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HEPATITIS C ASSAYS: OPERATIONAL CHARACTERISTICS

(PHASE I)

REPORT 1

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BLOOD SAFETY AND CLINICAL TECHNOLOGY
WORLD HEALTH ORGANIZATION
GENEVA

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1. SUMMARY

In 1998, WHO implemented a programme for the evaluation of performance and major operational characteristics of commercially available assays for detection of antibodies to Hepatitis C (HCV). This first report presents the findings of the Phase I evaluations of five anti-HCV assays conducted between September and November, 1999. The anti-HCV assays evaluated included:

- Advanced Quality™ One Step HCV Test (Bionike Inc.)
- HCV TRI-DOT (J. Mitra & Co. Ltd.)
- Serodia® HCV (Fujirebio Inc.)
- HCV SP•T (Genelabs Diagnostics Pte. Ltd)
- SeroCard™ HCV (Trinity Biotech plc)

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 HCV 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. Annex 1 shows the anti-HCV confirmatory testing algorithm used to characterize the WHO HCV evaluation panel. Annexes 2 and 3 show respectively, the list of evaluated HCV assays and addresses of manufacturers for 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 (ELISA) and assays using other technologies. Copies of this report are available on request from the Blood Transfusion Safety Team, Department of Blood Safety and Clinical Technology, 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 of WHO; the WHO Collaborating Centre on Transfusion Transmissible Infections, Sexually Transmitted and Blood Borne Virus Laboratory (SBVL), Virus Reference Division, Central Public Health Laboratory, 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.

In the first instance, the evaluations will be conducted in two phases, the first using a limited panel of well characterised 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 characterisation, in addition to being tested in the assays under evaluation at the field sites. 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, 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, low titre and worldwide performance panels is assessed.

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 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, etc., to help select assays appropriate to local needs.

3. LABORATORY ASPECTS OF HCV TESTING

3.1 A brief overview

The Hepatitis C virus is the major cause of the disease formerly known as non-A non-B post transfusion hepatitis. Since the introduction in 1990 of anti-HCV screening of blood donations in most industrialised countries, the incidence of this infection in transfusion recipients in this part of the world has been significantly reduced.

HCV, a single stranded RNA virus, is a member of the family *Flaviviridae.* Six major genotypes (1-6) and a series of subtypes of HCV have been identified. Genotypes 1-3 show a worldwide distribution while genotypes 4 and 5 appear predominantly in Africa and genotype 6 in Asia. Following the discovery of HCV and the sequencing of its genome in 1989, the first generation of HCV ELISAs was produced using recombinant proteins complementary to the NS4 region of the HCV genome as antigens. These assays showed limited sensitivity and specificity. Second generation tests, which included recombinant or synthetic antigens from the putative core and non-structural regions NS3 and NS4 resulted in a marked improvement in sensitivity and specificity. The third generation tests include antigens from the NS5 region of the genome, in addition to those used in second generation assays. Third generation tests have improved sensitivity, though this has been shown to be more likely due to the improvements to the core and NS3 antigens rather than the inclusion of the NS5 antigen. However, despite these improvements, the time between infection with HCV and the appearance of detectable antibodies (window period) is generally more than 40 days (Schreiber et al, 1996; Barrera et al, 1995). It is anticipated that test kits will undergo further improvement in the future.

The laboratory diagnosis of HCV infection is usually made on the basis of the detection of circulating antibodies. Serological tests for detecting antibodies to HCV are generally classified as **screening tests** or **confirmatory tests**. Screening tests provide the presumptive identification of antibody-positive specimens, whilst confirmatory tests are used to confirm that specimens found reactive with a particular screening test contain antibodies specific to HCV. Several screening tests may be used in a testing algorithm to determine a final sero-status. These second and/or third line tests are generally referred to as **supplemental tests**.

The most widely used HCV 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 industrialised countries. However many blood transfusion services in resource limited countries process only limited numbers of specimens. Hence individual tests would be more appropriate. Recently, 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 process low numbers of specimens daily.

When a single screening test is used for testing in a population with a very low prevalence of HCV infection, the probability that a person is infected when a reactive test result is obtained (i.e., the positive predictive value) is low, since the majority of people with reactive results are not infected (ie the positive results are false). This problem occurs even when a test of excellent quality and having high specificity is used. Accuracy can be improved if additional supplemental or confirmatory test(s), of equal or higher specificity, are used to retest all those samples found reactive by the screening test. Screening and supplemental tests, to be used in an HCV confirmatory strategy, must be selected carefully to ensure that common false reactivity between these assays does not occur.

Confirmatory assays that are commercially available for the diagnosis of HCV include line/strip immunoassays and assays using Nucleic Acid Amplification Technologies (NAT).

Line/strip immunoassays have individual recombinant or synthetic antigens applied as separate lines to the solid phase. Therefore the different antigens to which antibodies in a specimen are reacting can be distinguished. The application of established confirmation patterns of reactivity observed permits greater specificity. Examples of such assays include RIBA HCV 3.0 SIA (Chiron Corporation) and Inno-LIA HCV antibody III (Innogenetics).

The interpretation of results from different anti-HCV assays poses difficulties in a significant number of specimens including those collected from individuals suspected of seroconversion. In these cases, qualitative confirmatory NAT assays can provide useful additional information particularly as current commercial versions of these assays are capable of detecting HCV RNA down to 100 copies/ml. On the other hand, NAT assays are technically demanding, require sophisticated laboratory infrastructure and a negative result does not exclude HCV infection. In addition there is some evidence that not all genotypes of HCV are detected equally by NAT assays.

Companies are developing ELISAs for the detection of HCV core antigen. These assays can be used in addition to anti-HCV assays and may provide a valuable tool in the identification of individuals undergoing HCV seroconversion. These assays may be more appropriate as a supplement to anti-HCV antibody tests than using NAT for screening blood donations in countries with limited resources.

3.2 Quality assurance

All laboratories carrying out HCV tests, should have a well-functioning quality assurance programme. It is most important that quality assurance procedures be stringently applied 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 participate in 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

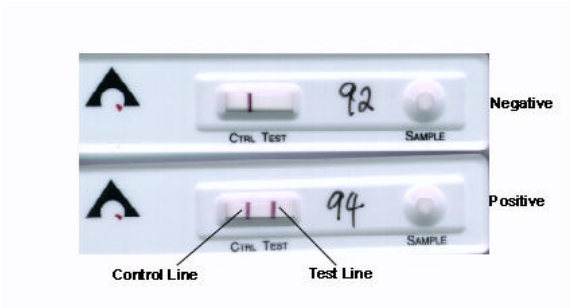
4.1 Assays (test kits) evaluated

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. Three of the five rapid tests are immunochromatographic or immunofiltration assays for the qualitative detection of anti-HCV. The end point of these is based on the visual detection of bound protein A/colloidal gold conjugate.

Advanced Quality ä One Step HCV Test (Bionike Inc.)

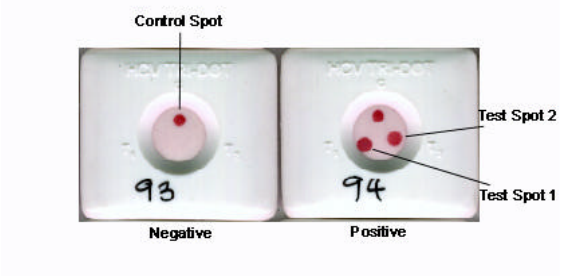
A colloidal gold, rapid immunochromatographic assay for the qualitative detection of anti-HCV.



HCV TRI-DOT (J. Mitra & Co. Ltd.)

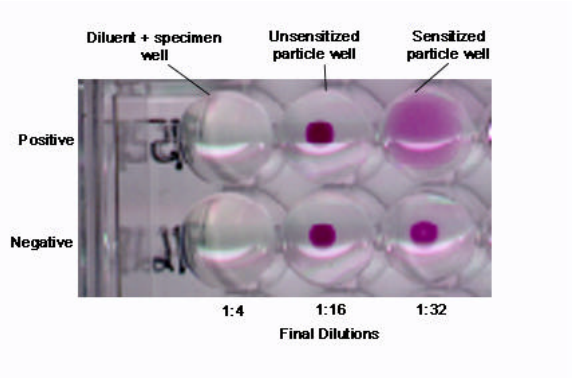
A rapid, protein A immunofiltration assay for the qualitative detection of anti HCV.

Note: The new version of this assay, HCV TRI-DOT version 4.0, has been evaluated by WHO and found to have improved specificity (details will be in included in report 2).



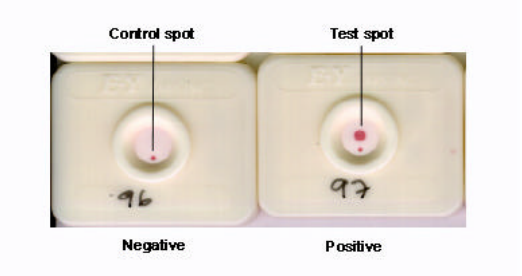
Serodiaâ HCV (Fujirebio Inc.)

A microtitre particle agglutination assay for the qualitative detection of anti-HCV.



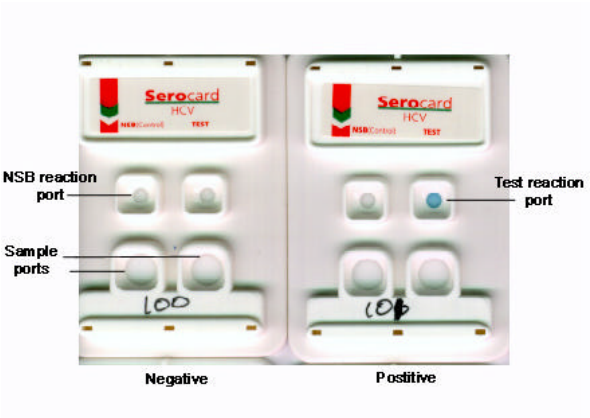
HCV SP· T (Genelabs Diagnostics Pte. Ltd)

A rapid protein A gold immunofiltration assay for the qualitative detection of anti-HCV.



SeroCardä HCV (Trinity Biotech plc)

A rapid test for qualitative detection of anti-HCV based on the enzyme linked immunosorbent assay principle.



4.2 Evaluation panels

4.2.1 WHO HCV panel

The phase I evaluations reported here were carried out using a panel of 257 sera (as shown in *Table A*), of which 39 were from Africa, 57 from Asia, 105 from Europe and 56 from South America. The panel contained 68 specimens positive for HCV. All specimens were stored in aliquots at -30ºC and were thawed at least once and not more than twice.

Table A: Composition of WHO HCV Panel: Phase 1

Origin of specimens	Anti-HCV positive specimens	Anti-HCV negative specimens	Total
Africa	1	38	39
Asia	20	37	57
Europe	40	65	105
Latin America	7	49	56
Total	68	189	257

Characterization of the WHO HCV Panel

For characterization, specimens in the WHO reference panel were screened by two reference ELISAs, Ortho 3.0 Enhanced SAVe (Ortho Clinical Diagnostics) and Monolisa anti-HCV Plus (Bio-Rad formerly Sanofi Diagnostics Pasteur). Specimens negative by both ELISAs were considered anti-HCV negative. Specimens showing reactivity with either or both ELISAs were further characterized using Chiron HCV RIBA 3.0. This algorithm, used for determination of the anti-HCV status of specimens in the WHO HCV reference panel, is shown diagrammatically in Annex 1.

When results of the reference ELISAs and the HCV RIBA 3.0 were all positive, a specimen was considered anti-HCV positive. When the initial ELISA results were discordant, specimens having negative HCV RIBA 3.0 results were considered anti-HCV negative. Similarly, specimens showing discordant ELISA reactivity and a positive HCV RIBA 3.0 were considered anti-HCV positive. Specimens with discordant ELISA results and indeterminate HCV RIBA 3.0 were excluded from the panel.

The results of the reference assays were interpreted according to the manufacturers' instructions.

4.2.2 Seroconversion panels

A seroconversion panel is a series of specimens, sequentially collected over a period of time, from an individual developing antibodies due to a primary infection.

Four commercial seroconversion panels, PHV 907, PHV 908, PHV 911 and PHV 913 [Boston Biomedica Inc.(BBI)] were tested on each of the five simple/rapid assays evaluated. These panels consisted of a total of 39 specimens collected from 4 individuals during seroconversion.

4.2.3 Performance panels

Additionally, one HCV worldwide performance panel containing 20 members, WWHV 301 (BBI) and one anti-HCV low titre performance panel containing 15 members, PHV 104 (BBI) were tested.

4.3 Laboratory testing

All testing was performed according to the manufacturer's instructions. The specimens in the WHO HCV panel were randomised 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 HCV panel which gave initial results discordant from the reference results, were retested in duplicate. The results which occurred 2 out of 3 times were recorded as the final results. Samples from commercial panels giving discordant results were not retested.

4.4 Analysis

4.4.1 Sensitivity, specificity confidence limits (CL) and predictive values of anti-HCV 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 HCV 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 the ability of the assay under evaluation to identify correctly specimens that contain antibody to HCV (reference assays positive). Thus, sensitivity is the number of true positive specimens recognized by the assay under evaluation as positive (a), divided by the number of specimens identified by the reference assays as positive (a+c), expressed as a percentage.

Specificity : Is the ability of the assay under evaluation to identify correctly specimens that do not contain antibody to HCV (reference assays negative). Thus specificity is the number of true negative

specimens recognized by the assay under evaluation as negative (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage.

NOTE: Specimens which 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 antibody to HCV. 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 HCV 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 antibody to HCV. 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 HCV 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 HCV infection in the population from which the person comes. In general, the higher the prevalence of HCV 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 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. However, this effect only becomes apparent at prevalence of 80% and above.

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 with early seroconversion panels using the assays under evaluation were compared with those obtained using Ortho HCV 3.0 Enhanced SAVe, the assay arbitrarily designated the reference for determination of relative sensitivity in these panels. For each seroconversion series (panel) the first specimen in the sample sequence to become reactive with Ortho HCV 3.0 Enhanced SAVe was assigned the value "0". Results from the assays under evaluation were compared with Ortho HCV 3.0 Enhanced SAVe 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 each of the assays under evaluation. For example, if an assay became reactive two specimens earlier in a series than Ortho HCV 3.0 Enhanced SAVe, the value assigned for that series in that assay was -2. Similarly, if an assay became reactive one specimen later than Ortho HCV 3.0 Enhanced SAVe, the value assigned was +1. The assigned values over the 4 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 panels.

The number of samples correctly identified in the worldwide and low titre performance panels was determined by comparison with the anti-HCV status assigned (expected results) following testing and interpretation of the combined reference tests, the Ortho HCV 3.0 Enhanced SAVe and Chiron HCV RIBA 3.0. The HCV RIBA data were provided by BBI.

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

Table 1 summarises the general characteristics of the assays. 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 respectively. Performance of the assays under evaluation on early seroconversion panels, world-wide performance and low titre panels is given in Tables 6, 7 and 8 respectively. The relative performance of the assays under evaluation compared to the reference tests on seroconversion panels is shown in Figure 1 while Figure 2 represents the comparison in performance on all three types of commercial panel. Explanatory notes are provided at the end of the assay evaluation tables.

ASSAY EVALUATIONS

Table 1 General characteristics and operational aspects: Simple/rapid assays

NAME	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard ä HCV
Company	Bionike, Inc., San Francisco, USA	J. Mitra & Co. Ltd. New Delhi, India	Fujirebio Inc., Tokyo Japan	Genelabs Diagnostics, Pte. Ltd., Singapore	Trinity Biotech plc, Bray, Ireland
Assay type	immunochromatographic	immunofiltration	particle agglutination	immunofiltration	immunochromatographic
Solid phase	membrane	membrane	gelatin particles	membrane	membrane
Antigen type	recombinant proteins	recombinant proteins synthetic peptides	recombinant proteins	recombinant proteins	synthetic peptides
Specimen type	Serum/plasma	Serum/plasma	Serum/plasma	Serum/plasma	Serum/plasma and Whole Blood
Number of tests per kit	40	10, 50, 100	100, 220	20, 100	40
Lot numbers 1-2 Expiry dates 1-2	RD90328-8 - RD90508-8 March '00 - May '00	HCD04059 - HCD03059 April '00 - April '00	BG 90201 - BG 90402 Feb '00 - April. '00	9FK101 March '00	G 09820 March '00
Shelf life at (°C)	18 months (2 - 30)	12 months (2 - 8)	12 months (2 - 10)	6 - 8 months (25±3)	16 months (2 - 8)
Volume of serum needed (µl) Final dilution of serum	4 1:50	45 none	25 1:32	45 none	80 none
Total time to perform the assay (h. min.)	0.06	0.09	2.45	0.09	0.19
Reading	visual	visual	visual	visual	visual
Price/test (US$)	1.20	2.00	4.50	2.50	2.25

Table 2 Comparison of assay results with reference tests

NAME	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard ä HCV
Final Sensitivity % (95 CL)* n = 68	97.1 (89.8 - 99.6)	100.0 (94.7 -100.0)	100.0 (94.7 -100.0)	100.0 (94.7 -100.0)	98.5 (92.1 - 100.0)
Initial Specificity % (95 CL)*	94.7 (90.5 - 97.4)	79.4 (72.9 - 84.9)	99.5 (97.1 -100.0)	92.6 (87.9 - 95.9)	97.9 (94.7 - 99.4)
Final Specificity % (95 CL)* n = 189	96.3 (92.5 - 98.5)	91.5 (86.6 - 95.1)	99.5 (97.1 -100.0)	93.7 (89.2 - 96.7)	100.0 (98.1 - 100.0)
Indeterminate results%	1.2	3.1	0	1.9	0
Initial inter-reader variability%	8.2	Test spot 1: 15.6 Test spot 2: 3.1	0.8	6.2	4.7
PPV 0.1%	21.0	10.6	66.9	13.7	100.0
5.0%	58.0	38.2	91.3	45.3	100.0
10.0%	74.5	56.7	95.7	63.6	100.0
NPV 0.1%	100.0	100.0	100.0	100.0	100.0
5.0%	99.8	100.0	100.0	100.0	99.9
10.0%	99.7	100.0	100.0	100.0	99.8

* 95% confidence limits

Table 3 Detailed operational aspects: Simple/rapid assays

NAME	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard ä HCV
Dimension (cm) of kit : w-l-h	13.5 - 22.3 - 9 (40 tests)	10 - 10 - 6 (10 tests) 13.5 - 16 - 9.5 (50 tests) 14.5 - 24.3 - 9 (100 tests)	13.2-9.7-6.4 (100 tests) 12.6-9.2-8.4 (220 tests)	19 - 28 - 11 (100 tests)	14 - 20 - 10 (40 tests)
Storage conditions (°C)	unopened kit: (2 - 30) opened kit: test cards (2 - 30) diluent (4)	2 - 8	2 - 10	unopened kit (25±3) opened kit (2 - 8)	2 - 8
Incubation temperature (°C)	2 - 30	20 - 25	15 - 30	25 ± 3	room temperature
Reading endpoint stability (h.min)	1.00	>24 .00	2.00	0.10	0.05 12.00 (with stop solution)
Stability after dilution/ reconstitution/opening at (°C)					
- antigen	expiry date (2 - 30)	expiry date (2 - 8)	Reconstituted particles 7	expiry date (2 - 8)	expiry date (2 - 8)
- controls	not applicable	expiry date (2 - 8)	days	6 months (4); 1 month	not applicable
- sample diluent	expiry date (4)	not applicable	(2-10)	(RT)	not applicable
- conjugate	not applicable	expiry date (2 - 8)	expiry date (2 - 10)	expiry date (2 - 8)	expiry date (2 - 8)
- substrate	not applicable	not applicable	expiry date (2 – 10)	1 month (4); 5 days	expiry date (2 - 8)
- wash buffer	not applicable	expiry date (2 - 8)	not applicable	(RT)	expiry date (2 - 8)
			not applicable	not applicable	
			not applicable	expiry date (2 - 8)	
Number of sera per run minimum - maximum	1 - 16	1 - 8	1 - 60	1 - 8	1 - 8
Number of controls per test run	not supplied	2	1	3	not supplied
- negative	1*	1	0	1	0
- cut-off/weak positive	0	0	0	1	0
- positive	1*	1	1	1	0
- blank	0	0	1	0	0
internal: reagent control	yes	yes	yes	yes	no
sample addition control	yes	no	no	yes	no
	* recommended by manufacturer				

Table 3 (continued). Detailed operational aspects: Simple/rapid assays

NAME	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard® HCV
Estimated time to perform one run: h.min (number of sera)	0.06 (16)	0.09 (8)	2.45 (60)	0.09 (8)	0.19 (8)
Equipment needed but not provided in the kit:[1]					
- washer	-	-	-	-	-
- incubator (water-bath)	-	-	-	-	-
- spectrophotometric reader	-	-	-	-	-
- refrigerator (storage)	+	+	+	+	+
- agitator , rocker	-	-	-	-	-
- aspiration device	-	-	-	-	-
- negative and/or positive controls	+	-	-	-	-
- automatic pipette (µl)	+	-	+	-	-
- multichannel (µl)	-	-	-	-	-
- disposable tips	+	-	+	-	-
- dilution tubes/rack,microtiterplate	+	-	+	-	-
- distilled or deionised water	-	-	-	-	-
- plate covers	-	-	-	-	-
- graduated pipette; cylinder (ml)	-	-	+ 1.0, 5.0, 10.0	-	-
- sulphuric acid/sodium hydroxide/HCl	-	-	-	-	+/-
- absorbent paper	-	-	-	-	-
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	-	-	-	-	-
- timer	+	-	+	-	+
Definition of positive results	pink test band and pink control band	light to dark pink dot in the test and control spots	agglutination of antigen particles; no agglutination of control particles	distinct red test dot; visible control dot	blue colour in test port of greater intensity than colour in control port
Definition of grey zone	not applicable	not applicable	not applicable	not applicable	not applicable

+ : 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 HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard ä HCV
Number of steps in the test procedure: - 1-2 steps - 3-5 steps - >5 steps	 6 3 1	6	6	3	3	1
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	1	2
Total (out of possible 10)		10	10	7	6	5
Comments on the test kit		-results may not appear for up to 5 minutes	none	none	none	-procedure is complicated - controls not provided. WHO recommends regular use of controls with S/R assays.

Table 4b Calculation of ease of performance: Simple/rapid assays

NAME	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard ä HCV
Need to prepare:					
- antigen	1[1]	1	0	1	1
- substrate	1	1	1	1	1
- wash solution	1	1	1	1	1
- conjugate	1	1	1	0	1
- predilution of serum	0[2]	1	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
- antigen	1	1	0	1	1
- controls	1	1	0	0	1
- sample diluent	1	1	1	1	1
- conjugate	1	1	1	0	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	1	0	1	1
- dilution - tubes, rack/microtiter plate	0	1	0	1	1
- distilled or deionised water	1	1	1	1	1
- plate covers	1	1	1	1	1
- graduated pipette ,cylinder	1	1	0	1	1
- sulphuric acid/sodium hydroxide	1	1	1	1	0
Technicians appraisal of the test kit[3] (rating/10)	10	10	7	6	5
Total (out of possible 30)	27	30	21	23	24
Ease of performance: - less easy < 20 - easy 20 ≤ x < 25 - very easy $ 25	very easy	very easy	easy	easy	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: Simple/rapid assays

NAME	Score	Advanced Quality™ One Step HCV Test	HCV TRI-DOT	SERODIA® HCV	HCV-SP· T	SeroCard® HCV
1. Sensitivity						
- 100%	5					
- 98 - 100%	3	0	5	5	5	3
- <98%	0					
2. Specificity						
- >98%	5					
- 95 - 98%	3	3	0	5	0	5
- <95%	0					
3. Incubation temperature						
- room t°	3	3	3	3	3	3
- other than room t°	1					
4. Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	3	2	2	2	3
- < 6 months	1					
5. Storage at						
- ambient t° possible - opened kit	5					
- ambient t° possible -unopened kit	2	2	1	1	2	1
- 2-8° C required	1					
6. Price per test US$						
- ≤ 1.0	3					
- 1.0> x ≤ 2.0	2	2	2	1	1	1
- > 2.0	1					
7. Ease of performance						
- very easy	5					
- easy	3	5	5	3	3	3
- less easy	1					
8. Rapidity of performance:1 serum						
- <10 min	3					
- 10 - 30 min	2	3	3	1	3	3
- > 30 min	1					
9. Washer/agitator						
- not needed	3	3	3	3	3	3
- needed	1					
10. Reading						
- visual: inter-reader variability # 3	5					
inter-reader variability > 3	3	3	3	5	3	3
- reading equipment	1					
Total (out of a possible 40)		27	27	29	25	28
Suitability for use in small laboratories:						
- less suitable < 23		suitable	suitable	suitable	suitable	suitable
- suitable 23 ≤ x ≤ 30						
- very suitable > 30						

Table 6 Performance on seroconversion panels : Simple/rapid assays

Panel ID	BLEED DATE	Days since first bleed	PCR[1] copies/ml	Reference ELISAs ELISA 1[2]	ELISA 2[3]	SR[1]	SR[2]	SR[3]	SR[4]	SR[5]	RIBA 3.0[4] RESULT	c100 (p) (NS4)	c33c (NS3)	c22 (p) CORE	NS5 (NS5)	SOD
PHV907- 01	06.04.96	0	$3x10^6$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-02	10.04.96	4	$2x10^6$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-03	13.04 96	7	$1x10^6$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-04	19.04.96	13	$1x10^6$	0.2	0.3	**pos**	**pos**	neg	**pos**	**pos**	ind	-	-	1+	-	-
-05	24.04.96	18	$1x10^6$	0.9	**1.8**	pos	pos	**pos**	pos	pos	ind	-	±	4+	-	-
-06	27.04.96	21	$1x10^6$	**1.3**	2.9	pos	pos	ind	pos	pos	**pos**	-	1+	4+	-	-
-07	17.09.96	164	nt	7.8	8.2	pos	pos	pos	pos	pos	nt	nt	nt	nt	nt	nt
PHV908 -01	26.01.96	0	$2x10^5$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-02	29.01.96	3	$3x10^5$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-03	31.01.96	5	$3x10^5$	0.1	0.1	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-04	06.02.96	11	$1x10^5$	0.1	0.6	neg	neg	**pos**	neg	neg	neg	±	±	-	-	-
-05	08.02.96	13	$2x10^5$	0.2	0.7	neg	neg	pos	neg	neg	**pos**	1+	1+	-	-	-
-06	14.02.96	19	$1x10^5$	0.8	**2.2**	neg	neg	pos	neg	neg	pos	2+	2+	-	-	-
-07	20.02.96	25	$5x10^4$	**4.5**	3.8	neg	**pos**	pos	neg	neg	pos	3+	4+	-	-	-
-08	22.02.96	27	$8x10^4$	5.6	4.7	neg	pos	pos	neg	neg	pos	3+	4+	-	-	-
-09	27.02.96	32	$1x10^5$	7.3	5.8	**pos**	pos	pos	ind	neg	pos	3+	4+	-	-	-
-10	01.03.96	35	$2x10^4$	7.4	6.3	pos	pos	pos	neg	neg	pos	3+	4+	-	-	-
-11	07.03.96	41	$1x10^4$	8.2	7.2	pos	pos	pos	**pos**	neg	pos	3+	4+	-	-	-
-12	11.03.96	45	$2x10^4$	8.5	7.2	pos	pos	pos	pos	neg	pos	3+	4+	-	-	-
-13	14.03.96	48	$2x10^5$	8.6	7.0	pos	pos	pos	pos	neg	pos	3+	4+	-	-	-
PHV911- 01	30.10.96	0	$>5x10^5$	0.0	0.0	ind	neg	neg	neg	neg	neg	-	-	-	-	-
-02	02.11.96	3	$>5x10^5$	0.1	0.0	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-03	13.11.96	14	$>5x10^5$	**1.0**	**1.5**	**pos**	ind	**pos**	neg	neg	**pos**	-	1+	3+	-	-
-04	20.11.96	21	$>5x10^5$	6.9	6.9	pos	**pos**	pos	**pos**	**pos**	pos	2+	4+	4+	-	-
-05	23.11.96	24	$>5x10^5$	8.9	7.2	pos	pos	pos	pos	pos	pos	3+	4+	4+	-	-
PHV913 -01	27.02.97	0	$>5x10^5$	0.1	0.0	**pos**	neg	neg	neg	neg	neg	-	-	-	-	-
-02	01.03.97	2	$>5x10^5$	0.3	0.1	pos	neg	neg	**pos**	neg	neg	-	-	-	-	-
-03	06.03.97	7	$>5x10^5$	**3.0**	**1.5**	pos	**pos**	neg	pos	**pos**	ind	-	-	2+	-	-
-04	08.03.97	9	$>5x10^5$	2.9	1.7	pos	pos	neg	pos	pos	ind	-	±	2+	-	-

1: HCV RNA Roche Amplicor PCR - data supplied by Boston Biomedica Inc.
2: ELISA 1 - Monolisa anti-HCV plus (Biorad Pasteur)
3: ELISA 2 - Ortho HCV 3.0 enhanced SAVe -short incubation (Ortho Diagnostics)
4: Ortho anti-HCV RIBA 3.0 (Ortho Diagnostics) - data supplied by Boston Biomedica Inc.

SR1 : Advanced Quality™ One Step HCV Test
SR2 : HCV TRI-DOT
SR3 : SERODIA® HCV
SR4 : HCV-SP•T
SR5 : SeroCard™ HCV

Table 7 Performance on world-wide performance panel: Simple/rapid assays

Panel ID	Origin	Murex HCV[1] Serotyping 1'- 6	Innogenetics[2] INNO-LiPA	Expected Result	PCR[3] copies /ml	Reference ELISA's ELISA 1[4]	ELISA 2[5]	SR[1]	SR[2]	SR[3]	SR[4]	SR[5]	RIBA 3.0[6] RESULT	c100(p) (NS4)	c33c (NS3)	c22(p) CORE	NS5 (NS5)	SOD
WWHV301-01	Argentina	1	1b	POS	$>5x10^5$	9.3	8.2	pos	pos	pos	pos	pos	pos	3+	4+	4+	3+	-
-02	Argentina	Not typable	1b	POS	$3x10^5$	9.5	9.5	pos	pos	pos	pos	pos	pos	-	2+	4+	-	-
-03	Argentina	3	3a/b	POS	$6x10^4$	10.4	8.5	pos	pos	pos	pos	pos	pos	4+	3+	4+	4+	-
-04	Argentina	2	2a/c	POS	$9x10^4$	10.4	9.4	pos	pos	pos	pos	pos	ind	4+	4+	4+	4+	3+
-05	Argentina	Neg	Not tested	NEG	nt	0.0	0.0	neg	neg	neg	neg	neg	neg	-	-	-	-	-
-06	Uganda	4	4c/d	POS	$4x10^3$	11.8	7.4	pos	pos	pos	pos	pos	pos	4+	4+	+/-	-	-
-07	Uganda	2	Not typable	POS	bld*	8.3	7.6	pos	pos	pos	pos	pos	pos	-	4+	+/-	2+	-
-08	Ghana	Neg	Not tested	NEG	nt	0.2	0.1	neg	**pos**	neg	neg	neg	neg	-	-	-	-	-
-09	China	1	1b,2a/c	POS	$3x10^3$	9.8	8.0	pos	pos	pos	pos	pos	pos	4+	4+	4+	2+	-
-10	China	1	2	POS	$4x10^3$	11.4	8.9	pos	pos	pos	pos	pos	pos	4+	4+	4+	2+	-
-11	China	1	1b	POS	$4x10^3$	7.3	7.8	pos	pos	pos	pos	**ind**	pos	2+	4+	+/-	-	-
-12	China	2	2	POS	$2x10^4$	10.2	8.8	pos	pos	pos	pos	pos	pos	4+	4+	4+	4+	-
-13	China	1,2	1a/b, 2a/c	POS	$1x10^5$	11.0	9.4	pos	pos	pos	pos	pos	pos	4+	4+	4+	4+	-
-14	Egypt	3	3a	POS	$1x10^5$	9.5	8.9	pos	pos	pos	pos	pos	pos	2+	2+	4+	-	-
-15	Egypt	4	4	POS	$6x10^4$	10.5	8.9	pos	pos	pos	pos	pos	pos	2+	4+	4+	-	-
-16	Egypt	4	4h	POS	$2x10^4$	11.5	8.8	pos	pos	pos	pos	pos	pos	4+	4+	3+	4+	-
-18	USA	1	1b	POS	$>5x10^5$	9.4	8.6	pos	pos	pos	pos	pos	pos	1+	3+	3+	-	-
-19	USA	Not typable	1a	POS	$3x10^5$	5.9	5.3	pos	pos	pos	**neg**	pos	pos	1+	4+	-	-	-
-20	USA	1	1a	POS	$1x10^4$	8.5	7.6	pos	pos	pos	pos	pos	pos	1+	4+	4+	2+	-

1: Murex HCV Serotyping 1 - 6 -data supplied by Boston Biomedica Inc.
2: Innogenetics INNOLiPA - data supplied by Boston Biomedica Inc.
3: HCV RNA Roche Amplicor PCR - data supplied by Boston Biomedica Inc.
4:ELISA 1 - Monolisa anti-HCV plus (Biorad Pasteur)
5:ELISA 2 - Ortho HCV 3.0 enhanced SAVe -short incubation (Ortho Diagnostics)
6: Ortho anti-HCV RIBA 3.0 (Ortho Diagnostics) - data supplied by Boston Biomedica Inc.
*: bld – below limit of detection

SR1 : Advanced Quality™ One Step HCV Test
SR2 : HCV TRI-DOT
SR3 : SERODIA® HCV
SR4 : HCV-SP•T
SR5 : SeroCard™ HCV

Note: Sample WWHV301-17 was deleted from the analysis because the RIBA result was indeterminate and the PCR was below limit of detection.

Table 8 Performance on low titre performance panel: Simple/rapid assays

Panel ID	Expected Result	PCR[1] Copies /ml	Reference ELISA's ELISA 1[2]	ELISA 2[3]	SR[1]	SR[2]	SR[3]	SR[4]	SR[5]	RIBA 3.0[4] RESULT	c100(p) (NS4)	c33c (NS3)	c22(p) CORE	NS5 (NS5)	SOD
PHV104-01	POS	bld*	0.6	1.9	pos	pos	pos	pos	**neg**	pos	1+	+/-	1+	-	nt
-02	POS	$1x10^5$	12.3	9.0	pos	pos	pos	pos	pos	pos	4+	4+	+/-	2+	nt
-04	POS	$6x10^5$	10.7	9.0	pos	pos	pos	pos	**neg**	pos	4+	4+	-	4+	nt
-05	POS	$1x10^5$	0.7	2.4	pos	pos	pos	pos	pos	pos	+/-	1+	3+	-	nt
-06	POS	$1x10^5$	11.0	8.1	pos	pos	pos	pos	pos	pos	+/-	4+	3+	4+	nt
-07	POS	$1x10^4$	8.4	7.8	pos	pos	pos	pos	**neg**	pos	4+	4+	-	4+	nt
-08	POS	bld	2.8	1.8	**neg**	**neg**	pos	**neg**	**neg**	pos	2+	1+	-	-	nt
-09	POS	bld	3.5	3.7	**neg**	pos	pos	**ind**	**neg**	pos	+/-	1+	2+	+/-	nt
-10	POS	$2x10^4$	4.5	4.4	ind	pos	pos	ind	**neg**	ind	+/-	2+	-	+/-	nt
-11	POS	bld	2.2	2.1	**neg**	pos	pos	pos	pos	pos	2+	3+	2+	+/-	nt
-12	NEG	nt	0.0	0.1	neg	**ind**	neg	neg	neg	neg	-	-	-	-	nt
-13	POS	$2x10^4$	2.0	2.5	**neg**	**ind**	pos	**neg**	**neg**	pos	3+	1+	-	+/-	nt
-14	POS	bld	3.2	2.8	pos	**neg**	pos	pos	**neg**	pos	1+	2+	+/-	-	nt
-15	POS	$1x10^5$	8.2	7.1	pos	pos	pos	pos	**neg**	pos	4+	4+	+/-	-	nt

1: HCV RNA Roche Amplicor PCR - data supplied by Boston Biomedica Inc.

2: ELISA 1 - Monolisa anti-HCV plus (Biorad Pasteur)

3: ELISA 2 - Ortho HCV 3.0 enhanced SAVe -short incubation (Ortho Diagnostics)

4: Ortho anti-HCV RIBA 3.0 (Ortho Diagnostics) - data supplied by Boston Biomedica Inc.

*: bld – below limit of detection

SR1 : Advanced Quality™ One Step HCV Test

SR2 : HCV TRI-DOT

SR3 : SERODIA® HCV

SR4 : HCV-SP•T

SR5 : SeroCard™ HCV

Note: Sample PHV104-3 was deleted from the analysis because the RIBA result was indeterminate and the PCR was below limit of detection

Explanatory Notes for Tables 1 - 8 and Figures 1- 2

Table 1.	**General characteristics and operational aspects of the assays**.
Specimen type	The SeroCard HCV (Trinity Biotech plc) may be used with whole blood samples. 80µl of whole blood, either as a fingerprick sample or whole blood anti-coagulated with heparin, EDTA or citrate is required. The performance of the assay with whole blood samples was not assessed in this evaluation.
Shelf life at (°C)	The unopened kits of two assays, the Advanced Quality™ One Step HCV Test (Bionike Inc.) and the HCV -SP•T (Genelabs Diagnostics Pte Ltd) may be stored at room temperature. After opening, the kit must be stored at 2 - 8°C.
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. - 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 prices stated are the catalogue prices and therefore indicative.
Table 2.	**Comparison of the results of the assays with reference tests**
Sensitivity	calculated as described on page 7 of this document.
Specificity	calculated as described on page 7 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	Simple/rapid assays - test results which could not be interpreted as clearly positive or negative were considered indeterminate.

Explanatory Notes for Tables 1 - 8 and Figures 1- 2

Inter-reader variability	calculated as described on page 9 of this document.
Note	The two values given in the table for the HCV TRI-DOT refer to the separate interpretation of the test spots.
Table 3.	**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.
Number of controls per test run	
Internal control	The following assays all have a control spot or line on all their test devices which checks that sample has been added, the procedure is followed correctly and reagents function correctly: ADVANCED Quality™ One Step HCV Test (Bionike Inc.) and HCV SP•T (Genelabs Diagnostics Pte. Ltd.) - The following assays have a control spot or line on all their test devices which check that reagents function correctly and the procedure has been correctly followed: HCV TRI-DOT (J.Mitra & Co. Ltd.). - The control particles of the SERODIA® HCV (Fujirebio Inc.) check for non-specific agglutination of the gelatin particles. -The SeroCard™ HCV (Trinity Biotech plc.) assay has a control port to assess the degree of colour that may develop due to non-specific binding of proteins present in the sample
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 and 4b.	**Calculation of ease of performance of the assay**
	The criteria for this calculation are given in the respective tables.

Explanatory Notes for Tables 1 - 8 and Figures 1- 2	
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** 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 antibody to HCV earlier than other assays of higher specificity. Caution should be taken when reviewing seroconversion performance of assays tested only in 4 panels.
Table 7.	**Results of the assays on world-wide performance panel** A world panel including different genotypes was tested in each assay. Any results which were different from the expected results are shown in bold type. Sample WWHV301-17 was deleted from the analysis because the RIBA result was indeterminate and the PCR was below limit of detection.
Table 8.	**Results of the assays on a low titre panel** A panel of samples with low anti-HCV titre were tested in each assay. Any results which were different from the expected results are shown in bold type. Sample PHV104-3 was deleted from the analysis because the RIBA result was indeterminate and the PCR was below limit of detection.
Figure 1.	**Relative performance on seroconversion panels** Four seroconversion panels (BBI), each containing several samples taken at different time intervals early in the infection period (window period), were tested with the simple and/or rapid anti- HCV test kits. The results obtained with these assays were compared to those of the combined outcome of the reference tests (seepage 21 of this report); the difference in days of the first sample of a panel to become positive with test X as compared to the first positive result with the reference tests. If a test gave a positive result earlier than the reference tests the number of days difference in detection were rated as negative; if the test became positive later the number of days were rated as positive. The mean of the difference in time period for a test to become positive as compared to the reference tests was calculated and plotted on a yardstick. The 95% confidence limits of the mean were also calculated. These limits should be interpreted with caution as only 4 panels were tested.
Figure 2.	**Relative performance of the assays on seroconversion, world-wide performance and low titre panels** Assay performance was compared on the three types of panels 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 world-wide and low titre panels. The maximum possible number of correctly identified specimens was 14 for the lowtitre panel and 19 for the world-wide panel.

Figure 1: Relative Performance in Seroconversion Panels as compared to the reference assay (Ortho HCV 3.0)

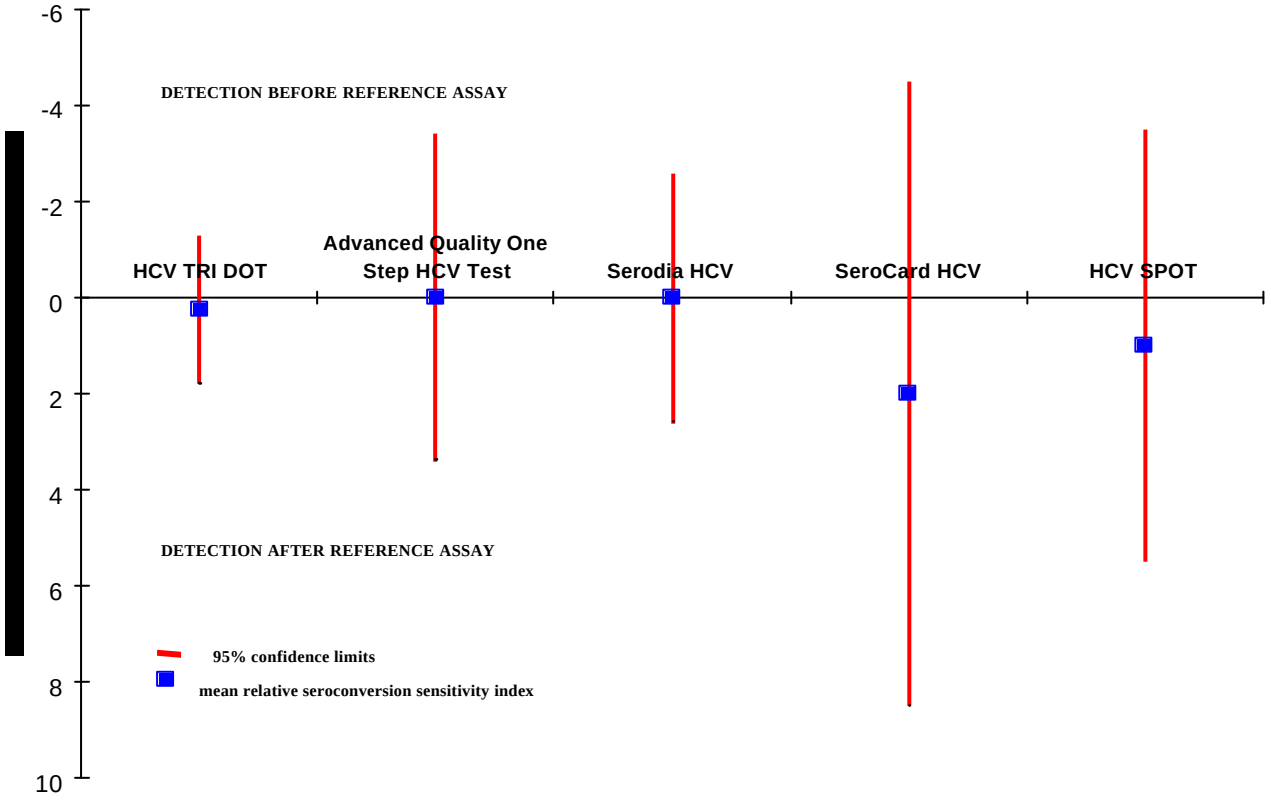
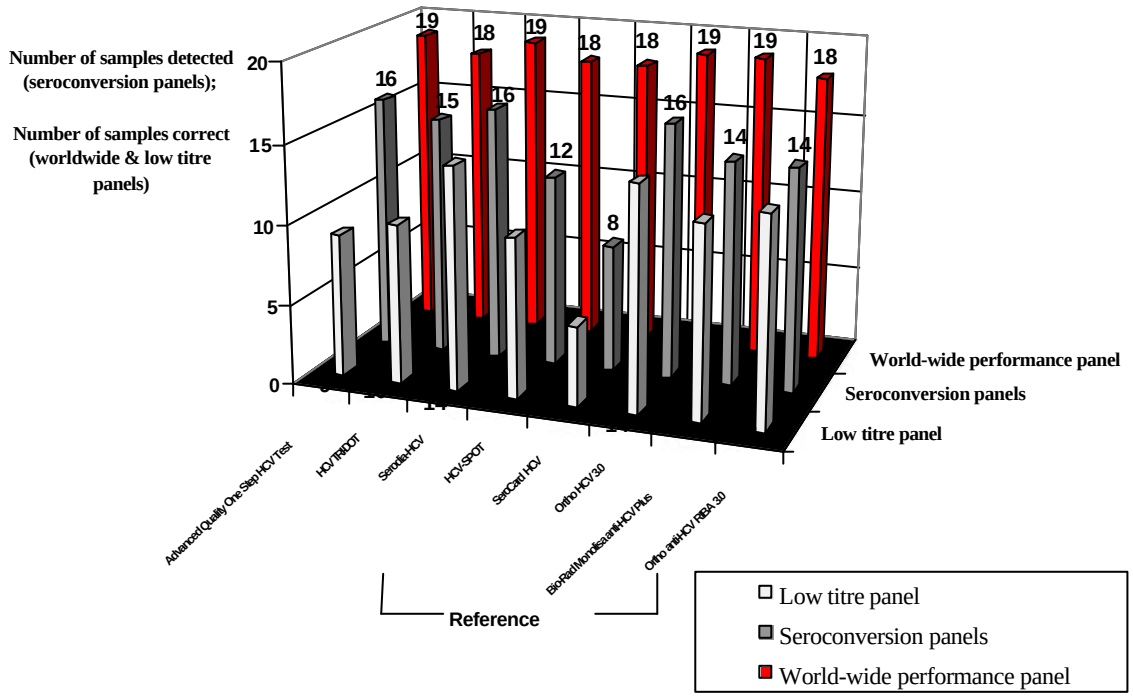
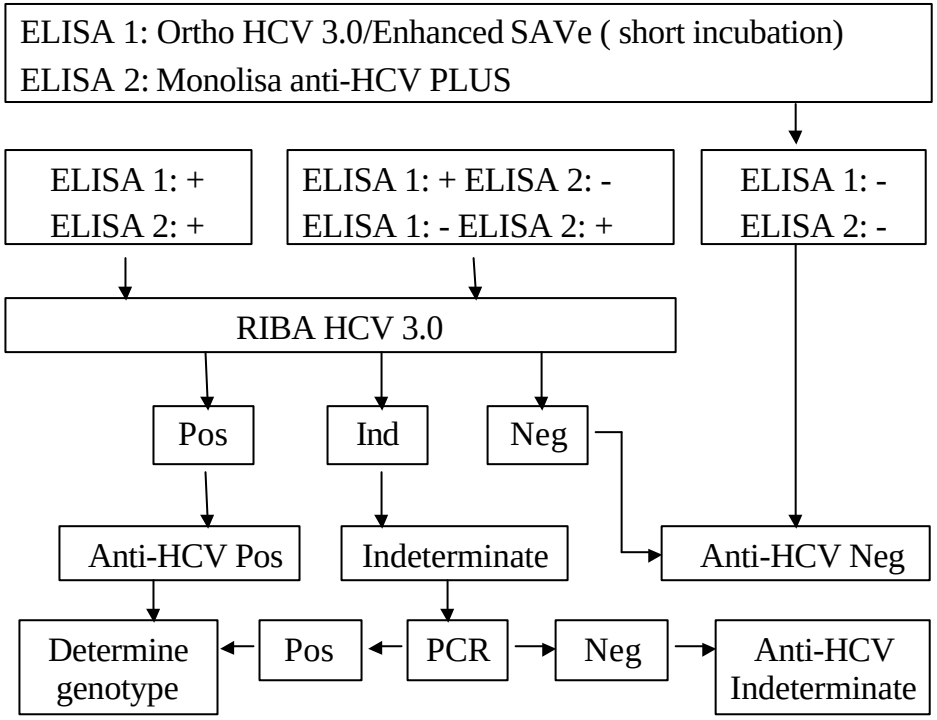


Figure 2. Relative Performance in Seroconversion, Worldwide and Low Titre Panels



6. ANNEXES

ANNEX 1: Algorithm for characterization of the WHO HCV panel



ANNEX 2 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 HCV Test (Bionike Inc.)	1	1.20 (99)	97.1 (89.8 - 99.6)	96.3 (92.5 - 98.5)	1.2	8.2	VE	2-30
HCV TRI-DOT (J. Mitra & Co. Ltd.)	1	2.00 (99)	100.0 (94.7 - 100.0)	91.5 (86.6 - 95.1)	3.1	15.6 (test spot 1) 3.1 (test spot 2)	VE	2-8
Serodia® HCV (Fujirebio Inc.)	1	4.50 (99)	100.0 (94.7 - 100.0)	99.5 (97.1 - 100.0)	0.0	0.8	E	2-10
HCV SP•T (Genelabs Diagnostics Pte Ltd.)	1	2.50 (99)	100.0 (94.7 - 100.0)	93.7 (89.2 - 96.7)	1.9	6.2	E	25 " 3
SeroCard™ HCV (Trinity Biotechplc)	1	2.25(99)	98.5 (92.1 - 100.0)	100.0 (98.1 - 100.0)	0.0	4.7	E	2-8

Legend for Annex 2

a: Operational Characteristics of Hepatitis C Assays (Phase I) Report 1 -

b: Prices are those quoted by the manufacturer at the time of the evaluation. The prices stated are the catalogue prices and therefore indicative.

c, d, e : Sensitivity, specificity and 95% confidence limits were calculated as described on page 7 and 8 of this document

f: Indeterminate results were calculated as described in the explanatory notes on page 23 of this document

g: Inter-reader variability was calculated as described on page 9 of this document.

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 List of assay manufacturers' addresses

Bio-Rad Pasteur ., 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

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

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; E-mail: 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; E-mail: genelabs@pacific.net.sg
Website: www.genelabs.com.sg
Europe: Halle de Frêt, P. O. Box 1015, 1215 Geneva 15 Airport, Switzerland.
Tel: +41 22 788 1908; Fax +41 22 788 1986; E-mail: salesgva@genelabs.ch

Innogenetics N.V./S.A., Technologie Park 6, B-9052 Zwijnaarde, Gent, Belgium.
Tel: +32 9 329 1623; Fax +32 9 245 7623; Website: www.innogenetics.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; E-mail: jmitra@ndb.vsnl.net.in

Ortho Clinical Diagnostics N.V., Antwerpseweg 19/21, B-2340 Beerse, Belgium.
Tel: +32 14 60 02 11; Fax: + 32 14 61 51 58;

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

7. REFERENCES

Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. N Engl J Med 1996;334(26):1685-90.

Barrera JM, Francis B, Ercilla G, Nelles M, Achord D, Darner J, Lee SR. Improved detection of anti-HCV in post-transfusion hepatitis by a third-generation ELISA. Vox Sang 1995;68:15-18.

8. ACKNOWLEDGEMENT

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