

WHO/BCT/BTS/01.4

Distr.: General

Orig.: English

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

REPORT 1

MAY 2001



**BLOOD SAFETY AND CLINICAL TECHNOLOGY
WORLD HEALTH ORGANIZATION**

Copyright © World Health Organization, 2001

This document is issued by WHO for general distribution.

The document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. This document may not be reproduced, in part or in whole, without written approval from the contact person listed below. This document must not be used in conjunction with commercial or promotional purposes.

Neither WHO nor the Central Public Health Laboratory, London, UK warrant or represent that the evaluations conducted with the Hepatitis B test kits referred to in this document are accurate, complete and/or error-free. WHO and the Central Public Health Laboratory disclaim all responsibility and liability for any use made of the data contained herein.

The mention of specific companies and of certain manufacturers' products does not imply that they are endorsed or recommended by WHO in preference to others of a similar nature that are not mentioned.

Contact: Dr. G. Vercauteren, Blood Safety and Clinical Technology – WHO – 20, Avenue Appia – 1211 Geneva 27 – Switzerland.

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

1.	Summary	1
2.	Background information	2
3.	Laboratory aspects of HBsAg testing	2
	3.1 A brief overview	2
	3.2 Quality assurance	4
	3.3 Safety	4
4.	Materials and methods	4
	4.1 Assays (test kits) evaluated	4
	4.2 Evaluation panels	8
	4.2.1 WHO HBsAg panel	8
	4.2.2 Seroconversion panels	9
	4.2.3 Performance panel	9
	4.3 Laboratory testing	9
	4.4 Analysis	9
	4.4.1 Sensitivity, specificity, confidence limits (CL) and predictive values of HBsAg tests	9
	4.4.2 Inter-reader variability	11
	4.4.3 Sensitivity in seroconversion panels	11
	4.4.4 Sensitivity in performance panel	11
	4.4.5 Additional analyses	11
5.	Assay evaluations	12
	Group 1: Simple/rapid assays	14
	Table 1. General characteristics and operational aspects	14
	Table 2. Comparison of the results of assays with reference tests	15
	Table 3. Detailed operational aspects	16
	Table 3.(cont.) Detailed operational aspects	17
	Table 4a. Technician's appraisal of the test kit	18
	Table 4b. Calculation of ease of performance	19
	Table 5. Suitability for use in small laboratories	20

Table 6.	Results on seroconversion panels	21
Table 7.	Results on low titre performance panel	22
Group 2: Simple/rapid assays		23
Table 8.	General characteristics and operational aspects	23
Table 9.	Comparison of the results of assays with reference tests	24
Table 10.	Detailed operational aspects	25
Table 10.(cont.)	Detailed operational aspects	26
Table 11a.	Technician's appraisal of the test kit	27
Table 11b.	Calculation of ease of performance	28
Table 12.	Suitability for use in small laboratories	29
Table 13.	Results on seroconversion panels	30
Table 14.	Results on low titre performance panel	31
Explanatory notes for Tables 1-14 and Figures 1 and 2		32
Figure 1.	Relative performance in seroconversion panels as compared to the reference assay (Monolisa Ag HBs Plus)	35
Figure 2.	Relative performance in seroconversion and low titre panels	36
6.	Annexes	37
	Annex 1. Algorithm for characterization of the WHO HBsAg panel	37
	Annex 2. Cumulative list of assays evaluated; currently commercially available	38
	Annex 3. Cumulative list of assay manufacturers' addresses	40
7.	Additional Reading	41
8.	Acknowledgements	41

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 first report presents the findings of the Phase I evaluations of 10 HBsAg assays conducted during September to November 1999 and January to May 2001. The HBsAg assays evaluated included:

Group 1: Tables 1 – 7

- ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)
- Determine™ HBsAg (Abbott Laboratories)
- Doublecheck™ HBs Antigen (Orgenics)
- Genelabs Diagnostics Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)
- HEPACARD (J.Mitra & Co. Ltd.)

Group 2: Tables 8-14

- ImmunoComb® II HBsAg 90' (Orgenics)
- SERODIA® -HBs.PA (Fujirebio Inc.)
- Uni-Gold™ HBsAg (Trinity Biotech plc)
- GENEDIA® HBsAg Rapid Device (Green Cross Life Science Corp)
- HEP B STAT-PAK *ULTRA FAST* (Chembio Diagnostic Systems Inc.)

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 WHO HBsAg panel, the cumulative list of assays evaluated and the addresses of manufacturers of the assays evaluated.

This first report contains Phase I assessments of simple/rapid tests only. Subsequent reports will be issued on a regular basis and will include results on enzyme linked immunosorbent assays (ELISAs) and assays using other technologies. Copies of these reports are available on request from the Department of Blood Safety and Clinical Technology (BCT), 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 Blood Safety and Clinical Technology, WHO; the WHO Collaborating Centre on Transfusion Transmissible Infections, Sexually Transmitted and Blood Borne Virus Laboratory (SBVL), Virus Reference Division, Central Public Health Laboratories, 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, i.e. 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. The clinical course of an HBV infection includes an incubation period (generally 4 - 12 weeks), acute illness (2 weeks – 3 months) and recovery for individuals who resolve their infection. Many HBV infections in adults are without the classical symptoms of jaundice. Individuals in whom HBsAg is present in their blood for more than six months are considered to be chronically infected with HBV. Serological profiles of acute and chronic HBV infections are presented in Figures A, B and C below.

Figure A: Acute HBV infection

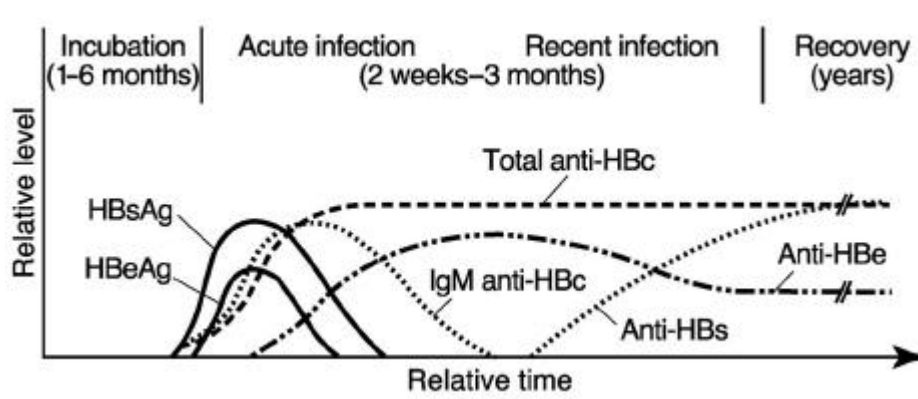


Figure B: Chronic HBV infection (HBeAg positive)

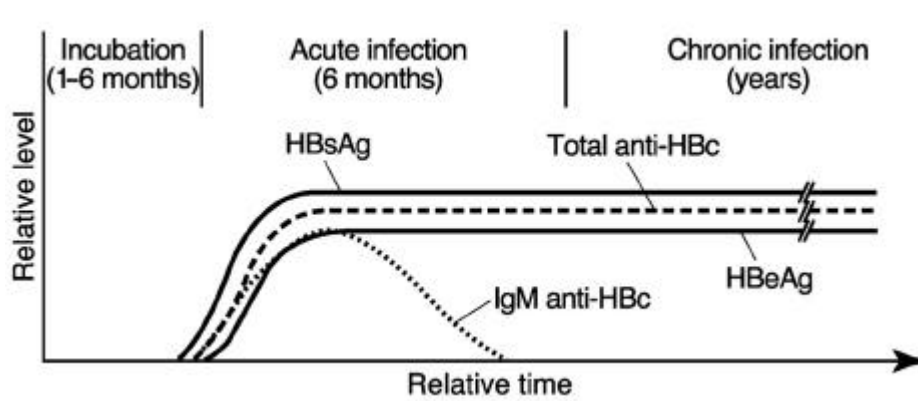
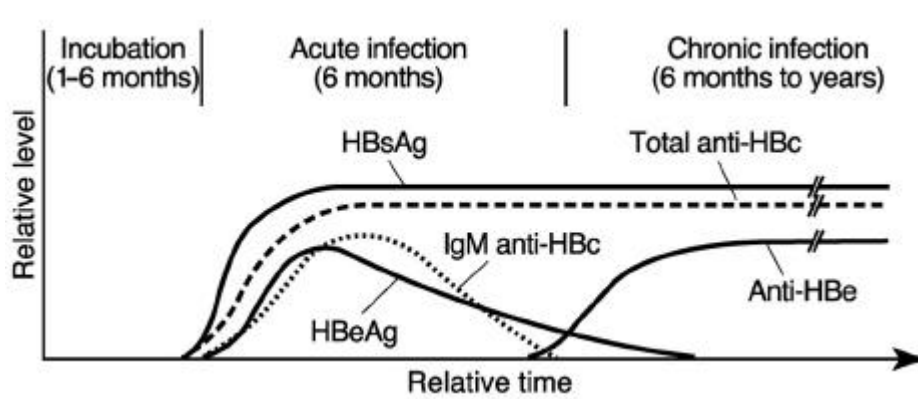


Figure C: Chronic HBV infection (HBeAg negative)



The most commonly used diagnostic and blood screening markers sought is Hepatitis B surface antigen (HBsAg). 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 using 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 and anti-

HBs. The presence of HBeAg indicates an individual is of higher infectivity, and seroconversion to anti-HBe correlates with reduced infectivity. In an acute infection it 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 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. A positive result is indicated by the appearance of a coloured dot or line, or shows an agglutination pattern. While most of these tests can be performed in less than 10 minutes, other simple tests are less rapid and their performance requires 30 minutes to 2 hours. The results are read visually. 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.

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 serum or plasma 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 Biosafety Guidelines for Diagnostic and Research Laboratories Working With HIV, Geneva, World Health Organization, 1991 (WHO AIDS Series 9) and Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, Geneva, World Health Organization, 1997 (WHO/EMC/97.3).

4. MATERIALS AND METHODS

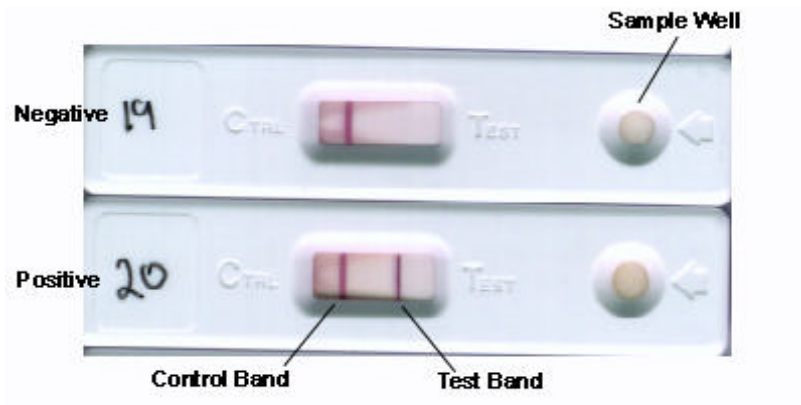
4.1 Assays (test kits) evaluated

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 each of the assays under evaluation, accompanied by a picture showing the appearance of a positive and negative result, is shown below.

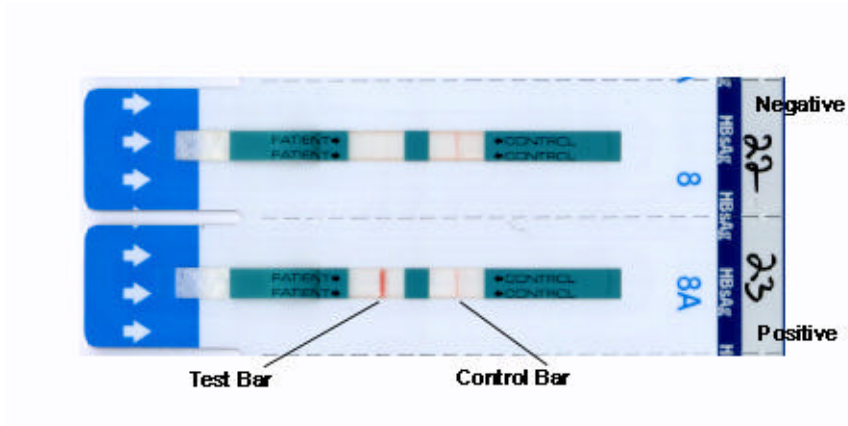
ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)

Is a one step, immunochromatographic assay for the detection of HBsAg in human serum.



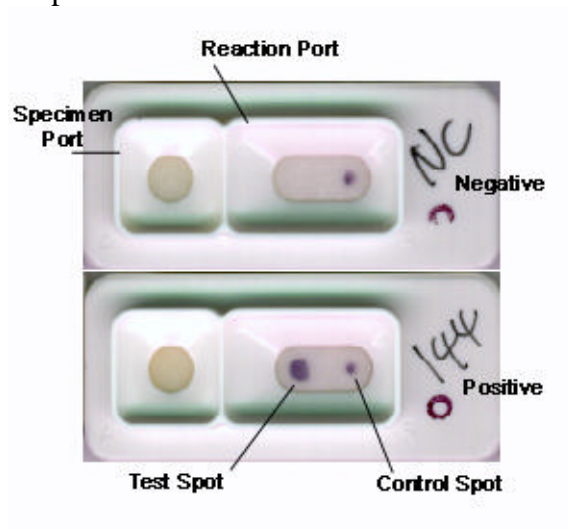
Determine™ HBsAg (Abbott Laboratories)

Is a visually read, qualitative immunoassay for the detection of HBsAg in human serum, plasma and whole blood.



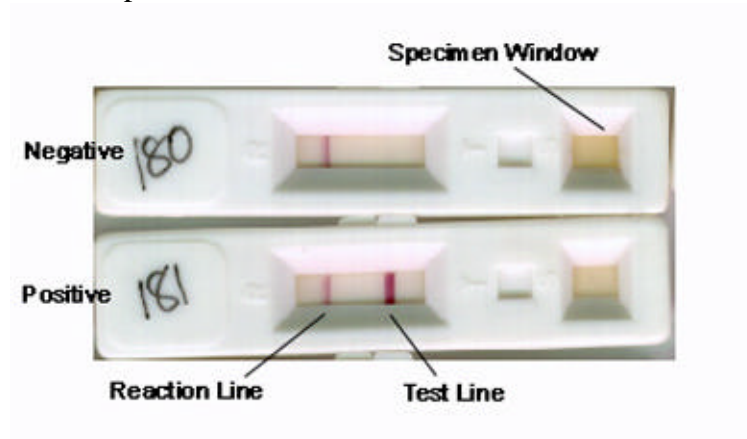
Doublecheck™ HBs Antigen (Orgenics)

Is a qualitative immunochromatographic/filtration assay for the detection of HBsAg in human serum and plasma.



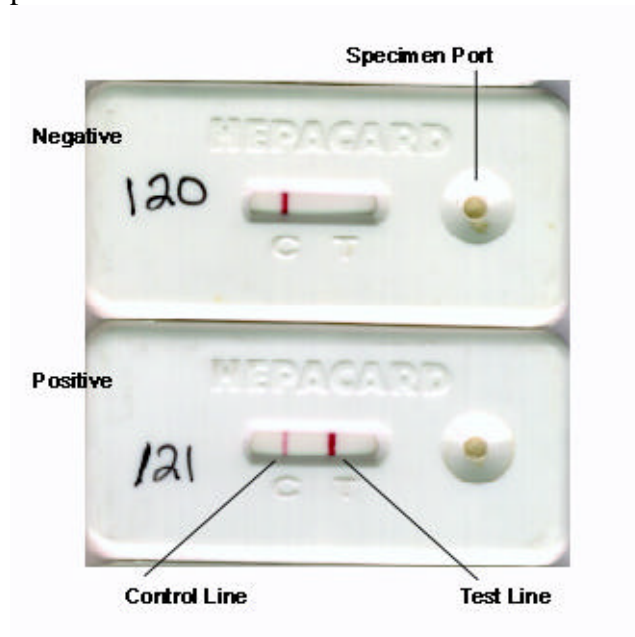
Genelabs Diagnostics Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)

Is a qualitative, one step immunochromatographic assay for the detection of HBsAg in human serum or plasma.



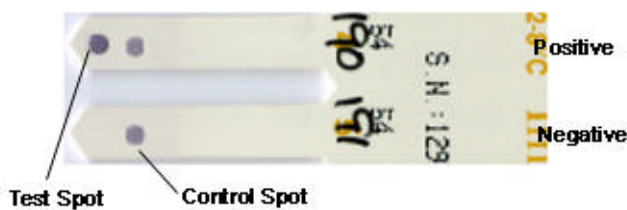
HEPACARD (J.Mitra & Co. Ltd.)

Is a qualitative, one step enzyme immunoassay for the detection of HBsAg in human serum or plasma.



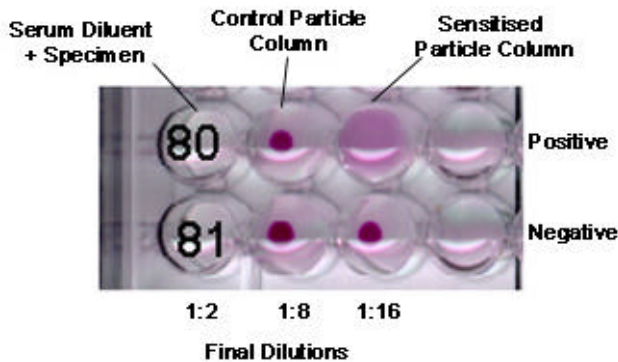
ImmunoComb® II 90' (Orgenics)

Is a solid phase enzyme immunoassay for the qualitative detection of HBsAg in human serum and plasma.



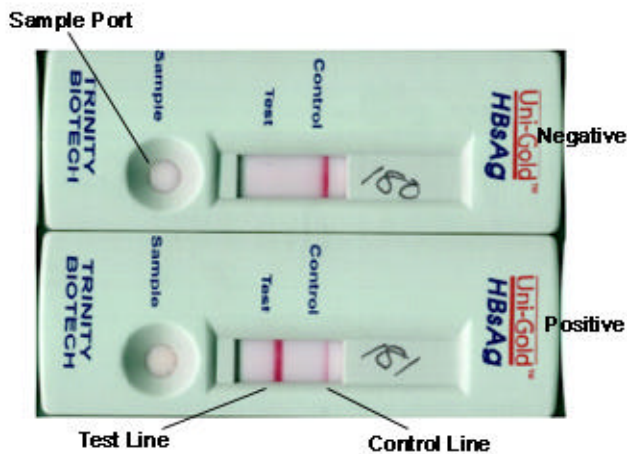
SERODIA® -HBs.PA (Fujirebio Inc.)

Is a particle agglutination assay for the qualitative detection of HBsAg. It may be used in both qualitative (described in this report) and quantitative procedures. The agglutination test is performed in a curved bottom microtitre plate.



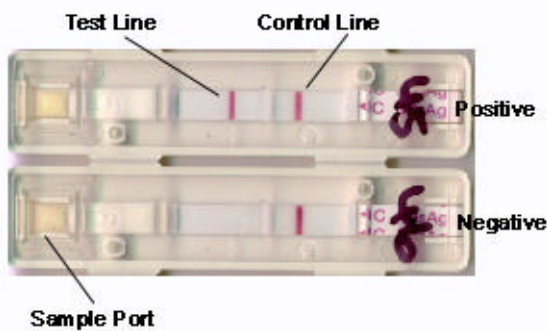
Uni-Gold™ HBsAg (Trinity Biotech plc)

Is a qualitative immunochromatographic assay for the detection of HBsAg in human serum, plasma or whole blood.



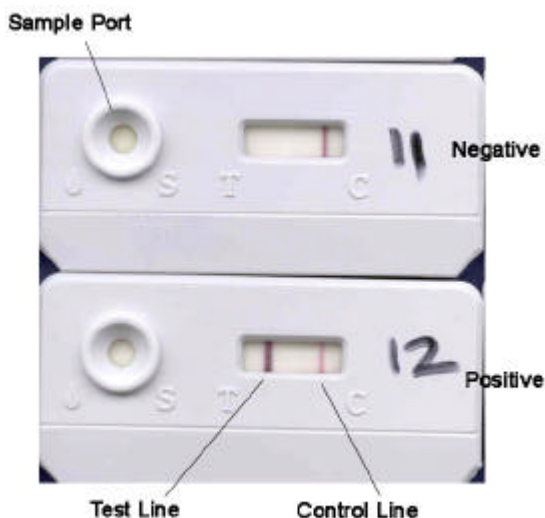
GENEDIA® HBsAg Rapid Device (Green Cross Life Science Corp)

Is an immunochromatographic assay, designed for the qualitative determination of HBsAg in human serum.



HEP B STAT-PAK *ULTRA FAST* (Chembio Diagnostic Systems Inc.)

Is a one step screening test for the detection of HBsAg in serum.



4.2 Evaluation panels

4.2.1 WHO HBsAg panel

The phase I evaluations reported here were carried out using a panel of 277 sera (as shown in Table A), of which 60 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	17	43	60
Asia	30	30	60
Europe	30	67	97
Latin America	22	38	60
Total	99	178	277

Characterization of WHO HBsAg panel

For characterization, specimens in the WHO reference panel were screened by ELISAs, Hepanostika HBsAg Uniform II (Organon Teknika) 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 (Organon Teknika). 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. Five commercial seroconversion panels, PHM901, PHM902, PHM903, PHM907 and PHM910 [Boston Biomedica Inc. (BBI)] were tested on each of the ten simple/rapid assays evaluated. These panels consisted of a total of 43 specimens collected from 5 individuals during seroconversion.

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. Visual interpretations of the results were made independently by three technicians. When the three technicians interpreted the results 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.

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	

$$\text{Sensitivity} = a/(a+c)$$

$$\text{Specificity} = d/(b+d)$$

$$\text{Positive predictive value} = a/(a+b)$$

$$\text{Negative predictive value} = d/(c+d)$$

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

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

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:

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

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 Inter-reader variability

The inter-reader variability was calculated as the percentage of specimens for which initial test results were differently interpreted (i.e. positive, negative or indeterminate) by the independent readers.

4.4.3 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 5 seroconversion series were averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence limits were determined.

4.4.4 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.

4.4.5 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 10 test kits evaluated have been divided into 2 groups. Results from the first 5 test kits (Group 1) are presented in tables 1-7, and results from the second 5 test kits (Group 2) are presented in tables 8-14. Tables 1 and 8 summarize the general characteristics of the assays. Results of the assays evaluated as compared to the reference tests are given in Tables 2 and 9; Tables 3 and 10 provide 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 for the Group 1 kits and in tables 11a, 11b and table 12 for the Group 2 kits. Performance of the assays under evaluation on early seroconversion panels, and low titre panels is given in Tables 6 and 7, and 13 and 14 respectively. The relative performance of all 10 of the 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 commercial panels. Explanatory notes are provided at the end of the assay evaluation tables.

ASSAY EVALUATIONS

Group 1: Simple/rapid assays

Table 1. General characteristics and operational aspects

NAME	ADVANCED QUALITY^ä One Step HBsAg Test	Determine ^ä HBsAg	Doublecheck^ä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Company	Bionike, Inc., San Francisco, USA	Abbott Laboratories, Dainabot Co. Ltd, Tokyo, Japan	Orgenics, Yavne, Israel	Genelabs Diagnostics, Pte. Ltd., Singapore	J. Mitra & Co. Ltd, New Delhi, India
Assay type	immunochromatographic	immunochromatographic	immunochromatographic	immunochromatographic	immunochromatographic
Antibody type	monoclonal	monoclonal	polyclonal monoclonal	monoclonal	polyclonal monoclonal
Solid phase	membrane	chromatographic strip	membrane	membrane	nitrocellulose strip
Specimen type	serum/plasma	serum/plasma/whole blood	serum/plasma	serum/plasma	serum/plasma
Number of tests per kit	40	100	40	20	20, 50, 100
Lot numbers evaluated (expiry date)	RD903100130 (Sept. '00) RD9051901 (Nov. '00)	46416U100 (Sept. '99)	990113 (Jan. '00)	FA-016 (Nov. '00) FA-017 (June '01)	HPC02059 (April '00) HPC03059 (April '00)
Shelf life at (°C)	18 months (2 - 30)	18 months (2 - 30)	12 months (2 - 8)	7 - 9 months (22 - 28)	12 months (2 - 8)
Volume of serum needed (µl) Final dilution of serum	200 none	50 none	150 5:2 (with diluent)	100 none	160 none
Total time to perform the assay: h. min. (number of sera)	0.35 (10)	0.18 (10)	0.17 (4) 0.20 (10)	0.25 (10)	0.25 (10)
Reading	visual	visual	visual	visual	visual
Price/test (US\$)	0.75	1.20	1.00	0.63	0.63

Table 2. Comparison of the assays with reference tests

NAME	ADVANCED QUALITYä One Step HBsAg Test	Determine ä HBsAg	Doublecheckä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Final Sensitivity % (95 CL)* n = 99	99.0 (94.5 – 100.0)	99.0 (94.5 – 100.0)	99.0 (94.5 – 100.0)	99.0 (94.5 – 100.0)	99.0 (94.5 – 100.0)
Initial Specificity % (95 CL)*	94.4 (89.9 – 97.3)	99.4 (96.9 – 100.0)	95.5 (91.3 – 98.0)	97.2 (93.6 – 99.1)	96.1 (92.1 – 98.4)
Final Specificity % (95 CL)* n = 178	95.5 (91.3 – 98.0)	99.4 (96.9 – 100.0)	96.1 (92.1 – 98.4)	97.8 (94.3 – 99.4)	97.8 (94.3 – 99.4)
Indeterminate results %	2.9	0	1.4	0.7	0.7
Initial inter-reader variability %	5.4	0	2.2	2.2	2.5
PPV 0.1%	18.2	62.5	20.4	31.3	31.3
5.0%	53.7	89.7	57.2	70.3	70.3
10.0%	71.0	94.8	73.8	83.3	83.3
NPV 0.1%	100.0	100.0	100.0	100.0	100.0
5.0%	99.9	99.9	99.9	99.9	99.9
10.0%	99.9	99.9	99.9	99.9	99.9

* 95 % Confidence Limits

Table 3. Detailed operational aspects

NAME	ADVANCED QUALITYä One Step HBsAg Test	Determine ä HBsAg	Doublecheckä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Dimension (cm) of kit : w-l-h	13.3 – 22.1 – 9.2 (40 tests)	16 – 27 – 15 (100 tests)	26 – 30.5 – 11 (40 tests)	12 – 12 – 6.5 (20 tests)	10.3 – 19.5 – 5.0 (20 tests) 10.3 – 21.0 – 11.0 (50 tests) 21.5 – 23.5 – 11.0 (100 tests)
Storage conditions (°C)	2 – 30	2 – 30	2 – 8	22 – 28	2 – 8
Incubation temperature (°C)	room temperature	room temperature	room temperature (22-26)	room temperature	room temperature
Reading endpoint stability (h.min)	0.35 ± 0.05	up to 24.00	8.00	≥ 24.00	≥ 24.00
Stability after dilution/ reconstitution/ opening at (°C)					
- antigen	expiry date (2 – 30)	expiry date (2 – 30)	expiry date (2 – 8)	expiry date (22 – 28)	expiry date (2 – 8)
- controls	not applicable	not applicable	expiry date (2 – 8)	not applicable	not applicable
- sample diluent	not applicable	not applicable	expiry date (2 – 8)	not applicable	not applicable
- conjugate	not applicable	not applicable	expiry date (2 – 8)	not applicable	not applicable
- substrate	not applicable	not applicable	expiry date (2 – 8)	not applicable	not applicable
- wash buffer	not applicable	not applicable	expiry date (2 – 8)	not applicable	not applicable
Number of sera per run minimum – maximum	1 – 10	1 – 30	1 – 4	1 – 10	1 – 10
Number of controls per test run	not supplied	not supplied	2	not supplied	not supplied
- negative	0	0	1	0	0
- cut-off/weak positive	0	0	0	0	0
- positive	0	0	1	0	0
- blank	0	0	0	0	0
internal control : reagent control	yes	yes	yes	yes	yes
: sample addition control	yes	yes	yes	yes	no

Table 3. (continued) Detailed operational aspects

NAME	ADVANCED QUALITY^ä One Step HBsAg Test	Determine ^ä HBsAg	Doublecheck^ä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Estimated time to perform one run: h. min (number of sera)	0.15 (1) 0.35 (10)	0.16 (1) 0.18 (10)	0.10 (1) 0.20 (10)	0.10 (1) 0.25 (10)	0.10 (1) 0.25 (10)
Equipment needed but not provided in the kit: ¹					
- washer	-	-	-	-	-
- incubator (water-bath)	-	-	-	-	-
- spectrophotometric reader	-	-	-	-	-
- refrigerator (storage)	-	-	+	-	+
- agitator , rocker	-	-	-	-	-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	+	+	-	+	-
- multichannel (µl)	-	-	-	-	-
- disposable tips	+	+	-	+	-
- dilution tubes/rack, microtiterplate	-	-	-	-	-
- distilled or deionised water	-	-	-	-	-
- plate covers	-	-	-	-	-
- graduated pipette; cylinder (ml)	-	-	-	-	-
- sulfuric acid/sodium hydroxide	-	-	-	-	-
- absorbent paper	-	-	-	-	-
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	-	-	-	-	-
- timer	+	+	+	+	+
Definition of positive results	distinct colored bands in both control and test regions	red bars in both control and patient windows	a blue/grey spot in both test and control regions	a purple/red line in both test and control regions	a distinct pink line in both test and control regions
Definition of grey zone	not applicable	not applicable	not applicable	not applicable	not applicable

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	ADVANCED QUALITY One Step HBsAg Test	Determine ä HBsAg	Doublecheckä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Number of steps in the test procedure: -1-2 steps -3-5 steps ->5 steps	6 3 1	6	6	3	6	6
Clarity of kit instructions: - good - needs improvement	2 1	2	2	2	2	2
Kit and reagent packaging and labelling: - good - needs improvement	2 1	2	2	2	2	2
Total (out of possible 10)	10	10	10	7	10	10
Comments on the test kit		very easy to set up	straight forward and easy to set up	none	very easy test to set up	easy to set up, volume in the dropper needs to be stated

Table 4b. Calculation of ease of performance

NAME	ADVANCED QUALITY ^ä One Step HBsAg Test	Determine ^ä HBsAg	Doublecheck ^ä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Need to prepare: -antigen -substrate -wash solution -conjugate -predilution of serum	1 ¹ 1 1 1 1	1 1 1 1 1	1 1 1 1 0	1 1 1 1 1	1 1 1 1 1
Stability after dilution/opening: (expiry date = 1; less = 0) -antigen -controls -sample diluent -conjugate -substrate -wash buffer -sufficient reagents -wash (yes =0; no = 1)	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1
Item needed but not provided in the kit: -reagent trough -automatic /multichannel pipette -dilution – tubes, rack/microtiter plate -distilled or deionised water -plate covers -graduated pipette ,cylinder -sulfuric acid/sodium hydroxide	1 0 ² 1 1 1 1 1	1 0 1 1 1 1 1	1 1 1 1 1 1 1	1 0 1 1 1 1 1	1 1 1 1 1 1 1
Technician’s appraisal of the test kit ³ (rating out of 10)	10	10	7	10	10
Total (out of possible 30)	29	29	26	29	30
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	very easy	very easy	very easy	very easy	very easy

NAME	ADVANCED QUALITY ¹ One Step HBsAg Test	Determine ² HBsAg	Doublecheck ² HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD

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	ADVANCED QUALITY ^a One Step HBsAg Test	Determine ^a HBsAg	Doublecheck ^a HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Sensitivity						
- 100%	5					
- 98 – 100%	3	3	3	3	3	3
- <98%	0					
Specificity						
- >98%	5					
- 95 – 98%	3	3	5	3	3	3
- <95%	0					
Incubation temperature						
- room t°	3	3	3	3	3	3
- other than room t°	1					
Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	3	3	2	2	2
- < 6 months	1					
Storage at						
- ambient t° possible (opened kit)	5					
- ambient t° possible (unopened kit)	2	5	5	1	5	1
- 2-8 °C required	1					
Price per test (US\$)						
- ≤ 1.0	3					
- ≤ 2.0	2	3	2	3	3	3
- > 2.0	1					
Ease of performance						
- very easy	5					
- easy	3	5	5	5	5	5
- less easy	1					
Rapidity of performance:1 serum						
- < 10 min	3					
- 10 – 30 min	2	2	2	2	2	2
- > 30 min	1					
Washer/agitator						
- not needed	3	3	3	3	3	3
- needed	1					
Reading						
- visual: inter-reader variability ≤ 3%	5					
: inter-reader variability > 3%	3	3	5	5	5	5
- reading equipment	1					

NAME	Score	ADVANCED QUALITY^ä One Step HBsAg Test	Determine^ä HBsAg	Doublecheck^ä HBs Antigen	Genelabs Diagnostics Rapid HBsAg Test	HEPACARD
Total (out of possible 40)		33	36	30	34	30
Suitability for use in small laboratories: - less suitable < 23 - suitable $23 \leq x \leq 30$ - very suitable > 30		very suitable	very suitable	Suitable	very suitable	suitable

Table 6. Results on seroconversion panels

Panel ID	Bleed Date	Days since first bleed	HBV DNA Detection PCR ¹	HBsAg conc ng/ml ²	Reference ELISA 1 ³ OD/CO	Reference ELISA 2 ⁴ OD/CO	SR1	SR2	SR3	SR4	SR5	
PHM901	-01	25.11.87	0	+	0.3	0.7	0.8	neg	neg	neg	neg	neg
	-02	27.11.87	2	+	0.6	0.8	2.0	neg	neg	neg	neg	neg
	-03	03.12.87	8	+	>2.5	4.1	19.9	neg	pos	neg	neg	neg
	-04	05.12.87	10	+	>2.5	7.1	20.9	pos	pos	neg	neg	neg
	-05	11.12.87	16	+	>2.5	21.3	181.8	pos	pos	pos	pos	pos
	-06	16.12.87	21	+	>2.5	29.1	181.8	pos	pos	pos	pos	pos
	-07	18.12.87	23	+	>2.5	21.3	181.8	pos	pos	pos	pos	pos
PHM902	-01	27.08.90	0	-	< 0.1	0.6	0.3	neg	neg	neg	neg	neg
	-02	29.08.90	2	-	< 0.1	0.5	0.3	neg	neg	neg	neg	neg
	-03	04.09.90	8	-	< 0.1	0.5	0.2	neg	neg	neg	neg	neg
	-04	06.09.90	10	-	< 0.1	0.7	0.1	neg	neg	neg	neg	neg
	-05	23.10.90	57	+	< 0.1	0.6	0.1	neg	neg	neg	neg	neg
	-06	25.10.90	59	+	< 0.1	0.6	0.3	neg	neg	neg	neg	neg
	-07	30.10.90	64	+	< 0.1	0.7	0.3	neg	neg	neg	neg	neg
	-08	01.11.90	66	+	< 0.1	0.6	0.4	neg	neg	neg	neg	neg
	-09	06.11.90	71	+	0.5	0.9	1.3	neg	neg	neg	neg	neg
	-10	08.11.90	73	+	0.5	1.0	1.5	neg	neg	neg	neg	neg
	-11	13.11.90	78	+	1.2	2.9	2.5	neg	neg	neg	neg	neg
	-12	15.11.90	80	+	2.2	2.2	3.5	neg	neg	neg	neg	ind
	-13	20.11.90	85	+	> 2.7	5.6	8.1	neg	pos	neg	neg	neg
	-14	23.11.90	88	+	> 2.7	5.3	12.5	ind	pos	ind	neg	neg
PHM903	-01	15.01.91	0	+	< 0.1	0.5	0.0	neg	neg	neg	neg	neg
	-02	18.01.91	3	+	< 0.1	0.5	0.1	neg	neg	neg	neg	neg
	-03	21.01.91	6	+	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-04	25.01.91	10	+	0.1	0.9	0.5	neg	neg	neg	neg	neg
	-05	29.01.91	14	+	1.0	1.1	1.4	neg	neg	neg	neg	neg
	-06	01.02.91	17	+	1.4	4.1	4.5	neg	pos	neg	neg	neg
PHM907	-01	26.02.91	0	-	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-02	01.03.91	3	-	< 0.1	0.6	0.1	neg	neg	neg	neg	neg
	-03	05.03.91	7	-	< 0.1	0.6	0.1	neg	neg	neg	neg	neg
	-04	08.03.91	10	-	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-05	10.04.91	43	+	< 0.1	0.4	0.4	neg	neg	neg	neg	neg
	-06	17.04.91	50	+	1.0	1.1	3.1	neg	neg	neg	neg	neg
	-07	19.04.91	52	+	> 2.6	3.6	6.4	neg	neg	neg	neg	neg
	-08	24.04.91	57	+	> 2.6	11.8	32.9	neg	pos	neg	pos	neg
	-09	29.04.91	62	+	> 2.6	25.5	188.7	pos	pos	neg	pos	pos
	-10	01.05.91	64	+	> 2.6	24.3	188.7	pos	pos	pos	pos	pos
PHM910	-01	31.08.90	0	-	0.2	0.6	0.1	neg	neg	neg	neg	neg
	-02	18.09.90	18	±	0.2	0.7	0.4	neg	neg	neg	neg	neg
	-03	05.10.90	35	+	0.9	0.9	1.4	neg	neg	neg	neg	neg
	-04	12.10.90	42	+	> 2.7	2.7	4.7	neg	neg	neg	neg	neg
	-05	16.10.90	46	+	> 2.7	5.1	7.6	pos	pos	neg	neg	neg
	-06	19.10.90	49	+	> 2.7	5.6	16.2	pos	pos	neg	neg	neg

¹ HBV DNA Detection PCR - data supplied by Boston Biomedica Inc.² HBsAg conc. ng/ml - data supplied by Boston Biomedica Inc.³ Reference ELISA 1 - Hepanostika HBsAg Uni-Form II (Organon Teknika)⁴ Reference ELISA 2 - Monolisa Ag HBs Plus (Bio-Rad)

SR1 : ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)

SR2 : Determine™ HBsAg (Abbott Laboratories)

SR3 : Doublecheck™ HBs Antigen (Orgenics)

SR4 : Genelabs Diagnostics Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)

SR5 : HEPACARD (J. Mitra & Co. Ltd.)

Table 7. Results on low titre performance panel

Panel ID	Expected Result	PCR copies/ml ¹	HBsAg conc. IU/ml ²	Reference ELISA 1 ³ OD/CO	Reference ELISA 2 ⁴ OD/CO	SR1	SR2	SR3	SR4	SR5
PHA105-01	POS	5x10 ³	0.3	2.0	2.0	neg	neg	neg	neg	neg
-02	POS	3x10 ³	0.8	2.5	5.9	neg	neg	neg	neg	neg
-03	POS	2x10 ³	0.3	2.0	1.5	neg	neg	neg	neg	neg
-04	POS	5x10 ⁴	0.3	1.3	1.7	neg	neg	neg	neg	neg
-05	POS	2x10 ⁴	0.3	1.9	2.5	neg	neg	neg	neg	neg
-06	POS	7x10 ⁴	0.6	4.0	3.9	neg	neg	neg	neg	neg
-07	POS	7x10 ³	0.1	0.7	1.1	neg	neg	neg	neg	neg
-08	POS	2x10 ⁴	0.2	1.1	1.8	neg	neg	neg	neg	neg
-09	POS	9x10 ³	0.2	1.3	1.8	neg	neg	neg	neg	neg
-10	POS	2x10 ³	0.3	1.8	1.2	neg	neg	neg	neg	neg
-11	NEG	NEG	NEG	0.4	0.3	neg	neg	neg	neg	neg
-12	POS	7x10 ³	0.3	3.2	2.9	neg	neg	neg	neg	neg
-13	POS	< 4x10 ²	0.6	6.7	4.2	neg	neg	neg	neg	neg
-14	POS	7x10 ³	0.2	1.5	1.3	neg	neg	neg	neg	neg
-15	POS	2x10 ⁴	0.2	1.5	2.7	neg	neg	neg	neg	neg

¹ PCR copies/ml - data supplied by Boston Biomedica Inc.

² HBsAg conc. IU/ml - data supplied by Boston Biomedica Inc.

³ Reference ELISA 1 - Hepanostika HBsAg Uni-Form II (Organon Teknika)

⁴ Reference ELISA 2 - Monolisa Ag HBs Plus (Bio-Rad)

SR1 : ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)

SR2 : Determine™ HBsAg (Abbott Laboratories)

SR3 : Doublecheck™ HBs Antigen (Orgenics)

SR4 : Genelabs Diagnostics Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)

SR5 : HEPACARD (J. Mitra & Co. Ltd.)

Group 2: Simple/rapid assays**Table 8. General characteristics and operational aspects**

NAME	ImmunoComb [®] II HBsAg 90'	SERODIA [®] -HBs.PA	Uni-Gold [®] HBsAg	GENEDIA [®] HBsAg Rapid Device	HEP B STAT-PAK <i>ULTRA FAST</i>
Company	Orgenics, Yavne, Israel	Fujirebio Inc., Tokyo Japan	Trinity Biotech plc, Bray, Ireland	Green Cross Life Science Corp, Seoul, Korea	ChemBio Diagnostic Systems, Inc, Medford USA
Assay type	immunodot	gelatin particle agglutination	immunochromatographic	immunochromatographic	immunochromatographic
Antibody type	monoclonal	polyclonal	affinity purified polyclonal	monoclonal	monoclonal and polyclonal
Solid phase	comb	gelatin particle	membrane	nitrocellulose membrane	membrane strip
Specimen type	serum/plasma	serum/plasma	serum/plasma/ whole blood	serum/plasma	serum/plasma
Number of tests per kit	36	100, 200, 1000	20	50, 100, 300, 500	20
Lot numbers evaluated (expiry date)	990328 (Dec. '99)	SP81002 (Oct. '99) SP90802 (Aug. '00)	G13906 (March '00)	3462034 (Aug. '01)	HB111500 (Oct '02) HB052200/1 (April '02)
Shelf life at (°C)	12 months (2 - 8)	12 months (2 – 10)	12 months (2 - 27)	12 months (2-30)	24 months (8-30)
Volume of serum needed (µl) Final dilution of serum	100 5:6 (with diluent)	25 1:8, 1:16	100 none	100 none	160 none
Total time to perform the assay: h. min (number of sera)	1.40 (10)	2.45 (60)	0.15 (10)	0.05 (10)	0.30 (10)
Reading	visual	visual	visual	visual	visual
Price/test (US\$)	0.90	1.30 (100, 200 tests/kit) 1.20 (1000 tests/kit)	2.1	0.35 (100 tests)	0.70

Table 9. Comparison of the assays with reference tests

NAME	ImmunoCombâ II HBsAg 90'	SERODIAâ - HBs.PA	Uni-Goldä HBsAg	GENEDIAâ HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Final Sensitivity % (95 CL)* n = 99	99.0 (94.5 - 100.0)	99.0 (94.5 - 100.0)	99.0 (94.5 - 100.0)	99.0 (94.5 - 100.0)	99.0 (94.5 - 100.0)
Initial Specificity % (95 CL)*	92.7 (87.8 - 96.1)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)
Final Specificity % (95 CL)* n = 178	95.5 (91.3 - 98.0)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)	100.0 (97.9 - 100.0)
Indeterminate results%	0.7	0	0	0	0
Initial inter-reader variability%	4.3	6.1	0	0.4	0.4
PPV 0.1%	18.2	100.0	100.0	100.0	100.0
5.0%	53.7	100.0	100.0	100.0	100.0
10.0%	71.0	100.0	100.0	100.0	100.0
NPV 0.1%	100.0	100.0	100.0	100.0	100.0
5.0%	99.9	99.9	99.9	99.9	99.9
10.0%	99.9	99.9	99.9	99.9	99.9

* 95 % Confidence Limits

Table 10. Detailed operational aspects

NAME	ImmunoCombâ II HBsAg 90'	SERODIAâ -HBs.PA	Uni-Goldä HBsAg	GENEDIAâ HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Dimension (cm) of kit : w-l-h	18 - 13 - 5.5 (36 tests)	13.2-9.7-6.4 (100 tests) 12.6-9.2-8.4 (200 tests)	14 - 23 - 10.5 (20 tests)	21.0 – 9.0 – 8.0 (100 tests)	12.5 – 16.4 – 8.8
Storage conditions (°C)	2 - 8	2 – 10	2 - 27	2 - 30	8 – 30
Incubation temperature (°C)	37	15 – 30	room temperature	room temperature	room temperature
Reading endpoint stability (h.min)	>24.00	2.00	0.30	not indicated	0.30
Stability after dilution/ reconstitution/opening at (°C)					
- antigen	expiry date (2 - 8)	Reconstituted: 7 days (2 - 10)	expiry date (2 - 27)	opened kits: 4 weeks (2-30)	expiry date (8-30)
- controls	expiry date (2 - 8)	Reconstituted: 7 days (2 - 10)	not applicable	not applicable	not applicable
- sample diluent	expiry date (2 - 8)	Expiry date (2 - 10)	not applicable	not applicable	not applicable
- conjugate	not applicable	not applicable	not applicable	not applicable	not applicable
- substrate	not applicable	not applicable	not applicable	not applicable	not applicable
- wash buffer	not applicable	not applicable	expiry date (2 - 27)	expiry date (2 - 30)	not applicable
Number of sera per run minimum – maximum	1 - 10 (1 comb)	1 – 60	1 - 10	1 - 60	1 - 20
Number of controls per test run	2	1	not supplied	not supplied	not supplied
- negative	1	0	0	0	0
- cut-off/weak positive	0	0	0	0	0
- positive	1	1	0	0	0
- blank	0	0	0	0	0
internal control: reagent control	yes	yes	yes	yes	yes
: sample addition control	yes	no	no	yes	no

Table 10. (continued) Detailed operational aspects

NAME	ImmunoCombâ II HBsAg 90'	SERODIAâ -HBs.PA	Uni-Goldâ HBsAg	GENEDIAâ HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Estimated time to perform one run: h.min (number of sera)	1.00 (1) 1.40 (10)	2.30 (1) 2.45 (60)	0.05 (1) 0.15 (10)	0.05 (1) 0.10 (10)	0.30 (1) 0.30 (10)
Equipment needed but not provided in the kit: ¹					
- 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)	-	+	-	-	-
- sulfuric acid/sodium hydroxide	-	-	-	-	-
- absorbent paper	+	-	-	-	-
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	-	-	-	-	-
- timer	+	+	+	+	+
Definition of positive results	a spot in both test and control regions	agglutination in sensitized particles, no agglutination in control particles	a pink/red line in both test and control regions	2 pink/red bands in the control and test regions	2 pink/purple lines in the control and test regions
Definition of grey zone	not applicable	not applicable	not applicable	not applicable	not applicable

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 11a. Technician’s appraisal of the test kit

NAME	Score	ImmunoCombâ II HBsAg 90’	SERODIAâ - HBs.PA	Uni-Goldä HBsAg	GENEDIAâ HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Number of steps in the test procedure: -1-2 steps -3-5 steps - >5 steps	6 3 1	1	3	6	6	6
Clarity of kit instructions: - good - needs improvement	2 1	2	2	2	2	2
Kit and reagent packaging and labelling: - good - needs improvement	2 1	2	2	2	2	2
Total (out of 10)		5	7	10	10	10
Comments on the test kit		mixing sample with sample diluent is not easy because the pipette tip cannot reach the bottom of the well	none	very easy and straight forward test to set up	very easy test to perform and read	very easy test to perform

Table 11b. Calculation of ease of performance

NAME	ImmunoCombâ II HBsAg 90'	SERODIAâ -HBs.PA	Uni-Goldâ HBsAg	GENEDIAâ HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Need to prepare:					
-antigen	1 ¹	0	1	1	1
-substrate	1	1	1	1	1
-wash solution	1	1	1	1	1
-conjugate	1	1	1	1	1
-predilution of serum	1	0	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
-antigen	1	0	1	0	1
-controls	1	0	1	1	1
-sample diluent	1	1	1	1	1
-conjugate	1	1	1	1	1
-substrate	1	1	1	1	1
-wash buffer	1	1	1	1	1
-sufficient reagents	1	1	1	1	1
-wash (yes =0; no = 1)	1	1	1	1	1
Item needed but not provided in the kit:					
-reagent trough	1	1	1	1	1
-automatic /multichannel pipette	0 ²	0	1	0	1
-dilution – tubes, rack/microtiter plate	1	0	1	1	1
-distilled or deionised water	1	1	1	1	1
-plate covers	1	1	1	1	1
-graduated pipette ,cylinder	1	0	1	1	1
-sulfuric acid/sodium hydroxide	1	1	1	1	1
Technician's appraisal of the test kit ³ (rating out of 10)	5	7	10	10	10
Total (out of possible 30)	24	20	30	28	30
Ease of performance:					
-less easy < 20					
-easy 20 ≤ x ≤ 25	easy	easy	very easy	very easy	very easy
-very easy > 25					

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 11a

Table 12. Suitability for use in small laboratories

NAME	Score	ImmunoCombâ II HBsAg 90'	SERODIA â -HBsPA	Uni-Goldâ HBsAg	GENEDIA â HBsAg Rapid Device	HEP B STAT-PAK ULTRA FAST
Sensitivity						
- 100%	5					
- 98 – 100%	3	3	3	3	3	3
- <98%	0					
Specificity						
- >98%	5					
- 95 – 98%	3	3	5	5	5	5
- <95%	0					
Incubation temperature						
- room t°	3	1	3	3	3	3
- other than room t°	1					
Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	2	2	2	3	3
- < 6 months	1					
Storage at						
- ambient t° possible opened kit	5					
- ambient t° possible unopened kit	2	1	1	5	5	5
- 2-8 °C required	1					
Price per test (US\$)						
- ≤ 1.0	3					
- >1.0 ≤ 2.0	2	3	2	1	3	3
- > 2.0	1					
Ease of performance						
- very easy	5					
- easy	3	3	3	5	5	5
- less easy	1					
Rapidity of performance:1 serum						
- < 10 min	3					
- 10 – 30 min	2	1	1	3	3	2
- > 30 min	1					
Washer/agitator						
- not needed	3	3	3	3	3	3
- needed	1					
Reading						
- visual: inter-reader variability ≤ 3	5					
: inter-reader variability > 3	3	3	3	5	5	5
- reading equipment	1					
Total (out of 40)		23	26	35	38	37
Suitability for use in small laboratories:						

NAME	Score	ImmunoCombâ II HBsAg 90'	SERODIA â -HBs.PA	Uni-Goldä HBsAg	GENEDIA â HBsAg Rapid Device	HEP B STAT-PAK <i>ULTRA FAST</i>
- less suitable < 23 - suitable $23 \leq x \leq 30$ - very suitable > 30		suitable	suitable	very suitable	very suitable	very suitable

Table 13. Results on seroconversion panels

Panel ID	Bleed Date	Days since first bleed	HBV DNA Detection PCR ¹	HBsAg conc ng/ml ²	Reference ELISA 1 ³ OD/CO	Reference ELISA 2 ⁴ ELISA 2 ⁴ OD/CO	SR6	SR7	SR8	SR9	SR10	
PHM901	-01	25.11.87	0	+	0.3	0.7	0.8	pos	neg	neg	neg	neg
	-02	27.11.87	2	+	0.6	0.8	2.0	pos	neg	neg	neg	neg
	-03	03.12.87	8	+	>2.5	4.1	19.9	pos	neg	neg	neg	neg
	-04	05.12.87	10	+	>2.5	7.1	20.9	pos	ind	ind	neg	pos
	-05	11.12.87	16	+	>2.5	21.3	181.8	pos	pos	pos	pos	nt*
	-06	16.12.87	21	+	>2.5	29.1	181.8	pos	pos	pos	pos	pos
	-07	18.12.87	23	+	>2.5	21.3	181.8	pos	pos	pos	pos	pos
PHM902	-01	27.08.90	0	-	< 0.1	0.6	0.3	neg	neg	neg	neg	neg
	-02	29.08.90	2	-	< 0.1	0.5	0.3	ind	neg	neg	neg	neg
	-03	04.09.90	8	-	< 0.1	0.5	0.2	ind	neg	neg	neg	neg
	-04	06.09.90	10	-	< 0.1	0.7	0.1	neg	neg	neg	neg	neg
	-05	23.10.90	57	+	< 0.1	0.6	0.1	pos	neg	neg	neg	neg
	-06	25.10.90	59	+	< 0.1	0.6	0.3	pos	neg	neg	neg	neg
	-07	30.10.90	64	+	< 0.1	0.7	0.3	pos	neg	neg	neg	neg
	-08	01.11.90	66	+	< 0.1	0.6	0.4	pos	neg	neg	neg	neg
	-09	06.11.90	71	+	0.5	0.9	1.3	pos	neg	neg	neg	neg
	-10	08.11.90	73	+	0.5	1.0	1.5	pos	neg	neg	neg	neg
	-11	13.11.90	78	+	1.2	2.9	2.5	pos	neg	neg	neg	neg
	-12	15.11.90	80	+	2.2	2.2	3.5	pos	neg	neg	neg	neg
	-13	20.11.90	85	+	> 2.7	5.6	8.1	pos	neg	neg	neg	neg
	-14	23.11.90	88	+	> 2.7	5.3	12.5	pos	neg	neg	neg	neg
PHM903	-01	15.01.91	0	+	< 0.1	0.5	0.0	neg	neg	neg	neg	neg
	-02	18.01.91	3	+	< 0.1	0.5	0.1	neg	neg	neg	neg	neg
	-03	21.01.91	6	+	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-04	25.01.91	10	+	0.1	0.9	0.5	neg	neg	neg	neg	neg
	-05	29.01.91	14	+	1.0	1.1	1.4	pos	neg	neg	neg	neg
	-06	01.02.91	17	+	1.4	4.1	4.5	pos	neg	neg	neg	neg
PHM907	-01	26.02.91	0	-	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-02	01.03.91	3	-	< 0.1	0.6	0.1	neg	neg	neg	neg	neg
	-03	05.03.91	7	-	< 0.1	0.6	0.1	neg	neg	neg	neg	neg
	-04	08.03.91	10	-	< 0.1	0.6	0.2	neg	neg	neg	neg	neg
	-06	17.04.91	50	+	1.0	1.1	3.1	pos	neg	neg	neg	neg
	-07	19.04.91	52	+	> 2.6	3.6	6.4	pos	neg	neg	neg	neg
	-08	24.04.91	57	+	> 2.6	11.8	32.9	pos	neg	ind	neg	neg
	-09	29.04.91	62	+	> 2.6	25.5	188.7	pos	pos	pos	pos	pos
	-10	01.05.91	64	+	> 2.6	24.3	188.7	pos	pos	pos	pos	pos
	PHM910	-01	31.08.90	0	-	0.2	0.6	0.1	neg	neg	neg	neg
-02		18.09.90	18	±	0.2	0.7	0.4	neg	neg	neg	neg	neg
-03		05.10.90	35	+	0.9	0.9	1.4	neg	neg	neg	neg	neg
-04		12.10.90	42	+	> 2.7	2.7	4.7	pos	neg	neg	neg	neg
-05		16.10.90	46	+	> 2.7	5.1	7.6	pos	neg	neg	neg	neg
-06		19.10.90	49	+	> 2.7	5.6	16.2	pos	neg	neg	neg	neg

¹ HBV DNA Detection PCR - data supplied by Boston Biomedica Inc.² HBsAg conc. ng/ml - data supplied by Boston Biomedica Inc.³ Reference ELISA 1 - Hepanostika HBsAg Uni-Form II (Organon Teknika)⁴ Reference ELISA 2 - Monolisa Ag HBs Plus (Bio-Rad)

SR6 ImmunoComb® II HBsAg 90'

SR7 SERODIA® -HBs.PA

SR8 Uni-Gold™ HBsAg

SR9 GENEDIA® HBsAg Rapid Device
SR10 HEP B STAT-PAK *ULTRA FAST*
*not tested

Table 14. Results on low titre performance panel

Panel ID	Expected Result	PCR copies/ml ¹	HBsAg conc. IU/ml ²	Reference ELISA 1 ³ OD/CO	Reference ELISA 2 ⁴ OD/CO	SR6	SR7	SR8	SR9	SR10
PHA105-01	POS	5x10 ³	0.3	2.0	2.0	pos	neg	neg	neg	neg
-02	POS	3x10 ³	0.8	2.5	5.9	pos	neg	neg	neg	neg
-03	POS	2x10 ³	0.3	2.0	1.5	pos	neg	neg	neg	neg
-04	POS	5x10 ⁴	0.3	1.3	1.7	ind	neg	neg	neg	neg
-05	POS	2x10 ⁴	0.3	1.9	2.5	pos	neg	neg	neg	neg
-06	POS	7x10 ⁴	0.6	4.0	3.9	pos	neg	neg	neg	neg
-07	POS	7x10 ³	0.1	0.7	1.1	pos	neg	neg	neg	neg
-08	POS	2x10 ⁴	0.2	1.1	1.8	pos	neg	neg	neg	neg
-09	POS	9x10 ³	0.2	1.3	1.8	pos	neg	neg	neg	neg
-10	POS	2x10 ³	0.3	1.8	1.2	pos	neg	neg	neg	neg
-11	NEG	NEG	NEG	0.4	0.3	neg	neg	neg	neg	neg
-12	POS	7x10 ³	0.3	3.2	2.9	pos	neg	neg	neg	neg
-13	POS	< 4x10 ²	0.6	6.7	4.2	pos	neg	neg	neg	neg
-14	POS	7x10 ³	0.2	1.5	1.3	pos	neg	neg	neg	neg
-15	POS	2x10 ⁴	0.2	1.5	2.7	pos	neg	neg	neg	neg

¹ PCR copies/ml - data supplied by Boston Biomedica Inc.

² HBsAg conc. IU/ml - data supplied by Boston Biomedica Inc.

³ Reference ELISA 1 - Hepanostika HBsAg Uni-Form II (Organon Teknika)

⁴ Reference ELISA 2 - Monolisa Ag HBs Plus (Bio-Rad)

SR6 ImmunoComb® II HBsAg 90'

SR7 SERODIA® -HBs.PA

SR8 Uni-Gold™ HBsAg

SR9 GENEDIA® HBsAg Rapid Device

SR10 HEP B STAT-PAK *ULTRA FAST*

Explanatory notes for Tables 1 - 14 and Figures 1 and 2

Tables 1 and 8

General characteristics and operational aspects of the assays

Sample	The Determine™ HBsAg (Abbott Laboratories) and Uni-Gold™ HBsAg (Trinity Biotech plc) may also be used with whole blood samples. For the Determine™ HBsAg assay 50 µl of EDTA anti-coagulated whole blood, either as a fingerprick or venous sample is required. EDTA capillary tubes for fingerprick samples are available from the company. For the Uni-Gold™ HBsAg, 30 µl of whole blood either as a fingerprick sample or whole blood anti-coagulated with EDTA or sodium citrate is required. The performance of these assays with whole blood samples was not assessed in this evaluation.
Final dilution of the serum	is 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. <ul style="list-style-type: none">- dipstick and comb assays, a complete comb (8-12 specimens including controls).- simple/rapid assays designed for individual tests, the number which can be run simultaneously
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. The price is indicative.

Tables 2 and 9

Comparison of the results of the assays with reference tests

Sensitivity	Calculated as described on pages 9-10 of this document.
Specificity	Calculated as described on page 10 of this document.
95% Confidence limits (CL)	Calculated as described on page 10 of this document
PPV and NPV	Calculated as described on pages 10-11 of this document
Indeterminate results	Simple/rapid assays - test results which could not be interpreted as clearly positive or negative were considered indeterminate.
Inter-reader variability	Calculated as described on page 11 of this document.

Explanatory notes for Tables 1 - 14 and Figures 1 and 2

Tables 3 and 10

Detailed operational aspects 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 in the correct order, and have performed properly.
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 summarized in the table

Tables 4a, 4b and 11a, 11b

Calculation of ease of performance of the assay

The criteria for this calculation are given in the respective tables.

Tables 5 and 12

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.

Tables 6 and 13

Performance of the assay on seroconversion panels

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

Explanatory notes for Tables 1 - 14 and Figures 1 and 2

specificity. Caution should be taken when reviewing seroconversion performance of assays tested in only 5 panels.

Tables 7 and 14

Results of the assays on a low titre panel

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

Figure 1

Relative performance in seroconversion panels

See section 4.4.3 on page 11 for an explanation of how these data were analyzed. The results for the 5 tested panels are in Tables 6 and 13. The 95% confidence limits should be interpreted with caution as only 5 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 positive samples correctly identified as positive in the low titre panel. Note that the expected result for sample PHA105-11 in the low titre panel was negative and therefore was not included in the analysis.

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

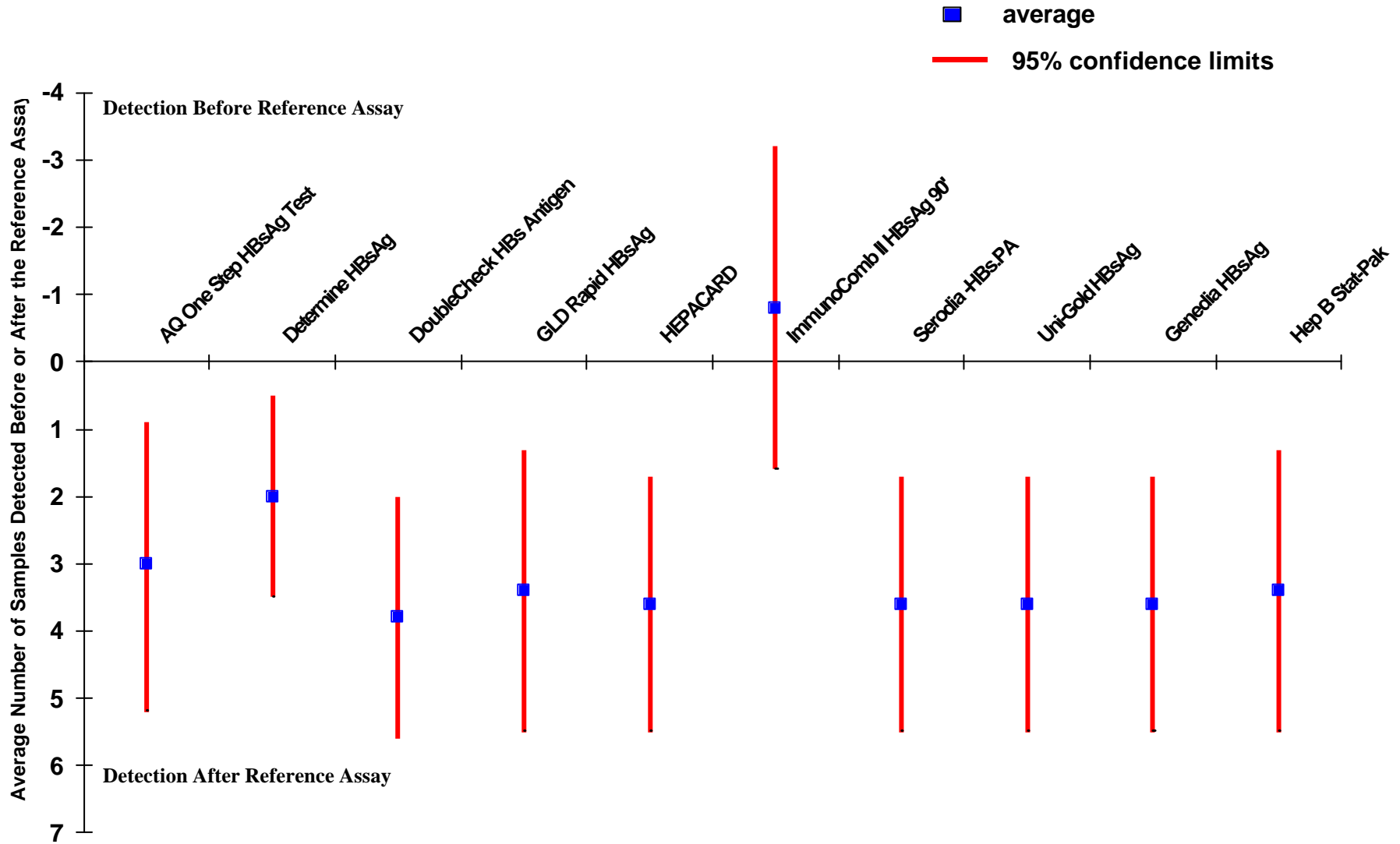
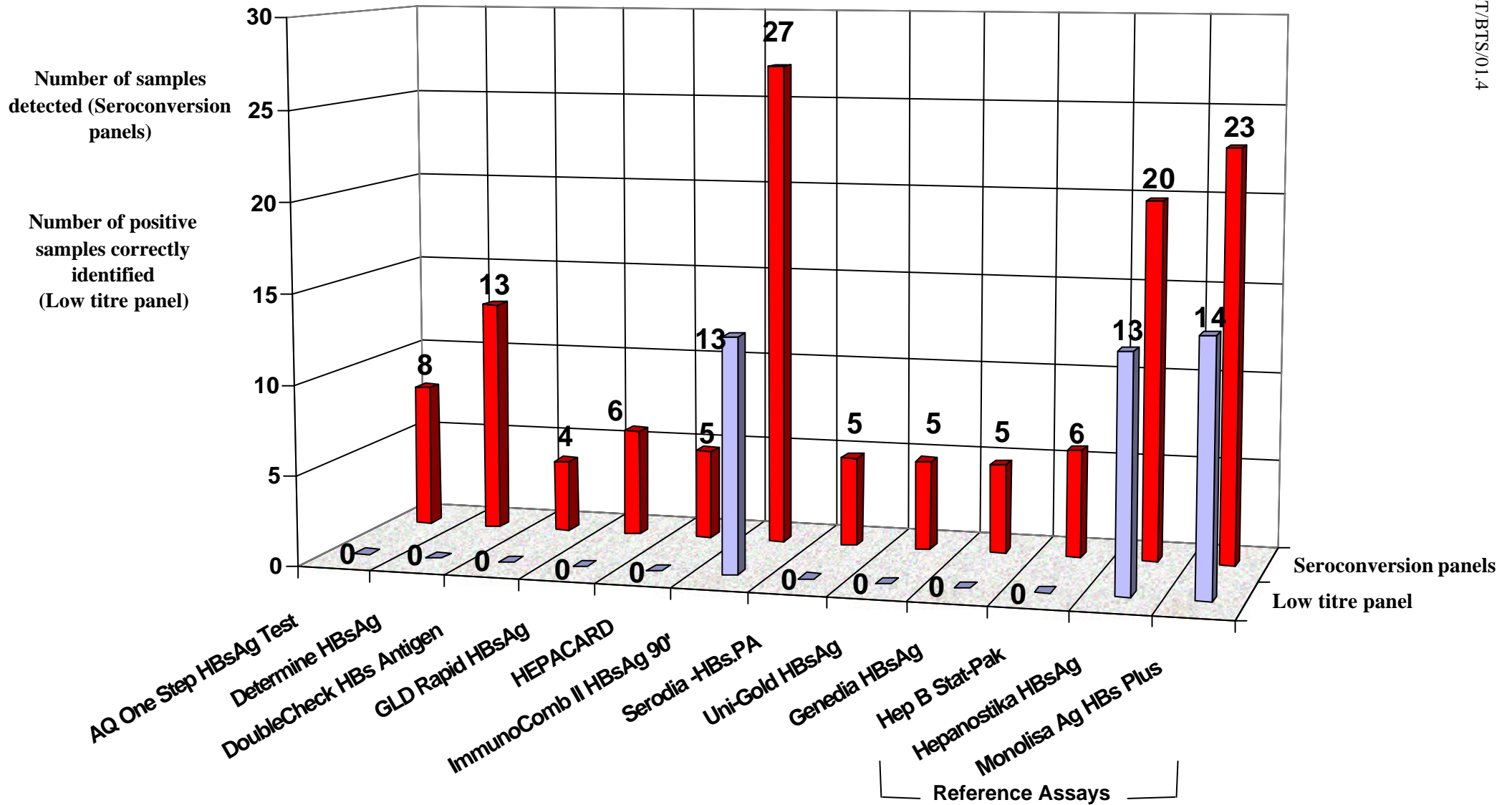
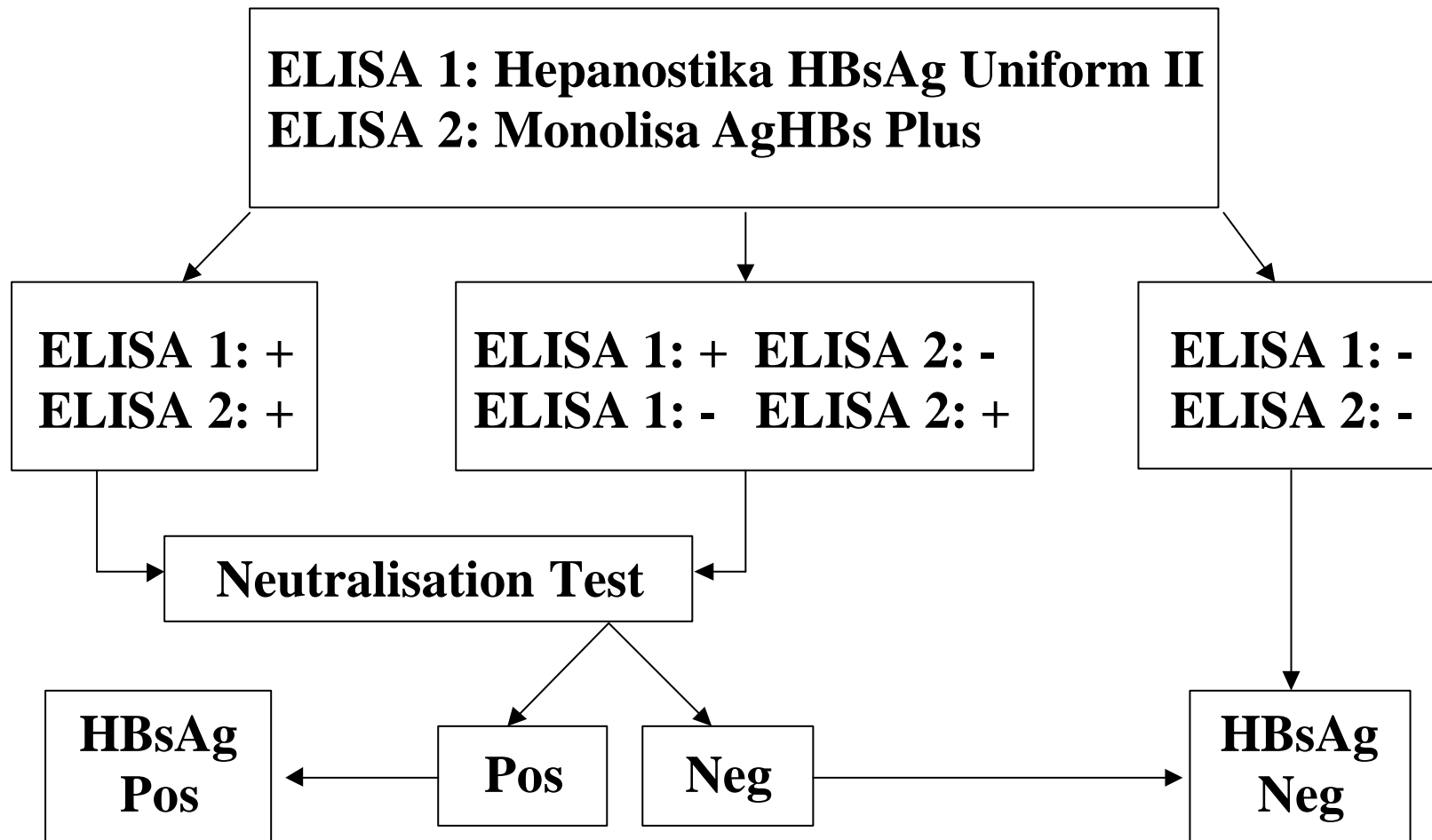


Figure 2. Relative performance in seroconversion and low titre panels



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)	99.0 (94.5 – 100.0)	95.5 (91.3 – 98.0)	2.9	5.4	VE	2-30
Determine™ HBsAg (Abbott Laboratories)	1	1.20 (1999)	99.0 (94.5 - 100.0)	99.4 (96.9 – 100.0)	0	0	VE	2-30
Doublecheck HBs Antigen (Organics)	1	1.00 (1999)	99.0 (94.5 - 100.0)	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)	99.0 (94.5 - 100.0)	97.8 (94.3 - 99.4)	0.7	2.2	VE	22-28
HEPACARD (J.Mitra & Co. Ltd.)	1	0.63 (1999)	99.0 (94.5 - 100.0)	97.8 (94.3 – 99.4)	0.7	2.5	VE	2-8
ImmunoComb® II HbsAg 90' (Organics)	1	0.90 (1999)	99.0 (94.5 – 100.0)	95.5 (91.3 – 98.0)	0.7	4.3	E	2-8
SERODIA® -HBs _s PA (Fujirebio Inc.)	1	1.30 (2001)	99.0 (94.5 – 100.0)	100.0 (97.9 – 100.0)	0.0	6.1	E	2-10
Uni-Gold™ HBsAg (Trinity Biotech plc)	1	2.1 (1999)	99.0 (94.5 – 100.0)	100.0 (97.9 – 100.0)	0	0	VE	2-27
GENEDIA® HBsAg Rapid Device (Green Cross Life Science Corp)	1	0.35(2001)	99.0 (94.5 – 100.0)	100.0 (97.9 – 100.0)	0	0	VE	2-30
HEP B STAT-PAK <i>ULTRA FAST</i> (Chembio Diagnostic Systems Inc.)	1	0.70 (2001)	99.0 (94.5 – 100.0)	100.0 (97.9 – 100.0)	0	0.4	VE	8-30

Legend for Annex 2.

- a: Operational Characteristics of Hepatitis B Surface Antigen Assays (PHASE I) Report 1
- 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 9-10 of this document.
- f: Indeterminate results were calculated as described in the explanatory notes on page 32 of this document
- g: Inter-reader variability was calculated as described on page 11 of this document.
- h: Ease of performance is defined in Tables 4b and 11b.
- i: Storage conditions listed are for unopened kits. See Tables 3 and 10 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

Bio-Rad, 3, boulevard Raymond Poincaré, 92430 Marnes La Coquette, France.

Tel: + 33 1 47 95 60 00; Fax: + 33 1 47 41 91 33; Website: www.bio-rad.com

Chembio Diagnostic Systems Inc., 3661 Horseblock Road, Medford, NY 11763, USA

Tel: +1 631 9241135; Fax: +1 631 9246033; Email: info@chembio.com

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

Fujirebio Europe BV, Takkebijsters 69c, 4817 BL Breda, The Netherlands.

Tel: +31 76 571 0440; Fax: +31 76 587 2181; Email: febv@xs4all.nl

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; Email: genelabs@pacific.net.sg

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; Email: salesgva@genelabs.ch

Green Cross Life Science Corp, 227-3, Gugal-li, Giheung-eup, Yongin-shi, 449-900, Kyunggi-do, Korea.

Tel: +82 31 280 6245; Fax: +82 31 280 6249

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; Email: jmitra@ndb.vsnl.net.in

Organon Teknika, Boseind 15, PO Box 84, 5280 AB Boxtel, The Netherlands.

Tel: +31 411 654911, Telefax: +31 411 654201

Organics, P.O. Box 360 Yavne 70650, Israel.

Tel: +972 8 942 9233; Fax: +972 8 943 8758; Website: www.organics.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 BCT 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, London, UK for supplying specimens to the WHO Hepatitis B Surface Antigen evaluation panel.

We acknowledge the 9 companies, Bionike Inc., Abbott Laboratories, Orgenics, Genelabs Diagnostics Pte Ltd., J.Mitra & Co. Ltd., Fujirebio Inc., Trinity Biotech plc, Green Cross Pharmaceutical Management Corp, and Chembio Diagnostic Systems Inc. for supplying the test reagents free of charge.
