 Bispot*	Orgenics	9	62	73.0	71.4-100	161	100	NA	223	92.5
GENIE II HIV-1/HIV-2	Bio-Rad	4	28	71.4	71.4	72	100	NA	100	92.0
SD Bioline HIV-1/2 Version 3.0	SD Standard Diagnostics	2	14	71.4	71.4	36	100	NA	50	92.0
RAPID 1-2-3 HEMA Express HIV	Hema Diagnostic Systems	1	7	71.4	71.4	18	100	NA	25	92.0
Total rapid tests		*48*	*334*	*72.4*	*71.4-100*	*858*	*99.5*	*88.9-100*	*1192*	*91.9*
Ab EIA										
Enzygnost anti-HIV-1/2/plus	Siemens	1	7	42.9		18	100		25	84.0
Ag/Ab EIAs										
Genscreen ULTRA HIV Ag-Ab*	Bio-Rad	29	203	92.6	57-100	522	99.6	94.4-100	725	97.6
Axsym HIV Ag/Ab Combo*	Abbott	4	25	100	NA	71	100	NA	96	100
Architect HIV Ag/Ab Combo*	Abbott	3	21	80.9	57-100	54	100	NA	75	94.7
Murex HIV Ag/Ab combination	Abbott Murex	4	28	100	NA	72	100	NA	100	100.0
Total Ag/Ab EIAs		*40*	*277*	*93.1*	*57-100*	*719*	*99.7*	*94.4-100*	*996*	*97.9*
HCV		72	421	80.0		1292	98.1		1713	93.7
Rapid tests										
Rapid anti-HCV test*	AccuBioTech	1	6	100	NA	19	100	NA	25	100
HCV TRI-DOT	J. Mitra & Co.	2	12	50.0	50	38	100	NA	50	88.0
Hexagon HCV*¶	Human	9	53	83.0	50-100	149	98.7	94.7-100	202	94.6
Immunocomb II HCV	Orgenics	2	12	58.3	50-66.6	38	97.4	94.7-100	50	88.0
Rapid Signal HCV Dipstrip	Orgenics	11	66	47.0	16.6-100	208	95.7	73.7-100	274	84.0
SD Bioline HCV*	SD Standard Diagnostics	3	18	72.2	50-100	56	96.4	94.7-100	74	90.5
One-step HCV test	Pistis	1	6	66.7	NA	19	100	NA	25	92.0
CYPRESS anti-HCV	Cypress Diagnostics	1	6	50.0	NA	19	100	NA	25	88.0
Total rapid tests		*30*	*179*	*63.7*	*16.6-100*	*546*	*97.4*	*73.7-100*	*725*	*89.1*
Ab EIAs										
INNOTEST HCV Ab*	Innogenetics	2	12	91.7	83.3-100	38	100	NA	50	98.0
Architect anti-HCV*	Abbott	3	18	100	NA	57	100	NA	75	100
Axsym HCV Version 3.0*	Abbott	5	30	100	NA	94	94.7	89.5-100	124	96.0
Anti HCV Elisa	Fortress Diagnostics	1	6	83.3	NA	19	100	NA	25	96.0
Murex anti-HCV Version 4.0*	Abbott Murex	5	30	96.7	83.3-100	95	98.9	94.7-100	125	98.4
Total Ab EIAs		*16*	*96*	*96.9*	*83.3-100*	*303*	*98.0*	*89.5-100*	*399*	*97.7*
Ag/Ab EIAs										
Monolisa HCV Ag-Ab ULTRA*	Bio-Rad	19	104	100	NA	310	99.6	94.7-100	414	99.7
Murex HCV Ag/Ab combination*	Abbott Murex	7	42	61.9	50-100	133	97.7	89.5-100	175	89.1
Total Ag/Ab EIAs		*26*	*146*	*89.0*	*50-100*	*443*	*99.1*	*89.5-100*	*589*	*96.6*
HBsAg		72	500	75.6		1289	97.5		1789	91.3
Rapid tests										
One-step HBsAg test	AccuBioTech	1	7	57.1	NA	18	100	NA	25	88.0
HEXAGON HBsAg	Human	9	62	41.9	14.2-71.4	161	99.4	94.4-100	223	83.4
Immunocomb II AgHBs	Orgenics	1	7	42.9	NA	18	100	NA	25	84.0
Determine HBsAg	Abbott	11	76	53.9	28.5-57.1	198	99.5	94.4-100	274	86.9
SD Bioline HBsAg	SD Standard Diagnostics	4	28	53.6	42.8-57.1	71	98.6	94.4-100	99	85.9
Rapid Signal HBs Ag Dipstrip	Orgenics	1	7	42.9	NA	18	100	NA	25	84.0
HEP-CHECK-1	Vedalab	1	7	14.3	NA	18	88.9	NA	25	68.0
One-step HBsAg test	Pistis	1	7	42.9	NA	18	100	NA	25	84.0
AgHBs STRIPS test	Taytec	1	7	42.9	NA	18	100	NA	25	84.0
One-step HBs Ag test strip	Plamatec	1	7	42.9	NA	18	100	NA	25	84.0
Total rapid tests		*31*	*215*	*47.4*	*14.2-71.4*	*556*	*99.1*	*94.4-100*	*771*	*84.7*
HBsAg EIAs										
Architect AgHBs qualitative*	Abbott	3	21	95.2	85.4-100	54	98.2	94.4-100	75	97.4
Axsym AgHBs Version 2*	Abbott	5	34	100	NA	88	78.4	61.1-100	122	84.4
HBsAg ELISA	Human	1	6	66.7	NA	17	100	NA	23	91.3
Hépanostika HBsAg Ultra	bioMérieux	1	7	85.7	NA	18	100	NA	25	96.0
Monolisa HBs Ag ULTRA	Bio-Rad	28	196	100	NA	502	98.6	88.8-100	698	99.0
Murex HBsAg Version 3.0	Abbott Murex	2	14	85.7	85.7	36	100	NA	50	96.0
HBs Ag Elisa Kit	Biorex Diagnostics	1	7	57.1	NA	18	100	NA	25	88.0
Total HBsAg EIAs		*41*	*285*	*96.8*	*85.4-100*	*733*	*96.3*	*61.1-100*	*1018*	*96.4*
All markers										
Rapid tests (n = 23)		109	728	62.8		1960	98.8		2688	89.0
EIAs (n = 13)		58	388	95.9		1054	96.9		1442	96.6
Ag/Ab EIAs (n = 6)		66	423	91.7		1162	99.5		1585	95.5
Total (n = 42)		*233*	*1539*	*79.1*		*4176*	*98.5*		*5715*	*93.3*

* 100% quality score in at least one lab.
† The result obtained for each positive sample was considered as correct when the sample/cut off (S/CO) value was equal to or above 0.9 for EIAs and, for rapid tests, when the result was expressed as “positive” or “doubtful.” One exception was made for Samples S17 and S22, which were considered as correct when the ratio was higher than 0.5 with Genscreen HIV Ag/Ab Ultra.
‡ The sensitivity was de ned as the percentage of correct results among the positive samples.
§ The speci city was de ned as the percentage of negative results among the samples negative for the marker.
|| The quality score was the percentage of correct results among all samples of the panel.
¶ One lab, which had obtained an unusually high rate (p = 0.02) of false-positive results (12/19), was excluded from the analysis.
NA = not applicable.

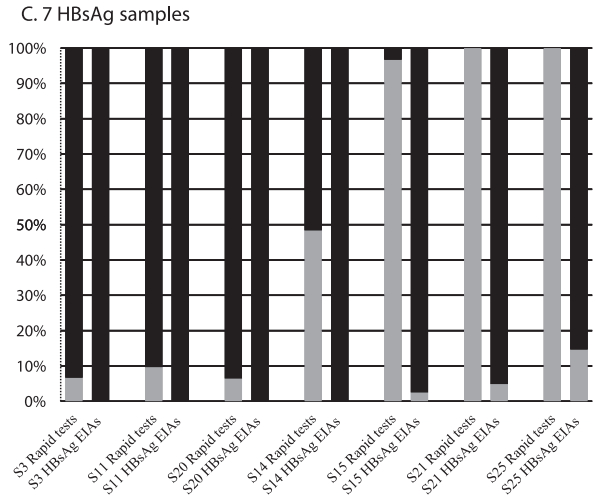
Figure 2: Proportions of positive (■) and negative (□) results according to the three categories of assays (rapid tests, Ab or Ag EIA, Ag/Ab EIAs).



(A) Results in seven anti-HIV–positive samples. HIV-1 samples were strongly (S10), intermediately (S2), and weakly (S17 and S22) positive and mixed with, respectively, anti-HCV (S6) and HBsAg (S11); Sample S7 was anti-HIV-2 positive. The total number of results obtained for each sample was 48 with rapid tests (46 for sample S7), one with Ab EIA, and 40 (39 for sample S2) with Ag/Ab EIAs.



(B) Results in six anti-HCV–positive samples. Sample S5 was strongly positive, S13 was intermediately positive, and S18 and S24 were weakly positive; Samples S6 and S20 were mixtures, with, respectively, anti-HIV and HBsAg. The total number of results obtained for each HCV-positive sample was 30 with rapid tests (29 for Sample S18); 16 with Ab EIAs and 23 for Samples S5, S20, and S6; and 25 for Sample S18 and S26 for Samples S13 and S14, with Ag/Ab EIAs.



(C) Results in seven HBsAg-positive samples. The HBsAg levels were higher than 100 ng/mL for Sample S3, 10 ng/mL for Sample S14, 1 ng/mL for Samples S21 and S15, and 0.2 ng/mL for Sample S25; Samples S11 and S20 were mixtures, with, respectively, anti-HIV and anti-HCV. The total number of results obtained for each HBsAg-positive sample was 31 with rapid tests (30 for Samples S3 and S15) and 41 (40 for Samples S3 and S15) with Ag EIAs.

Sensitivity of assays by virus

HIV screening

Assay sensitivities were similar within types of tests: from 71.4% to 73% for rapid tests and from 80.9% to 100% for Ag/Ab EIAs (Table 2). The percentage of positive results is illustrated in Fig. 2A. The four samples presenting a confirmatory pattern on HIV-1 Western blot were positive with all the rapid tests and EIAs, except two samples that were falsely negative in two labs using one Ab EIA and one Ag/Ab EIAs, respectively. The two samples with a low anti-HIV titer were mainly negative by rapid tests (only two centers using two rapid tests provided doubtful results). The HIV-2 sample was detected by all assays, except in three labs using Ag/Ab EIAs. Notably, all false-negative results obtained with Ag/Ab EIAs were provided by the same centers

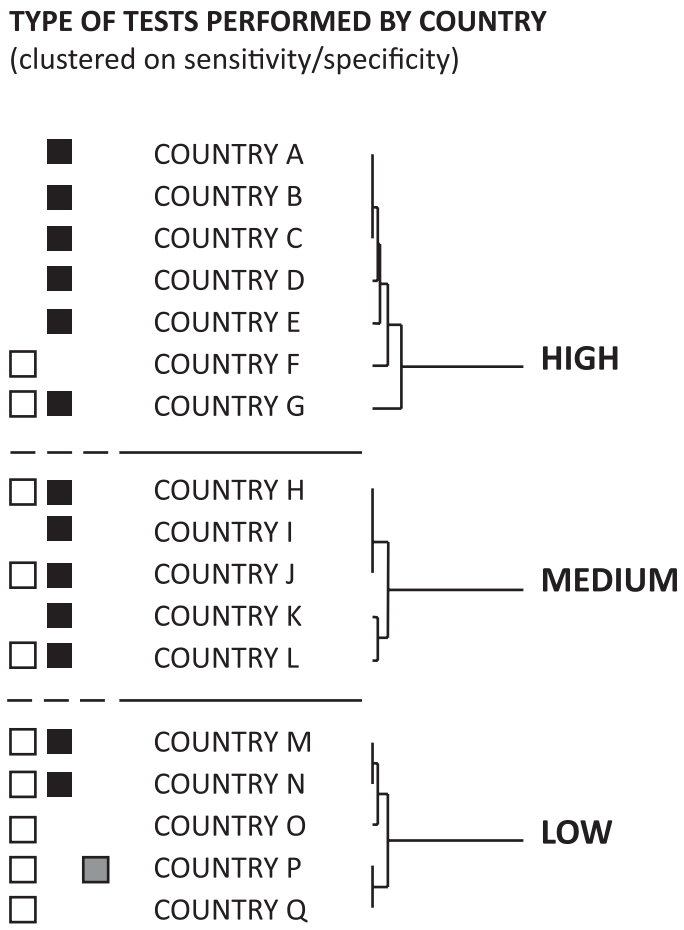
HCV screening

Assay sensitivities (Table 2) varied from 47.0% to 100% for rapid tests, from 83.3% to 100% for EIAs, and from 61.9% to 100% for Ag/Ab EIAs. Figure 2B illustrates the percentage of positive results. Samples with the highest Ab level were detected by all EIAs, although false-negative results were obtained with four rapid tests. Samples with the lowest HCV Ab level were more frequently negative, especially with rapid tests. In contrast to the Monolisa HCV Ag-Ab Ultra, which reached 100% sensitivity, the Murex HCV Ag/Ab combi test failed to detect several positive samples.

HBsAg screening

Assay sensitivity varied according to test type: from 14.3% to 57.1% for rapid tests and from 57.1% to 100% for EIAs. Two of three samples with a high HBsAg level went undetected by two rapid tests, while the third sample went undetected by one of the 11 labs using Determine HBsAg and by the lab using one-step HBsAg test strip. All but one of the samples (borderline with one rapid test) containing less than 1 ng/mL HBsAg were negative with rapid tests. The nine false-negative results were observed in five labs with five different assays (Fig. 2C).

Figure 3: Cluster analysis of laboratory test performance, by country and type of tests performed.



Countries are grouped by high, medium, and low performance (proportion of correct results for all three viral markers).
The types of tests performed by each country are indicated by the colored squares to the left:
(0) rapid tests;
(■) Ag/Ab EIA;
(■) Ag or Ab EIA.

Comparison of screening strategies

When the results of this QC study were analyzed from a blood screening point of view by considering each sample as a blood donation, three main strategies were identified: one (A) based on rapid tests for the three viruses (20 labs, mainly located in regional blood banks); one (B) based on Ag/Ab assays for HIV and HCV testing and a HBsAg EIA (20 labs, mainly in capitals); and one (C) based on an Ag/Ab assay for HIV, an EIA Ab for HCV, and an EIA for HBV (13 labs). Only 17 (28.3%) labs provided 100% of correct results, none used Strategy A, five used Strategy B, and 12 used Strategy C. The false-negative results accounted for a total of 321 samples: 115 HIV (of which 80% missed by rapid tests), 84 HCV (of which 77% missed by rapid tests), and 122 HBV (of which 92.6% missed by rapid tests) samples. Thus, rapid tests gave 262 (84%) false-negative results. Conversely, 64 negative samples were declared as positive (six HIV, 24 HCV, and 32 HBV, of which 36% were with rapid tests). We attempted to separate performance by country from performance by test type. We first used cluster analysis to group countries according to overall quality scores. Figure 3 shows three clusters: high-, medium-, and low-quality scores. However, when GEE analysis was repeated including both test type and country grouping, test type but not country group remained significantly associated with quality score. Most of the high-quality countries used Ag/Ab EIAs exclusively, while medium- and low-quality countries used either rapid tests only or a mixture of rapid and Ag/Ab EIAs. Finally, we clustered countries into three groups by per-capita GDP and performed a GEE analysis including test type and GDP group. For the HBV virus, countries with high GDP per capita performed significantly better than countries with medium and low GDP per capita, with an OR of 1.84 (95% CI, 1.14-2.97). However, for HCV, countries with high GDP per capita performed worse than their counterparts, with an OR of 0.40 (95% CI, 0.19- 0.84). Thus, we were unable to identify country-specific factors other than test type, which contributed significantly to quality.

DISCUSSION

This study measured infectious disease test performance in the blood bank setting in 17 African countries and found sensitivity values for HBV, HCV, and HIV screening that were much lower than expected.

We have demonstrated the poor overall sensitivity of blood screening, especially for HBV (75.6%), but also for HIV (81.4%). This was mainly due to the use of rapid tests, most markedly for HBV. Little variation in performance was observed among countries, showing the overriding influence of assay choice on quality of testing. The low percentage of assays achieving a 100% quality score is cause for concern, as only a few numbers of them reached this score in all labs using these assays. Interestingly, a 100% quality score was never achieved by some assays and only by 17.4% of the rapid tests, 46.2% of the Ag or Ab EIAs, and 100% of the Ag/Ab EIAs.

This could be due, at least in part, to interlaboratory variation, but the gap observed between Ag/Ab EIAs and rapid assays in terms of performance was mostly due to the increased capability of Ag/Ab EIAs to detect challenging samples. Many studies have reported the poor sensitivity of rapid tests for detection of anti-HIV,[7-9] anti-HCV,[10] and particularly of HBsAg.[6,7,11-13] Since almost none of the HBsAg rapid assays included in this study were able to detect less than 1 ng/mL HBsAg, most of their poor sensitivity is explained by an intrinsic failure of the assays themselves.

As they have been proven to significantly reduce the length of the window phase, Ag/Ab EIAs should be considered as a reliable alternative to NAT,14-19 especially in resource-limited countries. In our study, these assays provided overall better results than other assays, with, nevertheless, some failures in the detection of samples with weak reactivities.This was particularly observed for HCV with Murex assay, which missed a notable amount of samples compared to Monolisa, regardless of the laboratory. For the HIV Ag/Ab assays, the interlaboratory variation, manifested as false-negative results for weak samples, suggests technical problems within the laboratories rather than intrinsic problems with the tests.

Some results can inform the provision of practical advice to help the operators to conduct more accurate testing. The interlaboratory variations were markedly observed with rapid tests in samples presenting a low level of markers. This was probably related to the difficulty in reading weak reactions, which could be remedied by better training. In this regard, manufacturers should provide accurate instructions for interpreting results. In addition, the surprisingly low specificity observed for a widely used HBsAg EIA also supports operator error, prob- ably due to the programming of a wrong protocol in the machine. This also points to the need for better training and verification of all steps in the laboratory protocol.

If we analyze our data from a blood screening point of view, 321 infected units would have been transfused due to false-negative results, which corresponds to 5.6% of 5715 results of the study. Assuming that those that were missed by rapid tests (84%) would have been detected by EIAs, 270 viral contaminations (92 HIV, 65 HCV, and 113 HBV) would have been avoided. These figures probably overestimate the risk, since the number of positives, moreover weakly reactive for a larger proportion of them, was artificially enriched in this panel and the number of false negatives in real life would depend upon the prevalence of the virus.
While the sensitivity of screening assays has a direct impact on blood safety, a lack of specificity leads to an unnecessary loss of blood donations and waste collection. The impact on the blood supply is particularly worrisome in resource-limited countries, where the number of blood units collected can be up to 75 times less than in developed countries.[20] The reports on specificity performance of assays (especially for rapid tests) mainly concerned HIV and showed a range from 96% to 100% according to the assays, the studied populations, and the tested samples.[8,21-31]

The lowest specificity rate was observed for HBsAg detection and, surprisingly, with EIAs. As EIAs used in this study are CE marked, we can assume that the lowest specificities were due to failures in handling of assays, a minimum of 99.5% specificity being required for CE approval. These observations strengthen the need for appropriate training on the use of assay for the personnel of African blood banks.

Despite the participation of a large number of centers, this study has some limitations. First, there was a relatively limited number of positive samples within the panel, which, moreover, included samples that could be considered as not representative of African epidemiology regarding their genotypes. Moreover, due to the lack of such native samples in large volumes, we used dilutions of positive samples to mimic early infections. This might be considered as not appropriate for this design. However, the objective of the study was not to evaluate intrinsic performance of assays as already performed else-where,[13,32,33] but rather to compare screening strategies.
Second, inclusion of low-titer HBsAg samples might exaggerate the insensitivity observed with rapid tests which are known to be less sensitive than EIAs.[33] However, to our knowledge, there are no data on the HBsAg titers in the study population, and we can assume that the proportion of low HBsAg titers is high because of the decline of HBsAg due to infections occurring mainly at, or shortly after, birth.

Third, the labs that participated in this study were those which probably have the highest level of expertise. Thus, they are probably not representative of all centers involved in the screening of blood in participating countries. Consequently, the overall performance may have been overestimated. Finally, some labs did not adhere strictly to the protocol of performing a single operational series of tests, but this did allow us to evaluate such variations from standard practice.

In conclusion, these results led us to make several recommendations. First, the use of EIAs and especially Ag/Ab EIAs should be recommended over rapid tests whenever possible. Unfortunately, the implementation of such assays remains problematic in remote parts of Africa. If rapid tests must be used, because they are more affordable or available for whole blood testing, laboratory technicians must receive special training and a QC program must be implemented.

Second, we recommend better training of laboratory technicians and improved algorithms for test interpretation. Both measures have the advantage of being relatively inexpensive. Finally, this study points out the need for ongoing QC as an operational measure. Periodic external quality assessment studies are indispensable to maintain an acceptable level of transfusion safety, and international organizations could play an important role in helping African blood centers organize such QC assessments.

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CONFLICT OF INTEREST

All authors declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work, and no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1. Tagny CT, Mbanya D, Tapko JB, Lefrere JJ. Blood safety in sub-Saharan Africa: a multi-factorial problem. Transfusion 2008;48:1256-61.
2. Zou S, Dorsey KA, Notari EP, Foster GA, Krysztof DE, Musavi F, Dodd RY, Stramer SL. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepa- titis C virus infections among United States blood donors since the introduction of nucleic acid testing. Transfusion 2010;50:1495-504.
3. Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepati- tis C and HIV transfusion-transmitted infections in the 21st century. Vox Sang 2011;100:92-8.
4. Lefrere JJ, Dahourou H, Dokekias AE, Kouao MD, Diarra A, Diop S, Tapko JB, Murphy E, Laperche S, Pillonel J. Esti- mate of the residual risk of transfusion-transmitted human immunodeficiency virus (HIV) infection in sub-Saharan Africa: a multi-national collaborative study. Transfusion 2011;51:486-92.
5. van Hulst M, Smit Sibinga CT, Postma MJ. Health economics of blood transfusion safety - focus on sub-Saharan Africa. Biologicals (Review). 2010;38:53-8.
6. Laperche S, Boukatou G, Kouegnigan L, Nebie Y, Boulahi MO, Tagny CT, Yahaya R, Tapko JB, Murphy E, Lefrere JJ. Transfusion safety on the African continent: an interna- tional quality control of virus testing in blood banks. Transfusion 2009;49:1600-8.
7. Kshatriya R, Cachafeiro AA, Kerr RJ, Nelson JA, Fiscus SA. Comparison of two rapid human immunodeficiency virus (HIV) assays, Determine HIV-1/2 and OraQuick Advance Rapid HIV-1/2, for detection of recent HIV seroconversion. J Clin Microbiol 2008;46:3482-3.
8. Plate DK. Evaluation and implementation of rapid HIV tests: the experience in 11 African countries. AIDS Res Hum Retroviruses 2007;23:1491-8.
9. Beelaert G, Fransen K. Evaluation of a rapid and simple fourth-generation HIV screening assay for qualitative detection of HIV p24 antigen and/ or antibodies to HIV-1 and HIV-2. J Virol Methods 2010;168:218-22.
10. Desbois D, Vaghefi P, Savary J, Dussaix E, Roque-Afonso AM. Sensitivity of a rapid immuno-chromatographic test for hepatitis C antibodies detection. J Clin Virol 2008;41: 129-33.
11. Randrianirina F, Carod JF, Ratsima E, Chretien JB, Richard V, Talarmin A. Evaluation of the performance of four rapid tests for detection of hepatitis B surface antigen in Antananarivo, Madagascar. J Virol Methods 2008;151:294-7.
12. Seremba E, Ocama P, Opio CK, Kagimu M, Yuan HJ, Attar N, Thomas DL, Lee WM. Validity of the rapid strip assay test for detecting HBsAg in patients admitted to hospital in Uganda. J Med Virol 2010;82:1334-40.
13. Scheiblauer H, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, Prince A. Performance evaluation of 70 hepa- titis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. Vox Sang 2010;98:403-14.
14. Tagny CT, Mbanya D, Leballais L, Murphy E, Lefrere JJ, Laperche S. Reduction of the risk of transfusion-transmitted human immuno-deficiency virus (HIV) infection by using an HIV antigen/antibody combination assay in blood donation screening in Cameroon. Transfusion 2010;51:184-90.
15. Ly TD, Ebel A, Faucher V, Fihman V, Laperche S. Could the new HIV combined p24 antigen and antibody assays replace p24 antigen specific assays? J Virol Methods 2007; 143:86-94.
16. Tuke TW, Grant PR, Waite J, Kitchen AK, Eglin RP, Tedder RS. Hepatitis C virus window phase infections : closing the window on hepatitis C virus. Transfusion 2008;48:594-600.
17. Laperche S, Elghouzzi MH, Morel P, Asso-Bonnet M, Le Marrec N, Girault A, Servant-Delmas A, Bouchardeau F, Deschaseaux M, Piquet Y. Is an assay for simultaneous detection of hepatitis C virus core antigen and antibody a valuable alternative to nucleic acid testing? Transfusion 2005;45:1965-72.
18. Laperche S, Le Marrec N, Girault A, Bouchardeau F, Servant-Delmas A, Maniez-Montreuil M, Gallian P, Levayer T, Morel P, Simon N. Simultaneous detection of hepatitis C virus (HCV) core antigen and anti-HCV antibodies improves the early detection of HCV infection. J Clin Microbiol 2005;43:3877-83.
19. Laperche S. Antigen-antibody combination assays for blood donor screening: weighing the advantages and costs. Transfusion 2008;48:576-9.
20. Allain J, Vinelli E, Ayob Y. Blood safety in developing coun- tries. In: Barbara JA, Regan FA, Contreras MC, editors. Transfusion microbiology. London: Cambridge University Press; 2008. p. 369-80.
21. Aghokeng AF, Ewane L, Awazi B, Nanfack A, Delaporte E, Peeters M, Zekeng L. Evaluation of four simple/rapid assays and two fourth-generation ELISAs for the identification of HIV infection on a serum panel representing the HIV-1 group M genetic diversity in Cameroon. J Acquir Immune Defic Syndr 2004;37:1632-40.
22. Aidoo S, Ampofo WK, Brandful JA, Nuvor SV, Ansah JK, Nii- Trebi N, Barnor JS, Apeagyei F, Sata T, Ofori-Adjei D, Ish- ikawa K. Suitability of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus in Ghana, West Africa. J Clin Microbiol 2001; 39:2572-5.
23. Beelaert G, Vercauteren G, Fransen K, Mangelschots M, De Rooy M, Garcia-Ribas S, van der Groen G. Comparative evaluation of eight commercial enzyme linked immun- osorbent assays and 14 simple assays for detection of anti- bodies to HIV. J Virol Methods 2002;105:197-206.
24. Delaney KP, Branson BM, Uniyal A, Kerndt PR, Keenan PA, Jafa K, Gardner AD, Jamieson DJ, Bulterys M. Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies. AIDS 2006;20:1655-60.
25. Ferreira Junior OC, Ferreira C, Riedel M, Widolin MR, Barbosa-Junior A. Evaluation of rapid tests for anti-HIV detection in Brazil. AIDS 2005;19 Suppl 4:S70-5.
26. Holguin A. Evaluation of three rapid tests for detection of antibodies to HIV-1 non-B subtypes. J Virol Methods 2004; 115:105-7.
27. Koblavi-Deme S, Maurice C, Yavo D, Sibailly TS, N'Guessan K, Kamelan-Tano Y, Wiktor SZ, Roels TH, Chorba T, Nken- gasong JN. Sensitivity and specificity of human immunodeficiency virus rapid serologic assays and testing algorithms in an antenatal clinic in Abidjan, Ivory Coast. J Clin Microbiol 2001;39:1808-12.
28. Reynolds SJ, Ndongala LM, Luo CC, Mwandagalirwa K, Losoma AJ, Mwamba KJ, Bazepeyo E, Nzilambi NE, Quinn TC, Bollinger RC. Evaluation of a rapid test for the detec- tion of antibodies to human immunodeficiency virus type 1 and 2 in the setting of multiple transmitted viral sub-types. Int J STD AIDS 2002;13:171-3.
29. Rouet F, Ekouevi DK, Inwoley A, Chaix ML, Burgard M, Bequet L, Viho I, Leroy V, Simon F, Dabis F, Rouzioux C. Field evaluation of a rapid human immunodeficiency virus (HIV) serial serologic testing algorithm for diagnosis and differentiation of HIV type 1 (HIV-1), HIV-2, and dual HIV-1-HIV-2 infections in West African pregnant women. J Clin Microbiol 2004;42:4147-53.
30. van den Berk GE, Frissen PH, Regez RM, Rietra PJ. Evaluation of the rapid immunoassay determine HIV 1/2 for detection of antibodies to human immunodeficiency virus types 1 and 2. J Clin Microbiol 2003;41:3868-9.
31. Wesolowski LG, MacKellar DA, Facente SN, Dowling T, Ethridge SF, Zhu JH, Sullivan PS. Post-marketing surveil- lance of OraQuick whole blood and oral fluid rapid HIV testing. AIDS 2006;20:1661-6.
32. Scheiblauer H, Soboll H, Nick S. Evaluation of 17 CE-marked HBsAg assays with respect to clinical sensitivity, analytical sensitivity, and hepatitis B virus mutant detection. J Med Virol 2006;78:S66-S70.
33. Scheiblauer H, El-Nageh M, Nick S, Fields H, Prince A, Diaz S. Evaluation of the performance of 44 assays used in countries with limited resources for the detection of anti- bodies to hepatitis C virus. Transfusion 2006; 46:708-18.