HEPATITIS B SURFACE ANTIGEN ASSAYS: OPERATIONAL CHARACTERISTICS

(PHASE I)

REPORT 2

WORLD HEALTH ORGANIZATION

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DEPARTMENT OF ESSENTIAL HEALTH TECHNOLOGIES
WORLD HEALTH ORGANIZATION

WHO Library Cataloguing-in-Publication Data

World Health Organization.
HEPATITIS B SURFACE ANTIGEN ASSAYS: OPERATIONAL CHARACTERISTICS (Phase I) REPORT 2

1.Hepatitis B - diagnosis 2.Hepatitis B surface antigen - diagnostic use 3.Enzyme-linked immunosorbent assay 4.Reagent kits, Diagnostics

ISBN 92 4 159220 6 (NLM classification: WC 536)

Printed in France

Contact: Dr G. Vercauteren, Essential Health Technologies - WHO - 20, Avenue Appia - 1211 Geneva 27-Switzerland
This document is available on the internet at: www.who.int/eht/

HEPATITIS B SURFACE ANTIGEN ASSAYS: OPERATIONAL CHARACTERISTICS *(PHASE I)*

REPORT 2

1. Summary

In 1998, WHO implemented a programme for the evaluation of performance and major operational characteristics of commercially available assays for the detection of Hepatitis B surface antigen (HBsAg). This second report presents the findings of the Phase I evaluations of 5 HBsAg assays conducted between September 2001 and January 2004. The HBsAg assays evaluated included:

- Enzygnost HBsAg 5.0 (Dade Behring Inc)
- Equipar HBsAg One Step (Equipar Diagnostici)
- Genedia HBsAg ELISA 3.0 (Green Cross Life Science Corp)
- HEPALISA (J Mitra & Co)
- Murex HBsAg Version 3 (Abbott-Murex)

Section 2 of this report provides background information on the evaluations and the intended use of the evaluation results. Sections 3 and 4 present the laboratory aspects of HBsAg testing and describe the way in which the evaluations were conducted and the results analysed. The results and outcomes of the analyses of the assay evaluations are contained in the tables and figures in section 5. Annexes 1, 2 and 3 show, respectively, the algorithm for characterization of the WHO HBsAg panel, the cumulative list of assays evaluated and the addresses of the manufacturers of the assays evaluated.

This report contains Phase I assessments of enzyme linked immunosorbent assays (ELISAs). The previous report, Operational Characteristics Report 1 (WHO/BCT/BTS/01.4) contains the assessments of 10 simple/rapid HBsAg assays. Copies of these reports are available on request from the Department of Essential Health Technologies (EHT), World Health Organization, 1211 Geneva 27, Switzerland.

2. BACKGROUND INFORMATION

In 1998, the World Health Organization (WHO) Blood Safety and Clinical Technology Department, conscious of the need to advise Member States on laboratory aspects associated with Hepatitis B and Hepatitis C testing for blood transfusion safety, initiated a project to provide objective assessments of commercially available assays for detection of Hepatitis B surface antigen (HBsAg) and Hepatitis C (HCV) antibodies, similar to that which has existed for HIV since 1988. This continuing project is coordinated by the Department of Essential Health Technologies, WHO; the WHO Collaborating Centre on Transfusion Transmissible Infections, Evaluations and Standards Laboratory, Health Protection Agency, London, UK carries out the laboratory investigations. The aim of the project is to supply those responsible for deciding which tests to use, and potential users of tests, with enough comparative data to apply their own criteria and choose the best tests for their particular situation.

It is intended that the evaluations will be conducted in two phases, the first using a limited panel of well characterized specimens held at the WHO Collaborating Centre (reference

laboratory), the second in 3-4 field laboratories. Aliquots of the specimens used in the field evaluations will be sent to the reference laboratory for characterization. The purpose of this 2-phase approach is to expand the number, type and origin of specimens in the evaluation panels and to archive them for use in future evaluations.

The assessments focus on the operational characteristics of these assays, such as ease of performance and their sensitivity and specificity on a panel of well-characterized sera of diverse geographical origins, and indicate their suitability for use in small laboratories, eg many blood-collection centres in developing countries. In addition, the sensitivity of the assays on seroconversion and low titre specimens is being assessed.

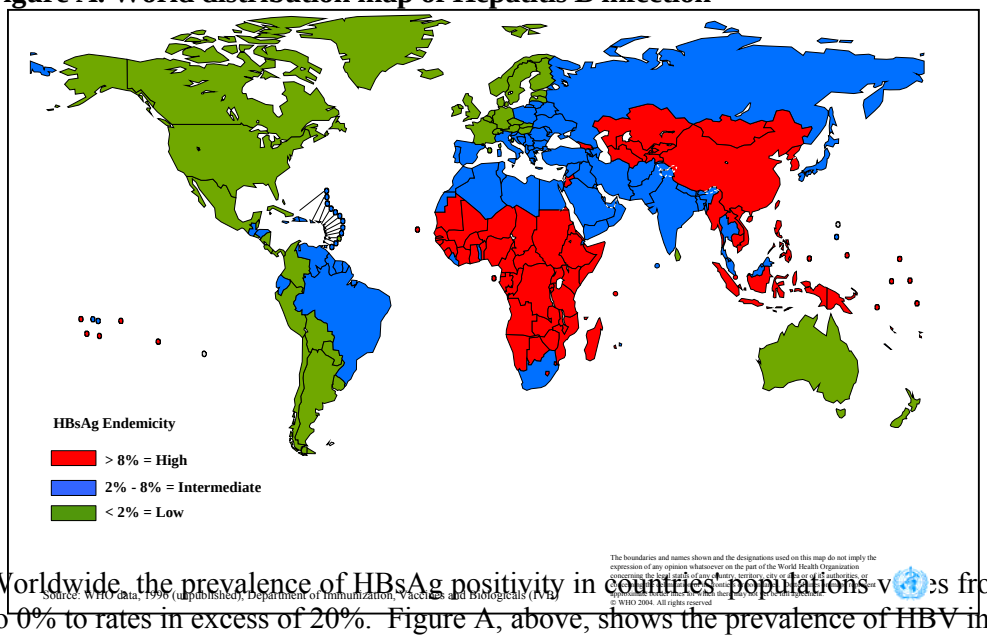
The findings of the assessments are published in the form of reports which are intended for use by health policy-makers, directors of blood banks, and managers of national prevention and surveillance programmes. They may be used in conjunction with consideration of other factors, such as experience with a given test, availability, cost, service and trouble-shooting provided locally by manufacturers, to help select assays appropriate to local needs.

3. LABORATORY ASPECTS OF HBsAg TESTING

3.1 A brief overview

Hepatitis B virus is a partially double-stranded circular DNA virus and is a member of the *Hepadnaviridae* family. The virus consists of a core capsid which contains viral DNA and this is surrounded by an envelope containing surface antigen (HBsAg). Both whole, intact virions and incomplete virus particles, consisting entirely of HBsAg, are produced during replication of HBV. The HBsAg particles vary greatly in morphology and are found in high concentrations in early acute infection and continue to be produced in chronic disease.

Figure A. World distribution map of Hepatitis B infection



Worldwide, the prevalence of HBsAg positivity in countries' populations varies from close to 0% to rates in excess of 20%. Figure A, above, shows the prevalence of HBV infections globally, divided into low (<2%), intermediate (2-7%) and high (>8%) prevalence areas.

HBV infection in industrialized countries occurs predominantly in adults where the clinical course most frequently seen is of acute infection, comprising an incubation period (generally 4 - 12 weeks), followed by an acute illness (2 weeks – 3 months) and recovery with no further sequelae, although up to 30% may show no or very mild symptoms in the acute phase. The serological profile is illustrated in Figure B.

In resource-poor settings, however, chronic infections frequently occur leading to a high prevalence of hepatitis B infection in such areas. High numbers of infants and young children are infected of whom approximately 90% of infants infected at birth and 30% of children aged under 5 do not clear the virus completely and continue to be chronically infected for the remainder of their lives.

Chronic infections may take one of two courses. The infected individual may remain HBeAg positive, a marker of viral replication which correlates with evidence of HBV DNA, for the remainder of the individuals' lives, see Figure C. Alternatively, after a number of years, chronically infected individuals may become HBeAg negative concomitant with the production of antibody to HBe, see Figure D. However, individuals in whom HBsAg is present in their blood for more than six months are considered to be chronically infected with HBV and are at a greater risk of development of cirrhosis and hepatocellular carcinoma.

HBsAg is the most commonly used marker of infection for diagnostic and blood screening. An individual positive for HBsAg is considered to be infected with HBV, and is therefore potentially infectious. Confirmation of a reactive HBsAg ELISA screening test is usually done by performing a neutralization test using a specific anti-HBs antiserum in the same screening ELISA. Where a simple/rapid HBsAg test is used and no neutralization reagents are available, confirmation of an acute or chronic infection for diagnostic purposes may be concluded based upon symptoms and appropriate monitoring tests. Other HBV markers which can be used diagnostically to monitor an HBV infection include HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs and HBV DNA. The presence of HBeAg indicates an individual is of higher infectivity, and seroconversion to anti-HBe correlates with reduced infectivity. In an acute infection this suggests that the infected person is progressing towards resolving their infection. Individuals who have seroconverted from HBsAg to anti-HBs have resolved their infection and are immune to further HBV infection.

The most widely used HBsAg screening tests worldwide are ELISAs as they are the most appropriate for screening large numbers of specimens on a daily basis, as is the case in blood transfusion services in industrialized countries. However, many blood transfusion services in resource limited countries only process limited numbers of specimens. Hence, individual tests would be more appropriate. Several simple, instrument and electricity-free screening tests have been developed including agglutination, immunofiltration (flow through) and immunochromatographic (lateral flow) membrane tests. In general, these simple/rapid (S/R) tests are most suitable for use in laboratories that have limited facilities and/or process low numbers of specimens daily. Care should be taken however to ensure that the test meets any regulatory specifications, eg in some countries the use of tests with a minimum detection level of 0.5ng/ml HBsAg for testing of donated blood is mandatory.

Figure B: Acute HBV infection

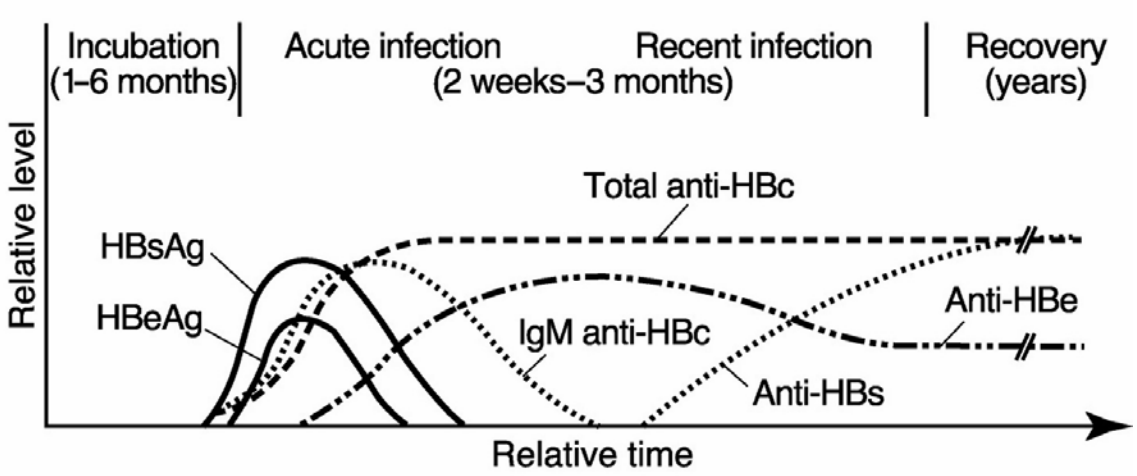


Figure C: Chronic HBV infection (HBeAg positive)

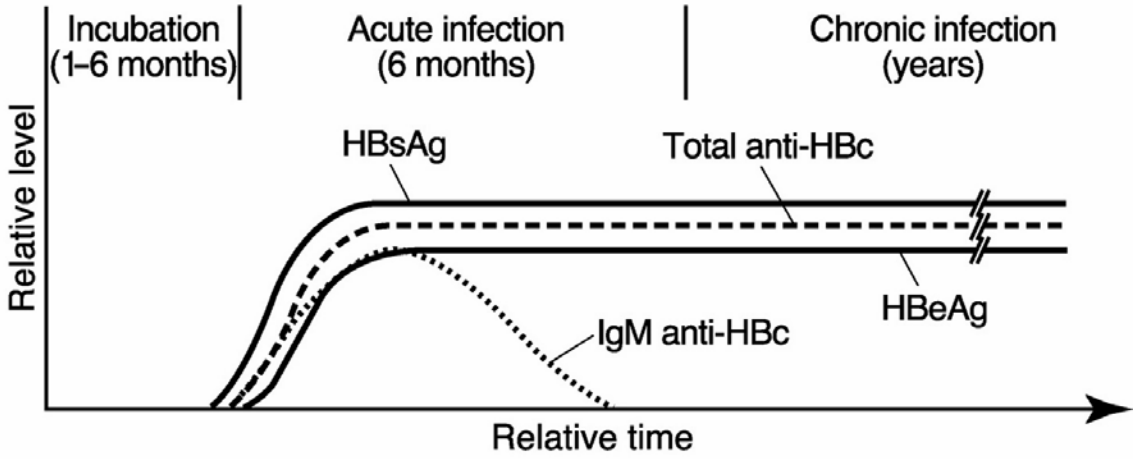
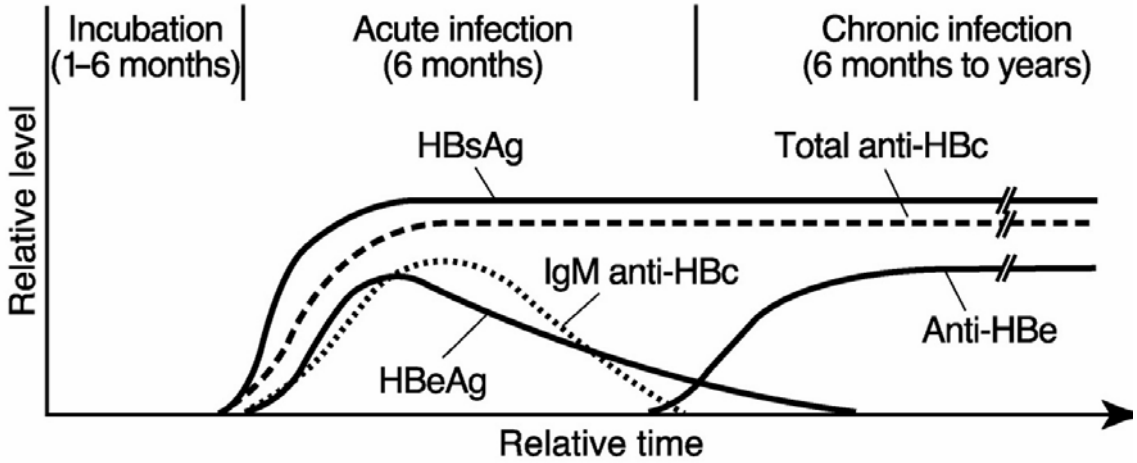


Figure D: Chronic HBV infection (HBeAg negative)



3.2 Quality assurance

All laboratories carrying out HBsAg tests should have a well-functioning quality assurance programme. It is most important that quality assurance procedures are stringently complied with so as to maximize the accuracy of the laboratory results. Procedures for detecting both (technical) laboratory and clerical errors must be included in all protocols. For example, procedures that guarantee the correct identification of initially reactive units of donated blood, which must be discarded, are essential to the maintenance of a safe blood supply. It is recommended that laboratories submit to an external quality assessment at least once a year.

3.3 Safety

The testing of all clinical specimens should be performed in such a manner as to minimize occupational risk. Guidelines for good laboratory practice have been developed that, if followed, will ensure safety and keep laboratory accidents to a minimum. For further details see the *Laboratory Biosafety Manual, second edition*, World Health Organization, Geneva, 1993 (ISBN 92 4 154450 3) and the Communicable Diseases Surveillance and Response section of the WHO website, www.who.int/csr , where information on laboratory biosafety and transport of infectious substances may be found.

4. MATERIALS AND METHODS

4.1 Assays (test kits) evaluated

Test kits for these assessments were kindly provided to WHO free of charge by each of the manufacturers of the assays under evaluation. The manufacturers were invited to visit the site at which the assessments were to be conducted in order to ensure correct performance of their assays.

A brief description of the principle of a typical 'antigen sandwich' ELISA assay, as deployed by the assays under evaluation, is described below and in Figure E accompanied by a picture showing the appearance of positive and negative results, Figure F.

The antibody, specific for the analyte, is immobilised onto the solid phase, usually 96-well polystyrene microtitre plate wells. The sample to be analysed is added to the well and any corresponding antigen is captured to form an antibody-antigen complex. A wash step may be included in the protocol at this point to remove any unbound molecules. An antibody labelled with an enzyme, or in some cases a co-enzyme, is added which binds to form an antibody-antigen-antibody/enzyme conjugate complex and is followed by a wash step. A substrate solution is added which will produce a colour change which is proportional to the amount of bound enzyme. Thus samples which do not contain the particular analyte will not form a complex and therefore no colour reaction will take place. Wells that contain samples that do contain the analyte will show a colour change corresponding to the number of individual complexes formed. The colour produced should be measured on a spectrophotometer to give a numerical reading which will allow comparison with the assay controls.

To confirm positive results in the HBsAg ELISA, a neutralization test should be carried out. A reagent containing anti-HBs is first added to the positive specimen. Two ELISA tests, as described above, are then carried out contemporaneously, one with the specimen only and one with the combined specimen/anti HBs. The results obtained are then

compared to determine if a reduction in reading is observed in the neutralized sample and thus confirmation of the initial positive result.

A number of variations of the ELISA technique may be employed; the indirect ELISA is normally used to detect antibody, the antibody capture assay is used to determine the class (eg IgM) of a particular antibody and the competitive ELISA is usually used for antibody detection whereby an absence of colour indicates a positive result.

Figure E. Diagram of generic 'antigen sandwich' ELISA for the detection of HBsAg

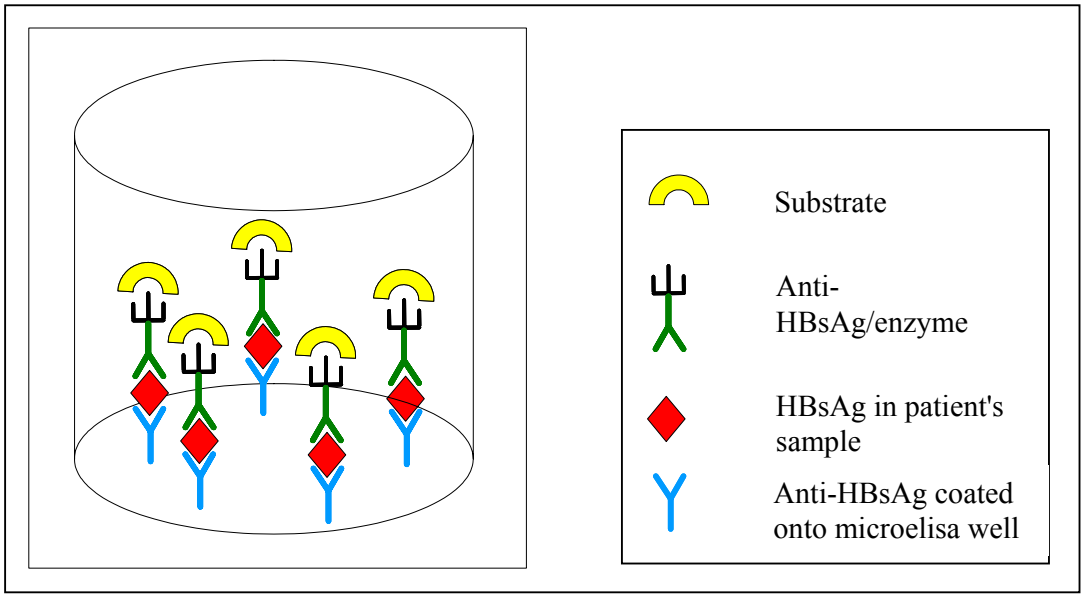
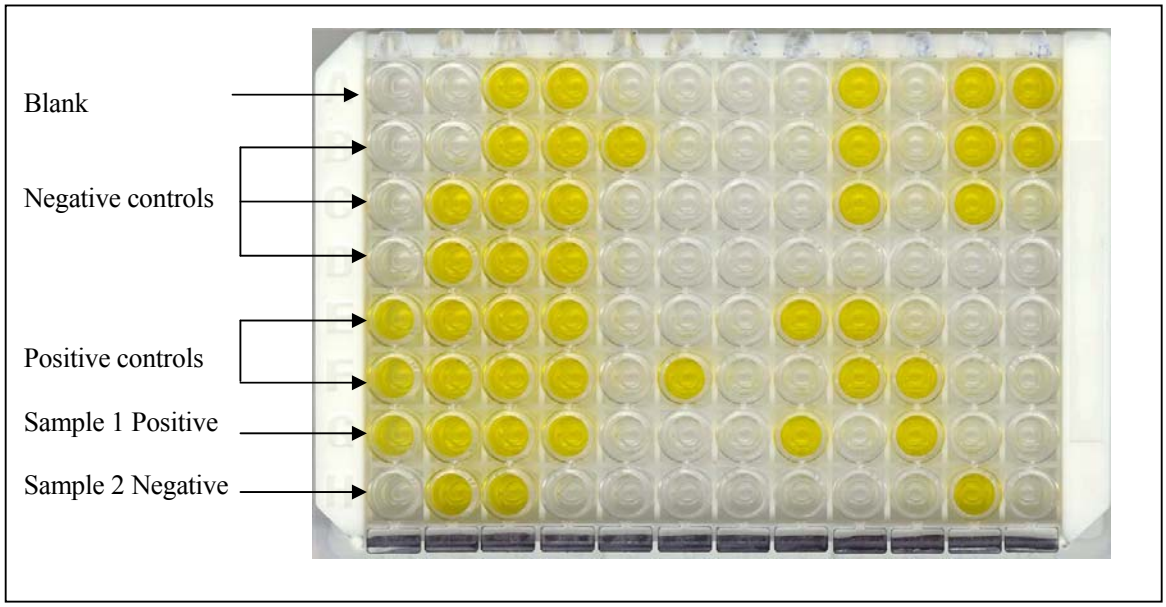


Figure F. Completed generic ELISA assay



4.2 Evaluation panels

4.2.1 WHO HBsAg panel

The phase I evaluations reported here were carried out using a panel of 276 sera (as shown in Table A), of which 59 were from Africa, 60 from Asia, 97 from Europe and 60 from South America. All specimens had been stored frozen in aliquots and thawed at least once and not more than three times.

Table A: Composition of WHO HBsAg panel: Phase I

Origin	HBsAg positive specimens	HBsAg negative specimens	Total
Africa	16	43	59
Asia	30	30	60
Europe	30	67	97
Latin America	22	38	60
Total	98	178	276

Characterization of WHO HBsAg panel

For characterization, specimens in the WHO reference panel were screened by two ELISAs, Hepanostika HBsAg Uniform II (bioMérieux) and Monolisa Ag HBs Plus (Bio-Rad). Specimens negative by both ELISAs were considered HBsAg negative. Specimens showing reactivity with either or both ELISAs were further characterized using the Hepanostika HBsAg Uniform II Confirmatory Assay (bioMérieux). When reactive results by the ELISAs were confirmed by the confirmatory (neutralization) assay, the specimen was considered HBsAg positive. This algorithm, used for the determination of the HBsAg status of specimens in the WHO HBsAg panel, is shown diagrammatically in Annex 1.

4.2.2 Seroconversion panels

In the context of these evaluations of HBsAg kits, a seroconversion panel is a series of specimens, sequentially collected over a period of time, from an individual developing HBsAg in response to primary HBV infection. Four commercial seroconversion panels, PHM902, PHM903, PHM907 and PHM910 [Boston Biomedica Inc. (BBI)] were tested on each of the five assays evaluated. These panels consisted of a total of 35 specimens collected from 4 individuals during seroconversion. Three further panels, PHM901, PHM916 and PHM928, were tested according to their availability at the time of each evaluation.

4.2.3 Performance panel

Additionally, one HBsAg low titre performance panel containing 15 members, PHA105 (BBI), was tested.

4.3 Laboratory testing

All testing was performed according to the manufacturer's instructions. The specimens in the WHO HBsAg panel were randomized before testing and all assay runs were performed by one operator. The results were read spectrophotometrically in a dedicated ELISA reader.

Specimens in the WHO HBsAg panel which gave initial results discordant from the reference results, were retested in duplicate. The result that occurred 2 out of 3 times was recorded as the final result. Samples from commercial panels giving discordant results were not repeated.

4.4 Analysis

4.4.1 Sensitivity, specificity, confidence limits (CL) and predictive values of HBsAg tests

The formula for calculation of sensitivity, specificity and predictive values is represented diagrammatically in Table B.

Table B Calculation of sensitivity, specificity and predictive values

		True HBsAg status		
		+	–	
Results of assay under evaluation	+	**a True-positives**	b False positives	a+b
	–	c False-negatives	**d True-negatives**	c+d
		a+c	b+d	

Sensitivity = a/(a+c) Positive predictive value = a/(a+b)
Specificity = d/(b+d) Negative predictive value = d/(c+d)

Sensitivity: Is a measure of the ability of the assay under evaluation to identify correctly sera that contain surface antigen to HBV (reference assays positive). Thus, sensitivity is the number of true positive sera recognized by the assay under evaluation as positive (a), divided by the number of sera identified by the reference assays as positive (a+c), expressed as a percentage.

Specificity: Is a measure of the ability of the assay under evaluation to identify correctly sera that do not contain surface antigen to HBV (reference assays negative). Thus, specificity is the number of true negative sera recognized by the assay under evaluation as negative (d), divided by the number of sera identified by the reference assays as negative (b+d), expressed as a percentage.
NOTE: Samples that gave indeterminate results with the assays under evaluation were included in the analyses.

Confidence Limits (CL): The 95% confidence limits are a means of determining whether observed differences in sensitivity or specificity between assays are significant or not. Exact 95% confidence limits for binomial proportions were calculated from the F-distribution (Armitage P. and Berry G. Statistical Methods in Medical Research, 2nd Edition. Blackwell Scientific Publications, Oxford, 1987, page 119).

Predictive Values:
The **positive predictive value (PPV)** is the probability that when the test is reactive, the specimen does contain surface antigen to HBV. This may be calculated in two ways:

1. using the simple formula a/(a+b) which will give an approximate value (see Table B).

2. using the more precise formula which takes the prevalence of HBV in the population into account

$$PPV = \frac{(\text{prevalence})(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1 - \text{prevalence})(1 - \text{specificity})}$$

The **negative predictive value (NPV)** is the probability that when the test is negative, a specimen does not have surface antigen to HBV. This may be calculated using:

1. the simple formula d/(c+d) which will give an approximate value (see Table B).

2. the more precise formula which takes the prevalence of HBV in the population into account:

$$NPV = \frac{(1 - \text{prevalence})(\text{specificity})}{(1 - \text{prevalence})(\text{specificity}) + (\text{prevalence})(1 - \text{sensitivity})}$$

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HBV infection in the population from which the person comes. In general, the higher the prevalence of HBV infection in the population, the greater the probability that a person testing positive is truly infected (i.e. the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e. the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false negative.

For calculating the positive and negative predictive values recorded in this report, the more precise formula at option 2 was used.

4.4.2 Sensitivity in seroconversion panels
The results obtained from early seroconversion panels using the assays under evaluation were compared with those obtained using Monolisa Ag HBs Plus; the assay arbitrarily designated the reference for determination of relative sensitivity in the panels. For each seroconversion series (panel) the first specimen in the sample sequence to become reactive

with Monolisa Ag HBs Plus was assigned the value "0". Results from the assays under evaluation were compared with Monolisa Ag HBs Plus by determining the difference between the specimen assigned value "0" and the relative position in the sample sequence of the first specimen which showed a reactive result with the assays under evaluation. For example, if an assay became reactive two specimens earlier in a series than Monolisa Ag HBs Plus, the value assigned for that series in that assay was -2. Similarly, if an assay became reactive one specimen later than Monolisa Ag HBs Plus, the value assigned was +1. The assigned values over the 4 seroconversion series, ie those tested in all five kits, were averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence limits were determined.

4.4.3 Sensitivity in performance panel

The number of samples correctly identified in the low titre performance panel was determined by comparison with the HBsAg status assigned (expected results) following interpretation of the combined reference tests, the Hepanostika HBsAg Uniform II, Monolisa Ag HBs Plus and the Hepanostika HBsAg Uniform II Confirmatory Assay.

4.4.4 Additional analyses

The technical aspects of the assays under evaluation were assessed by the technician who performed the testing. These assessments, along with other selected assay characteristics, contributed to an overall appraisal of each assay's suitability for use in small laboratories. To enable comparison between assays, an arbitrary scoring system was used to rate specified assay characteristics.

5. ASSAY EVALUATIONS

The results from the 5 test kits evaluated are presented in tables 1 to 7. Table 1 summarizes the general characteristics of the assays and the results of the assays evaluated as compared to the reference tests are given in table 2. Table 3 provides further details of operational aspects. Factors taken into account in the calculation of ease of performance and suitability for use in small laboratories are listed in tables 4a, 4b and table 5. Performance of the assays under evaluation on early seroconversion panels, and a low titre panel is given in tables 6 and 7, respectively. The relative performance of the 5 assays under evaluation, compared to the reference tests in seroconversion panels, is shown in Figure 1, while Figure 2 represents the comparison in performance in both seroconversion and low titre commercial panels. Explanatory notes are provided at the end of the assay evaluation tables.

ASSAY EVALUATIONS

Table 1. General characteristics and operational aspects

NAME	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA 3.0	HEPALISA	Murex HBsAg Version 3
Company	Dade Behring Inc, Deerfield, Illinois, USA	EQUIPAR Diagnostici, Saronno (Va), Italy	Green Cross Life Science Corp, Kyonggi-do, Korea.	J.Mitra & Co.Ltd, New Delhi, India	Abbott – Murex, Abbott Park, N Illinois, USA
Assay type	Two – step antigen sandwich ELISA	Direct sandwich ELISA	Antigen sandwich EIA	Direct sandwich ELISA	Direct sandwich ELISA
Antibody type	Monoclonal & Polyclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal
Solid phase	Microtitre wells	Microtitre wells	Microtitre wells	Microtitre wells	Microtitre wells
Specimen type	Serum/plasma	Serum/plasma	Serum/plasma	Serum/plasma	Serum/plasma
Number of tests per kit	192, 960	96	96, 192, 480	96, 192, 480	96, 480
Lot number evaluated Expiry date	33634 05/08/2004	72 28/02/2004	3170024 14/08/01	EHB01011, EHB12120 31/12/01, 30/11/01	H766310 01/06/04
Shelf life (°C)	15 months (2-8°C)	12 months (2-8°C)	12 months (2-8°C)	9 months (4-8°C)	12 months (2-8°C)
Volume of serum needed (µl) Final dilution of serum	100 4 in 5 with conjugate	150 None	100 4 in 5 with conjugate	100 none	75 3 in 4 with sample diluent
Total time to perform the assay, h. min. (no of tests)	3.00 (91+5 controls)	2.20 (91+5 controls)	2.45 (91+5 controls)	2.50 (91+5 controls)	2.50 (93+3 controls)
Reading wavelength (reference filter)	450nm (650nm)	450nm (630nm)	450nm (620nm)	450nm (630nm)	450nm (630nm)
Price/test US$ (kit size)	1.40 (192 tests/kit) 1.09 (960 tests7kit)	0.65 (96 tests/kit)	0.37 (96 tests/kit) 0.37(192 tests/kit) 0.35 (480 test/kit)	0.50 (96 tests/kit) 0.45 (192 tests/kit) 0.40 (480 test/kit)	1.00 (96 tests/kit) 0.90 (480 tests/kit)

Table 2. Comparison of the results of assays with reference tests

NAME		Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA 3.0	HEPALISA	Murex HBsAg Version 3
Final Sensitivity % (95 CL*) n = 98		100% (96.3 - 100%)	100% (96.3 - 100%)	100% (96.3 - 100%)	100% (96.3 - 100%)	100% (97.5 - 100%)
Initial Specificity % (95 CL*)		99.4 (96.9 - 100%)	89.9% (84.5 - 93.9%)	98.9% (96% - 99.9%)	97.8% (94.3 - 99.4%)	97.2% (93.6 - 99.1%)
Final Specificity % (95 CL*) n = 178		99.4 (96.9 - 100%)	98.3% (95.2 - 99.7%)	98.9% (96% - 99.9%)	100% (97.9 - 100%)	98.3% (95.2 - 99.7%)
Indeterminate results %		0%	0%	0%	0%	0%
PPV	1.0%	62.7%	37.3%	47.9%	100%	37.3%
	5.0%	89.8%	75.6%	82.7%	100%	75.6%
	10.0%	94.9%	86.7%	91.0%	100%	86.7%
NPV	1.0%	100%	100%	100%	100%	100%
	5.0%	99.9%	100%	100%	100%	100%
	10.0%	100%	100%	100%	100%	100%

* 95 % Confidence Limits

Table 3. Detailed operational aspects

NAME	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBSAg ELISA 3.0	HEPALISA	Murex HBsAg Version 3
Dimension (cm) of kit : w-l-h	21.0-34.0-10.0 (192 tests)	15.5 –14.0-9.5	12.5-23-7.5	12.6-19.0-9.0 (96 tests) 19.0-25.5-9.0 (192 tests) 31.5-38.2-9.0 (480 tests)	20.3–15.4–10.4 (480 tests)
Storage conditions (°C)	2-8°C	2-8°C	2-8°C	4-8°C	2-8°C
Incubation temperature (°C)	37 sample and conjugate 15-25 substrate	37 sample and conjugate Room temperature substrate	37 sample and conjugate 18-25 substrate	37 sample and conjugate 20-25 substrate	37 all incubations
Reading endpoint stability (h.min)	1 hour	One hour in the dark	Not indicated	Not indicated	15 minutes
Stability after dilution/ reconstitution/opening at (°C)					
- antibody coated wells	4 weeks (2-8)	Until desiccant turns pink	30 days (2-8)	30 days (4-8)	Expiry date (2-8)
- controls	4 weeks (2-8)/3mths (<20)	Expiry date (2-8)	Expiry date (4-8)	Expiry date (4-8)	Expiry date (2-8)
- sample diluent	N/A	N/A	Expiry date (4-8)	Expiry date (4-8)	Expiry date (2-8)
- conjugate	4 weeks (2-8)/2 days (15-25)	7 days (2-8)	5 days (2-8)	4 hours at (4-8)	Expiry date (2-8)
- substrate	5 days (2-8)/8 hrs (15-25)	4 hours (room temp)	2 hours (2-8)	Use immediately	2 days (2-8 or 15-25)
- wash buffer	1 week (2-8)/1 day (18-25)	21 days (2-8)	14 days (2-8)	2 months at 4-8	1 month (18-30)
Number of sera per run minimum – maximum	1-91	1-91	1-91	1-91	1-93
Number of controls per test run	5	5	5	5	3
- negative	3	3	3	3	2
- cut-off/weak positive	0	0	0	0	0
- positive	2	1	2	2	1
- blank	0	1	0	0	0
internal control:					
- reagent control	yes	no	yes	no	no
- sample addition control	no	no	no	no	yes

Table 3 (continued). Detailed operational aspects

NAME	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA 3.0	HEPALISA	Murex HBsAg Version 3
Estimated time to perform one run: h.min (number of sera)	3.00 (96)	2.20 (96)	2.45 (96)	2.50 (96)	2.50 (96)
Equipment needed but not provided in the kit:[1]	(for manual procedure)				
- washer	+	+	+	+	+
- incubator (water-bath)	+	+	+	+	+
- spectrophotometric reader	+	+	+	+	+
- refrigerator (storage)	+	+	+	+	+
- agitator , rocker, tray mixer	-	-	-	-	-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	+	+	+	+	+
- multichannel (µl)	+/-	+/-	+/-	+/-	+/-
- disposable tips	+	+	+	+	+
- dilution tubes/rack,microtiterplate	-	-	-	-	-
- distilled or deionised water	+	+	+	+	+
- plate covers	+	-	-	-	-
- graduated pipette; cylinder (ml)	+	+	+	+	+
- sulphuric acid/sodium hydroxide	-	- (HCl)	-	-	+
- absorbent paper	-	-	-	-	-
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	+/-	+/-	-	-	+/-
- timer	+	+	+	+	+
Definition of positive results	Test sample OD/CO≥1	Test sample OD/CO≥1	Test sample OD/CO≥1	Test sample OD/CO≥1	Test sample OD/CO≥1
Definition of grey zone	None	Cut off + 20%	None	None	None

[1] + : not provided in the kit but necessary to perform the test; - :provided in the kit or not necessary to perform the test; +/- : use is optional.

Table 4a. Technician's appraisal of the test kit

NAME	Score	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA	HEPALISA	Murex HBsAg Version 3
Number of steps in the test procedure: -1-2 steps -3-5 steps - >5 steps	 6 3 1	1	1	1	1	1
Clarity of kit instructions: - good - needs improvement	 2 1	1	2	2	2	2
Kit and reagent packaging and labelling: - good - needs improvement	 2 1	2	2	1	2	2
Total (out of 10)		4	5	4	5	5
Comments on the test kit		The kit was fairly easy to use and clearly labeled. However as the assay is normally performed on a bench top processor it was difficult to find the instructions for the manual procedure in the kit insert.	Overall the kit instructions were clear and all components were well labeled, however there was some difficulty following the washing instructions.	The kit instructions were a little confusing to follow. The labels were not stuck properly which was problematic as containers were similar and some reagents the same colour	Overall an easy test to follow and perform	The kit labelling and instructions were clear and easy to follow. Reverse pipetting is recommended in all steps (except sample addition), therefore extra care must be taken when adding reagents because the tips aren't changed between strips.

Table 4b. Calculation of ease of performance

NAME	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA	HEPALISA	Murex HBsAg Version 3
Need to prepare:					
-antigen	1[1]	1	1	1	1
-substrate	0[2]	0	0	0	0
-wash solution	0	0	0	0	0
-conjugate	1	0	0	0	1
-predilution of serum	1	1	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
-antigen	0	1	1	1	1
-controls	0	1	1	1	1
-sample diluent	1	1	1	1	1
-conjugate	0	0	0	0	1
-substrate	0	0	0	0	0
-wash buffer	0	0	0	1	0
-sufficient reagents	0	1	1	1	0
-wash (yes =0; no = 1)	0	0	0	0	0
Item needed but not provided in the kit:					
	0	0	0	0	0
-reagent trough	0	0	0	0	0
-automatic /multichannel pipette	1	1	1	1	1
-dilution tubes, rack/microtiter plate	0	0	0	0	0
-distilled or deionised water	1	1	1	1	1
-plate covers	0	0	0	0	0
-graduated pipette, cylinder	1	1	1	1	0
-sulphuric acid/sodium hydroxide					
Technician's appraisal of the test kit[3] (rating out of 10)	4	4	4	5	5
Total (out of possible 30)	10	13	13	14	14
Ease of performance: -less easy < 20 -easy $20 \leq x \leq 25$ -very easy > 25	Less easy	Less easy	Less easy	Less easy	Less easy

[1] 1 : positive rating: reagent needs no preparation; item provided in the kit.

[2] 0 : negative rating: reagent needs preparation; item not provided in the kit

[3] : see table 4a

Table 5. Suitability for use in small laboratories

NAME	Score	Enzygnost HBsAg 5.0	EQUIPAR HBsAg One Step	GENEDIA HBsAg ELISA	HEPALISA	Murex HBsAg Version 3
Sensitivity						
- 100%	5	3	5	5	3	5
- 98 – 100%	3					
- <98%	0					
Specificity						
- 100%	5	5	5	5	5	5
- 95 – 98%	3					
- <95%	0					
Incubation temperature						
- room t°	3	1	1	1	1	1
- other than room t°	1					
Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 yea	2	3	3	3	2	2
- < 6 months	1					
Storage at						
- ambient t° possible opened kit	5					
- ambient t° possible unopened kit	2	1	1	1	1	1
- 2-8 °C required	1					
Price per test (US$)						
- ≤ 1.0	3	2	3	3	3	3
- ≤ 2.0	2					
- > 2.0	1					
Ease of performance						
- very easy	5	1	1	1	1	1
- easy	3					
- less easy	1					
Rapidity of performance:1 serum						
- < 10 min	3	1	1	1	1	1
- 10 – 30 min	2					
- > 30 min	1					
Washer/agitator						
- not needed	3	1	1	1	1	1
- needed	1					
Reading						
- visual: inter-reader variability ≤ 3	5	1	1	1	1	1
: inter-reader variability > 3	3					
- reading equipment	1					
Total (out of 40)		19	22	22	19	21
Suitability for use in small laboratories: - less suitable < 23 - suitable 23 ≤ x ≤ 30 - very suitable > 30		Less suitable	Less suitable	Less suitable	Less suitable	Less suitable

Table 6. Results on seroconversion panels

Panel ID	Days since first bleed	HBV DNA Detection PCR[1]	HBsAg conc ng/ml[1]	Reference tests		ELISA 1	ELISA 2	ELISA 3	ELISA 4	ELISA 5
				REF 1[2] OD/CO	REF 2[2] OD/CO	OD/CO	OD/CO	OD/CO	OD/CO	OD/CO
PHM901 -01	0	+	0.3	0.7	0.8	NT	NT	0.95	0.16	NT
-02	2	+	0.6	0.8	**2.0**	NT	NT	**2.17**	0.61	NT
-03	8	+	>2.5	**4.1**	19.9	NT	NT	14.84	**8.47**	NT
-04	10	+	>2.5	7.1	20.9	NT	NT	20.53	8.26	NT
-05	16	+	>2.5	21.3	181.8	NT	NT	103.08	31.05	NT
-06	21	+	>2.5	29.1	181.8	NT	NT	103.08	32.90	NT
-07	23	+	>2.5	21.3	181.8	NT	NT	103.08	31.28	NT
PHM902 -01	0	-	< 0.1	0.6	0.3	0.18	0.2	0.68	0.11	0.42
-02	2	-	< 0.1	0.5	0.3	1.39	0.19	0.20	-0.04	0.42
-03	8	-	< 0.1	0.5	0.2	0.18	0.22	0.38	0.28	0.46
-04	10	-	< 0.1	0.7	0.1	1.39	0.22	0.52	0.04	0.46
-05	57	+	< 0.1	0.6	0.1	1.63	0.22	0.59	0.05	0.45
-06	59	+	< 0.1	0.6	0.3	1.70	0.23	0.61	-0.02	0.56
-07	64	+	< 0.1	0.7	0.3	0.51	0.26	0.69	0.07	0.62
-08	66	+	< 0.1	0.6	0.4	0.47	0.42	0.47	0.09	**1.19**
-09	71	+	0.5	0.9	**1.3**	**2.54**	0.99	**1.25**	0.44	2.79
-10	73	+	0.5	**1.0**	1.5	1.81	**1.17**	1.47	0.61	3.35
-11	78	+	1.2	2.9	2.5	5.69	3.26	2.79	0.86	9.61
-12	80	+	2.2	2.2	3.5	10.47	3.49	3.91	**1.37**	13.93
-13	85	+	> 2.7	5.6	8.1	20.35	7.95	7.25	2.68	25.32
-14	88	+	> 2.7	5.3	12.5	20.41	12.0	11.42	4.42	33.00
PHM903 -01	0	+	< 0.1	0.5	0.0	0.29	0.22	0.49	0.02	0.50
-02	3	+	< 0.1	0.5	0.1	0.41	0.42	0.39	0.00	0.57
-03	6	+	< 0.1	0.6	0.2	0.95	0.48	0.83	0.07	0.97
-04	10	+	0.1	0.9	0.5	**1.86**	0.65	0.71	0.02	**1.69**
-05	14	+	1.0	**1.1**	**1.4**	6.29	**1.60**	**1.68**	0.42	5.37
-06	17	+	1.4	4.1	4.5	17.59	2.52	3.75	**1.02**	11.62
PHM907 -01	0	-	< 0.1	0.6	0.2	0.21	0.26	0.53	-0.7	0.49
-02	3	-	< 0.1	0.6	0.1	0.20	0.26	0.47	-0.05	0.44
-03	7	-	< 0.1	0.6	0.1	0.18	0.25	0.44	0.07	0.45
-04	10	-	< 0.1	0.6	0.2	0.21	0.28	0.29	0.00	0.42
-06	50	+	1.0	**1.1**	**3.1**	**6.18**	**1.79**	**2.31**	0.37	**4.29**
-07	52	+	> 2.6	3.6	6.4	13.00	4.15	4.24	**1.07**	7.93
-08	57	+	> 2.6	11.8	32.9	59.27	21.63	21.66	8.46	35.59
-09	62	+	> 2.6	25.5	188.7	59.87	56.28	103.08	31.12	35.39
-10	64	+	> 2.6	24.3	188.7	59.89	56.97	103.08	31.60	35.80
PHM910 -01	0	-	0.2	0.6	0.1	0.22	0.31	0.66	-0.04	0.48
-02	18	±	0.2	0.7	0.4	0.38	0.35	0.89	0.02	0.60
-03	35	+	0.9	0.9	**1.4**	**3.69**	**1.26**	**1.54**	0.32	**3.56**
-04	42	+	> 2.7	**2.7**	4.7	23.84	5.32	4.81	**1.98**	17.84
-05	46	+	> 2.7	5.1	7.6	44.00	17.19	9.98	4.63	30.62
-06	49	+	> 2.7	5.6	16.2	51.24	11.42	15.17	7.02	35.39

[1] Data supplied by Boston Biomedica Inc.

[2] Reference ELISA test results obtained by WHO Collaborating Centre, HPA: REF 1 - Hepanostika HBsAg Uni-Form II (bioMérieux), REF 2 - Monolisa Ag HBs Plus (Bio-Rad)

NT = Not tested

NI = No information available

ELISA 1: ENZYGNOST HBsAg 5.0
ELISA 2: EQUIPAR HBsAg ONE-STEP
ELISA 3: GENEDIA HBsAg ELISA 3.0
ELISA 4: HEPALISA
ELISA 5: MUREX HBsAg VERSION 3

Table 6 cont. Results on early seroconversion panels

Panel ID	Days since first bleed	HBV DNA Detection PCR[1]	HBsAg conc ng/ml[1]	Reference tests		ELISA 1	ELISA 2	ELISA 3	ELISA 4	ELISA 5
				REF 1[2] OD/CO	REF 2[2] OD/CO	OD/CO	OD/CO	OD/CO	OD/CO	OD/CO
PHM916-01	0	-	<0.1	0.7	0.2	0.18	0.35	NT	NT	0.45
-02	7	-	<0.1	0.5	0.1	0.18	0.28	NT	NT	0.48
-03	41	-	<0.1	0.6	0.2	0.16	0.34	NT	NT	0.47
-04	43	-	<0.1	1.0	0.2	0.19	0.26	NT	NT	0.44
-05	48	+	<0.1	0.7	0.2	0.22	0.26	NT	NT	0.47
-06	50	+	<0.1	0.7	0.3	0.21	0.25	NT	NT	0.48
-07	55	+	<0.1	**1.1**	0.3	0.26	0.29	NT	NT	0.53
-08	57	+	<0.1	0.6	0.3	0.35	0.31	NT	NT	0.61
-09	62	+	0.2	0.7	0.6	**1.40**	0.52	NT	NT	**1.22**
-10	65	+	0.5	1.6	**1.4**	4.39	**1.03**	NT	NT	3.16
-11	69	+	2.3	2.1	4.9	17.57	1.49	NT	NT	15.16
PHM928-01	0	$<4x10^2$	NI	0.4	0.51	0.21	0.25	NT	NT	0.50
-02	2	$6x10^3$	NI	0.4	0.66	0.22	0.31	NT	NT	0.52
-03	7	$5x10^3$	NI	0.5	0.64	0.70	0.38	NT	NT	0.86
-04	9	$1x10^4$	NI	0.8	0.72	**1.13**	0.55	NT	NT	**1.27**
-05	14	$2x10^5$	NI	**2.2**	1.78	7.20	0.86	NT	NT	6.89
06	16	$2x10^5$	NI	2.8	**2.06**	6.35	0.80	NT	NT	9.52
07	21	$8x10^5$	NI	21.7	11.56	55.56	**10.14**	NT	NT	35.55

[1] Data supplied by Boston Biomedica Inc.
[2] Reference ELISA test results obtained by WHO Collaborating Centre, HPA: REF 1 - Hepanostika HBsAg Uni-Form II (bioMérieux), REF 2 - Monolisa Ag HBs Plus (Bio-Rad)
NT = Not tested
NI = No information available

ELISA 1: ENZYGNOST HBsAg 5.0
ELISA 2: EQUIPAR HBsAg ONE-STEP
ELISA 3: GENEDIA HBsAg ELISA 3.0
ELISA 4: HEPALISA
ELISA 5: MUREX HBsAg VERSION 3

Table 7. Results on low titre performance panel

Panel ID	Expected Result	PCR copies/ml[1]	HBsAg conc. IU/ml[1]	Reference tests		ELISA 1	ELISA 2	ELISA 3	ELISA 4	ELISA 5
				REF 1[2] OD/CO	REF 2[2] OD/CO	OD/CO	OD/CO	OD/CO	OD/CO	OD/CO
PHA105-01	POS	$5x10^3$	0.3	2.0	2.0	3.78	0.49	1.78	0.32	3.62
-02	POS	$3x10^3$	0.8	2.5	5.9	15.89	1.06	3.66	1.05	15.02
-03	POS	$2x10^3$	0.3	2.0	1.5	5.52	0.63	1.91	0.51	4.82
-04	POS	$5x10^4$	0.3	1.3	1.7	3.49	0.66	1.26	0.23	4.28
-05	POS	$2x10^4$	0.3	1.9	2.5	6.02	0.65	2.31	0.47	5.32
-06	POS	$7x10^4$	0.6	4.0	3.9	7.93	1.49	2.79	0.95	8.94
-07	POS	$7x10^3$	0.1	0.7	1.1	1.58	0.35	1.28	0.11	2.36
-08	POS	$2x10^4$	0.2	1.1	1.8	3.14	0.49	1.02	0.04	3.67
-09	POS	$9x10^3$	0.2	1.3	1.8	3.22	0.51	1.35	0.23	2.42
-10	POS	$2x10^3$	0.3	1.8	1.2	3.93	1.42	1.47	0.23	3.75
-11	NEG	NEG	NEG	0.4	0.3	0.24	0.52	0.57	-0.04	0.45
-12	POS	$7x10^3$	0.3	3.2	2.9	5.98	0.74	1.90	0.47	5.09
-13	POS	$< 4x10^2$	0.6	6.7	4.2	9.81	1.58	4.02	1.81	12.99
-14	POS	$7x10^3$	0.2	1.5	1.3	3.75	0.6	1.13	0.23	3.28
-15	POS	$2x10^4$	0.2	1.5	2.7	4.56	0.69	1.72	0.28	5.14

[1] Data supplied by Boston Biomedica Inc.
[2] Reference ELISA test results obtained by WHO Collaborating Centre, HPA: REF 1 - Hepanostika HBsAg Uni-Form II (bioMérieux), REF 2 - Monolisa Ag HBs Plus (Bio-Rad)

ELISA 1: ENZYGNOST HBsAg 5.0
ELISA 2: EQUIPAR HBsAg ONE-STEP
ELISA 3: GENEDIA HBsAg ELISA 3.0
ELISA 4: HEPALISA
ELISA 5: MUREX HBsAg VERSION 3

Explanatory Notes for Tables 1 - 7 and Figures 1 and 2

Table 1	**General characteristics and operational aspects of the assays**.
Sample type	The nature of specimen(s) that may be used in the assay.
Final dilution of the serum	The dilution of the serum in the test format, e.g. 10µl serum added to 200µl diluent gives a final dilution of 1:21.
Total time to perform the assay	Reflects the time needed to carry out 1 test run, i.e. the most economical use of the technique. For ELISAs, the number of samples + controls to complete 1 whole mictrotitre plate.
Price/test	As given at the time of the evaluation by the manufacturer, or converted to USD using the currency conversion rate at the time.
Table 2	**Comparison of the results of the assays with reference tests**
Sensitivity	Calculated as described on page 8 of this document.
Specificity	Calculated as described on page 8 of this document.
95% Confidence limits(CL)	Calculated as described on page 8 of this document
PPV and NPV	Calculated as described on page 8 of this document
Indeterminate results	Test results within stated ‘grey zone’ as defined by the manufacturer.

Explanatory Notes for Tables 1 - 7 and Figures 1 and 2

Table 3	**Detailed operational aspects of the assay**
Reading endpoint stability	Time within which results must be read after completion of the assay.
Minimum - maximum number of sera	- minimum number = 1 sample in addition to the required controls - maximum number = the maximum number of samples in addition to the required controls which can be simultaneously tested within the limits of the assay procedure.
Internal controls	Reagent control – if the assay has a reagent control, then the operator can tell visually if the reagents have been added. Sample addition control – if the assay has a sample addition control, then the operator can tell visually if the sample has been added to the test.
Definition of positive results	A sample is interpreted as positive according to the criteria set by the manufacturer and summarised in the table
Tables 4a, 4b	**Calculation of ease of performance of the assay**
	The criteria for this calculation are given in the respective tables.
Table 5	**Suitability of the assay for use in small laboratories**
	The criteria for this calculation are given in the respective table.
Note	These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.
Table 6	**Performance of the assay on seroconversion panels**

Explanatory Notes for Tables 1 - 7 and Figures 1 and 2

An assay's performance on the seroconversion panels should be viewed against both the sensitivity and specificity of the assay. Assays of relatively low specificity may appear to detect HBsAg earlier than other assays of higher specificity. Caution should be taken when reviewing seroconversion performance of assays tested only in limited numbers of panels.

Table 7 — **Results of the assays on a low titre panel**

A panel of samples with low HBsAg titre were tested in each assay. Any results which were different from the expected results are highlighted.

Figure 1 — **Relative performance in seroconversion panels**

See section 4.4.3 on page 10 for explanation of how this data was analyzed. The results for the 4 tested panels are on Tables 6 and 13. The 95% confidence limits should be interpreted with caution as only 4 panels were tested.

Figure 2 — **Relative performance of the assays on seroconversion and low titre panels**

Assay performance was compared on the two types of panel by summing the number of samples identified as positive on the seroconversion panels and summing the number of samples correctly identified as positive in the low titre panel.

Figure 1. Relative performance in seroconversion panels as compared to the reference assay (Monolisa Ag HBs PLUS)

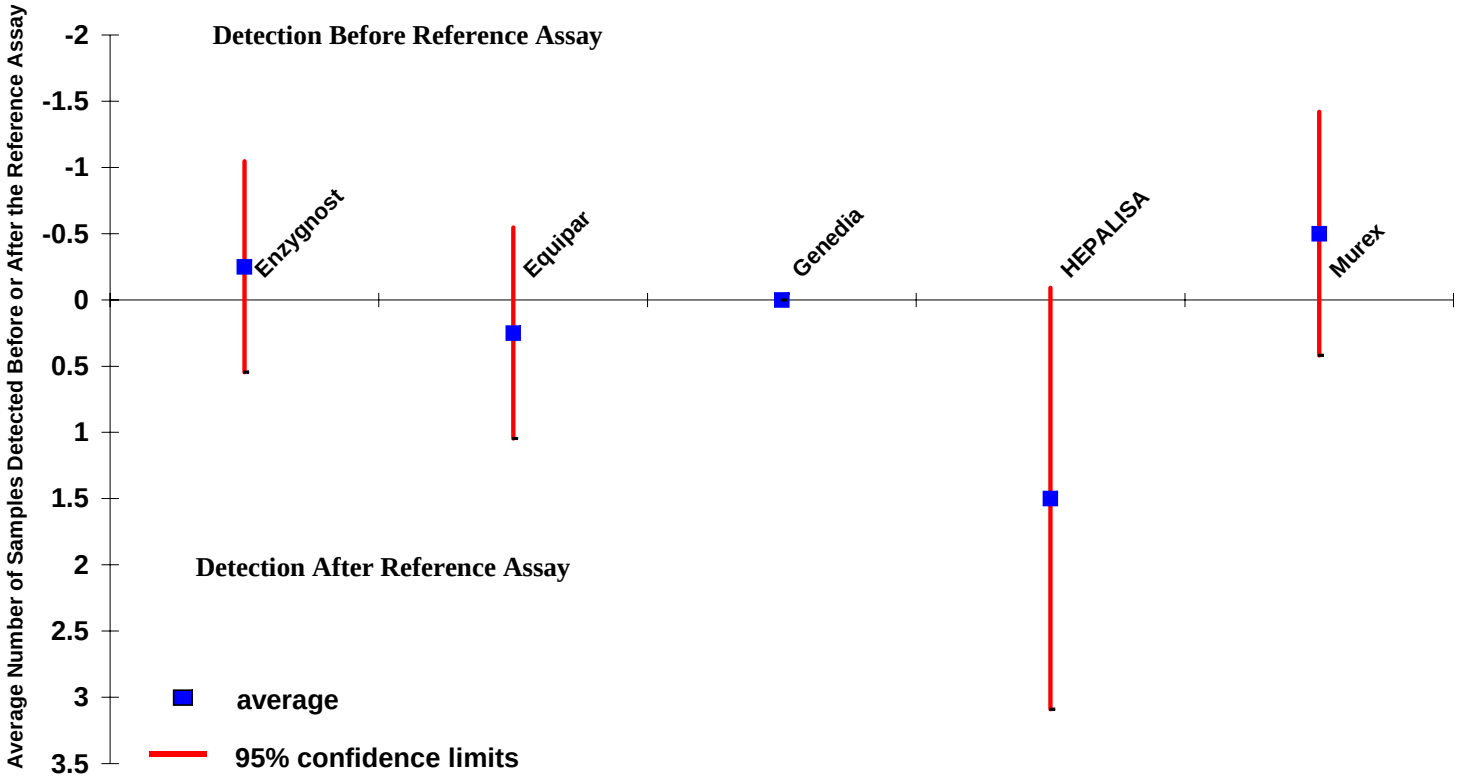
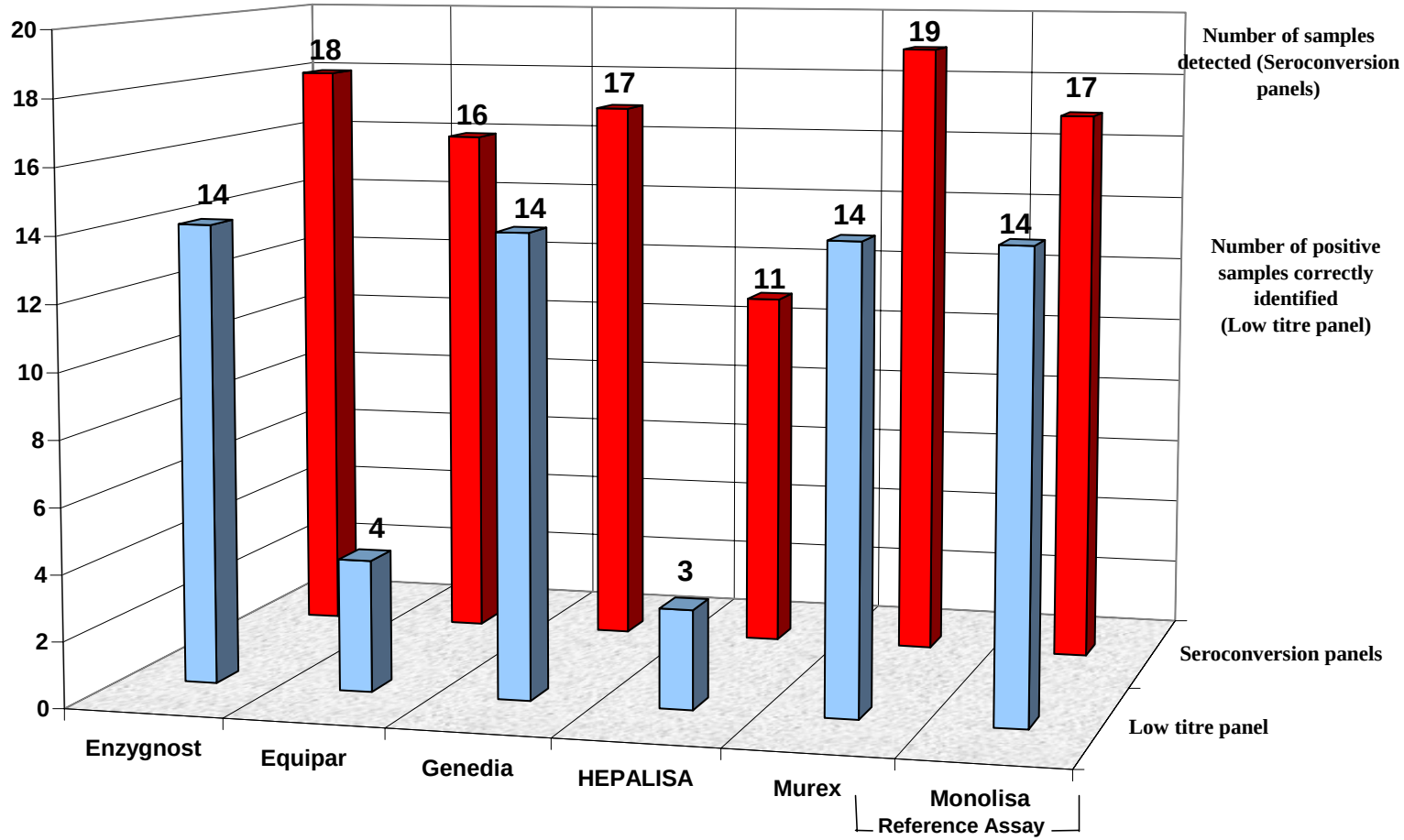
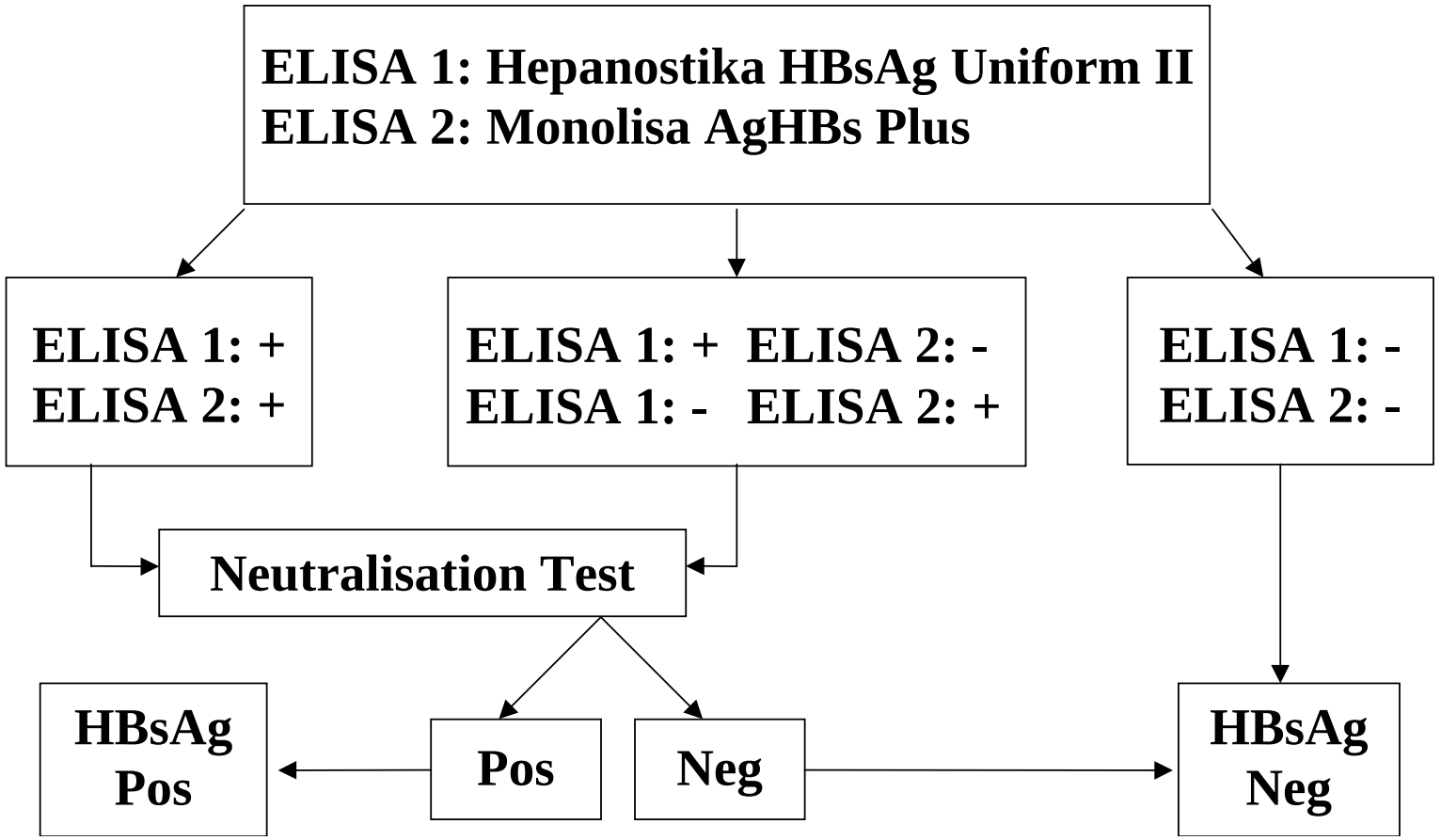


Figure 2. Relative performance in seroconversion and low titre panel



6. Annexes

Annex 1. Algorithm for characterization of the WHO HBsAg panel



Annex 2. Cumulative list of assays evaluated; currently commercially available

The names (and companies) of the assays evaluated to date under the WHO programme are listed in the table below. The number of the report in which each assay is covered is given, as well as cost per test; sensitivity and specificity with 95% confidence intervals, indeterminate results; initial inter-reader variability, ease of performance and suitability for use in small blood collection centres.

Simple/Rapid Assay (Company)	Report No [a]	Price/test[b] US$ (year)	Sensitivity[c] (%)[e]	Specificity [d] (%)[e]	Indeterminate results[f] (%)	Inter-reader variability[g] (%)	Ease of performance[h]	Storage conditions[i] (°C)
ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)	1	0.75 (1999)	100 (96.3 – 100)	95.5 (91.3 – 98.0)	2.9	5.4	VE	2-30
Determine™ HBsAg (Abbott Laboratories)	1	1.20 (1999)	100 (96.3 – 100)	99.4 (96.9 – 100)	0	0	VE	2-30
Doublecheck HBs Antigen (Orgenics)	1	1.00 (1999)	100 (96.3 – 100)	96.1 (92.1 – 98.4)	1.4	2.2	VE	2-8
Genelabs Diagnostics Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)	1	0.63 (1999)	100 (96.3 – 100)	97.8 (94.3 - 99.4)	0.7	2.2	VE	22-28
HEPACARD (J.Mitra & Co. Ltd.)	1	0.63 (1999)	100 (96.3 – 100)	97.8 (94.3 – 99.4)	0.7	2.5	VE	2-8
ImmunoComb® II HBsAg 90' (Orgenics)	1	0.90 (1999)	100 (96.3 – 100)	95.5 (91.3 – 98.0)	0.7	4.3	E	2-8
SERODIA® -HBs.*PA* (Fujirebio Inc.)	1	1.30 (2001)	100 (96.3 – 100)	100 (97.9 – 100)	0.0	6.1	E	2-10
Uni-Gold™ HBsAg (Trinity Biotech plc)	1	2.10 (1999)	100 (96.3 – 100)	100 (97.9 – 100)	0	0	VE	2-27
GENEDIA® HBsAg Rapid Device (Green Cross Life Science)	1	0.35 (2001)	100 (96.3 – 100)	100 (97.9 – 100)	0	0	VE	2-30

Annex 2 cont. Cumulative list of assays evaluated; currently commercially available

Simple/Rapid Assay (Company)	Report No [a]	Price/test[b] US$ (year)	Sensitivity[c] (%)[e]	Specificity [d] (%)[e]	Indeterminate results[f] (%)	Inter-reader variability[g] (%)	Ease of performance[h]	Storage conditions[i] (°C)
HEP B STAT-PAK *ULTRA FAST* (Chembio Diagnostic Systems Inc.)	1	0.70 (2001)	100 (96.3 – 100)	100 (97.9 – 100)	0	0.4	VE	8-30
Enzygnost HBsAg 5.0 (Dade Behring Inc)	2	1.09-1.40 (2004)	100 (96.3 – 100)	99.6 (97.9 – 100	0	NA	LE	2-8
Equipar HBsAg One Step (Equipar Diagnostici)	2	0.65 (2003)	100 (96.3 – 100)	98.3 (95.2 – 99.7)	0	NA	LE	2-8
Genedia HBsAg ELISA 3.0 (Green Cross Life Science Corp)	2	0.35-0.37 (2001)	100 (96.3 – 100)	98.9 (96.0 – 99.9)	0	NA	LE	2-8
HEPALISA (J Mitra & Co)	2	0.40-0.50 (2001)	100 (96.3 – 100)	100 (97.9 – 100)	0	NA	LE	4-8
Murex HBsAg Version 3 (Abbott-Murex)	2	0.90-1.00 (2003)	100 (96.3 – 100)	98.3 (95.2 – 99.7)	0	NA	LE	2-8

Legend for Annex 2.

a: 1: Operational Characteristics of Hepatitis B Surface Antigen Assays (PHASE I) Report 1
2: Operational Characteristics of Hepatitis B Surface Antigen Assays (PHASE I) Report 2

b: Prices are those quoted by the manufacturer at the time of the evaluation.

c, d, e: Sensitivity, specificity and 95% confidence limits were calculated as described on pages 8-9 of this document.

f: Indeterminate results were calculated as described in the explanatory notes on page 32 of Hepatitis B Surface Antigen Assays: Operational Characteristics (*PHASE I*) Report 1 and page 22 of this document.

g: Inter-reader variability was calculated as described on page 11 of Hepatitis B Surface Antigen Assays: Operational Characteristics (*PHASE I*) Report 1.

h: Ease of performance is defined in Table 4b.

i: Storage conditions listed are for unopened kits. See Table 3 for storage conditions of opened kits.

Annex 3. Cumulative list of assay manufacturers' addresses

Abbott Laboratories, D-09C9, Building AP6C4, 100 Abbott Park Road, Abbott Park, North Il. 60064-3500.
Tel: +1 847 9376100; Fax: +1 847 937 3559; Website: www.abbott.com

Bionike Inc., 1015 Grandview Drive, So. San Francisco, CA, 94080-4910 USA.
Tel: +1 415 737 7937; Fax: +1 650 737 5902; Website: www.bionike.com

bioMérieux sa, F-69280 Marcy l'Etoile, France.
Tel: +33 04 78 87 20 00; Fax: (+33) 04 78 87 20 90; Website: www.biomerieux.com

Chembio Diagnostic Systems Inc., 3661 Horseblock Road, Medford, NY 11763, USA
Tel: +1 631 924 1135; Fax: +1 631 9246033; Website: www.chembio.com

Dade Behring, Inc. 1717 Deerfield Road, Deerfield, Illinois 60015, USA
Tel: +1 847 267 5300; Fax +1 847 267 1066; Website: www.dadebehring.com

Equipar Diagnostici, 21/N Via G.Ferrari, 21047 Saronno (Va), Italy
Tel. +39 02960 5422; Fax. + 39 02960 7106; Website: www.equipar.it

Fujirebio Inc., FR Bldg., 62-5, Nihonbashi-Hamacho 2-Chome Chuo-Ku Tokyo 103-0007 Japan.
Tel: +81 3 5695 9217; Fax: +81 3 5695 9231; Website: www.fujirebio.co.jp

Genelabs Diagnostics Pte Ltd., 85, Science Park Drive, # 04-01 The Cavendish, Singapore Science Park, Singapore 118259.
Tel: +65 775 0008; Fax +65 775 4536; Website: www.genelabs.com.sg
Halle de Frêt, P. O. Box 1015, 1215 Geneva 15 Airport, Switzerland.
Tel: +41 22 788 1908; Fax +41 22 788 1986

Green Cross Life Science Corp, 227-3, Gugal-li, Giheung-eup, Yongin-shi, 449-900, Kyonggi-do, Korea.
Tel: +82 31 280 6245; Fax: +82 31 280 6249; Website: www.greencross.com

J. Mitra & Co. Ltd., A-180, Okhla Industrial Area, Phase-1, New Delhi-110 020, India.
Tel: +91 11 681 8971; Fax: +91 11 681 8970; Website: www.jmitra4u.com

Organon Teknika, see bioMérieux

Orgenics, P.O. Box 360 Yavne 70650, Israel.
Tel: +972 8 942 9233; Fax: +972 8 943 8758; Website: www.orgenics.com

Trinity Biotech plc, IDA Business Park, Bray, Co. Wicklow, Ireland.
Tel: +353 1276 9800; Fax: +353 1276 9888; Website: www.trinitybiotech.ie

7. ADDITIONAL READING

Additional information may be obtained by visiting the EHT section of the WHO website at www.who.int/bct and following the links to *Key Initiatives*, *HIV Diagnostics.* In addition to general information on diagnostics, assay evaluation reports for HIV, HCV and HBV are available as well as details of the WHO HIV Test Kit Bulk Procurement Scheme.

8. ACKNOWLEDGEMENTS

We would like to thank Mrs. S. Lloyd, National Blood Transfusion Service, Harare, Zimbabwe; Dr. E. Vinelli, National Blood Transfusion Service, Tegugialpa, Honduras; Dr. S. Tanprasert, National Blood Transfusion Service, Bangkok, Thailand and Dr. J. Parry, Central Public Health Laboratory, Health Protection Agency, London, UK for supplying specimens to the WHO Hepatitis B Surface Antigen evaluation panel.

We acknowledge the 5 companies, Abbott-Murex, Dade Behring Inc, Equipar Diagnostici, Green Cross Life Science Corporation and J Mitra & Co, for supplying the test reagents free of charge.

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