# Research/Recherche

## Rapid and simple hepatitis assays: encouraging results from a blood donor population in Zimbabwe

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*A rapid assay to detect antibodies to hepatitis C virus (HCV) in serum and two rapid/simple assays to detect hepatitis B surface antigen (HBsAg) in whole blood/serum were evaluated for their accuracy and suitability at the National Blood Transfusion Service, Harare, Zimbabwe. For this purpose, a total of 206 sera (196 routinely collected and 10 frozen) were tested using the HCV-SPOT (Genelabs Diagnostics), the SimpliRED HBsAg test (AGEN), and the Dipstick-HBsAg (PATH/Immuno-Chemical Laboratories). The results were compared with those obtained using a routine HBsAg enzyme immunoassay (EIA) (Auszyme, Abbott) and an HCV IgG second-generation EIA (Abbott). An HCV IgM test (Abbott) was used for samples that produced discordant results, and all HCV-reactive samples were confirmed using the INNO-LIA HCV Ab III synthetic peptide assay (Innogenetics).*

*Overall, the concordance between the HCV-SPOT and the HCV EIA was 97.6% (201/206). For the 193 sera that were true HCV negatives, the number of false positives was six with the HCV-SPOT test, while the HCV EIA produced three (specificity = 97.0% and 98.5%, resp.). Of these false positives, two were so in both tests. None of the false positives contained IgM antibodies to HCV, and there were no false negatives in the two HCV tests. The concordance between the two rapid HBsAg tests and the HBsAg EIA was 99.5% (205/206). All the rapid/simple tests were easy to perform and interpret, required no (or minimal) laboratory equipment, and could be taught easily to local laboratory personnel. The cost of these tests is equivalent to or less than that of routine EIA methods.*

## Introduction

Although a large variety of serological assays are available for diagnosing human immunodeficiency virus (HIV) infection (*1*), there are far fewer assays for detecting hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. An enzyme immunoassay (EIA) is most frequently used to detect HBV surface antigen (HBsAg) and antibodies to HCV. While EIAs are highly accurate and applicable in many situations, alternatives are available and may be more appropriate in some instances; for example, the testing of samples in remote laboratories — where facilities and capabilities are less than optimal, or a stable electricity supply is not available, and in instances where rapid results are required (emergency transfusions and transplantation services), where low-volume testing is performed, or where technical expertise is limited — may dictate the need for alternatives such as simple and rapid assays. Use of such assays can be of value in physicians' offices, emergency rooms, autopsy rooms, and in small blood centres.

As technology evolves, tests become simpler and faster, and attractive alternatives to routine methods emerge. For example, oral fluids (*3*), urine (*4*), and whole blood (*5*) have been examined in an effort to simplify collection of samples; their use can result in time and cost savings, and they have proved useful in several situations. The use of rapid and simple tests as alternatives to EIA has also been of value as a confirmatory strategy for HIV testing and can offer cost savings (*6*). However, new alternatives must be thoroughly evaluated and have comparable

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accuracy to existing methodologies. The testing of blood for HIV, HBV, and HCV is important, particularly for transfusion purposes.

Recently Constantine et al. reported the successful use of two rapid HCV assays in a high-prevalence population in Romania (*2*); these tests also require to be further evaluated in a low prevalence population. To the best of our knowledge, there have been no other reports on the use of rapid HCV assays. Simple tests to detect HBsAg are available, but accounts of the performance of the new generation of simple tests by independent investigators are scarce. The present article reports the accuracy and performance characteristics of several of these newer rapid hepatitis virus tests in a low prevalence, blood donor population in a developing country.

## Materials and methods

### *Serum samples*

A total of 206 serum samples that had been collected routinely as part of the normal services of the National Blood Transfusion Service, Harare, Zimbabwe were used in the study. Of these samples, 196 were freshly collected, while 10 had been stored frozen at –20 °C, and were from individuals who had tested reactive for HCV by EIA. These 10 archived sera were included to permit some assessment of the sensitivity of the HCV rapid test since the prevalence of HCV infection in the study population was low (0.4%). In addition, eight of the fresh sera had tested positive for HBsAg on the day prior to the start of the study, and were included for the same reason (the prevalence of HBsAg in the donor population is about 2%).

### *Testing procedures*

All the fresh serum samples, stored at 4 °C, were tested blind using rapid assays within 24 hours of being collected. Testing by EIA (Auszyme (Abbott, Chicago, IL, USA) for HBsAg; and HCV second-generation EIA for HCV (Abbott, Chicago, IL, USA)), was carried out on the previous day and the results were compared with those obtained with the rapid tests. The 10 frozen samples were tested blind along with the fresh samples. The rapid tests included the following: HCV-SPOT (Genelabs Diagnostics, Singapore) for HCV; and SimpliRED HBsAg test (AGEN, Brisbane, Australia) and the Dipstick-HBsAg (Program for Appropriate Technology in Health (PATH), Seattle, WA, USA/ Immuno-Chemical Laboratories, Bangkok-RIA) for HBsAg. All tests were performed as recommended by the manufacturers under local laboratory conditions in Harare. Any sample that yielded a discrepant result was retested blind in duplicate using all the tests to ensure that no errors had occurred. Repeat testing was performed in Harare for the rapid tests and at the University of Maryland for the EIA. All samples reactive for HCV by any test were also tested using the INNO-LIA HCV Ab III confirmatory test (Innogenetics, Ghent, Belgium) for resolution. Samples that gave discordant results using the HCV assays were further tested using an HCV EIA (Abbott, Wiesbaden, Germany) designed to detect IgM antibodies.

### *Description of the tests used*

***HCV-SPOT test.*** The HCV-SPOT test is a dot-blot assay that uses recombinant antigens absorbed on a membrane encased in a plastic device. No dilution of the sample or initial filtering is required, and the sample volume is 40 µl (one drop). The presence of antibodies to HCV is revealed using a protein-A–gold conjugate which turns red. The test incorporates a procedural control dot and does not require to be timed since the absorption procedure is preprogrammed for optimal performance. The procedure consists of sequential additions of blocking buffer, sample, wash buffer, conjugate, buffer, and stop solution. The presence of a large red dot in addition to the procedural control dot indicates a positive result. The HCV-SPOT test can be kept at room temperature for extended periods (6 months), and requires no laboratory equipment. In addition, the results can be stored as a permanent record.

***SimpliRED HBsAg test.*** The SimpliRED HBsAg test is an autologous red cell agglutination rapid assay in which the patient's erythrocytes act as the indicator when agglutinated by the presence of an anti-HBsAg antibody coupled to an antibody to red blood cell antigens (chemical conjugate of two monoclonal antibodies). The presence of HBsAg in the sample cross-links the antibodies on adjacent cells producing visible agglutination. The test uses one reagent and whole blood but serum can be used along with group O, Rh-negative red blood cells. In the study, 10 µl of patient's serum was mixed with 10 µl of group O, Rh-negative red blood cells and one drop of the conjugate reagent. The results are produced in 2 minutes, and only a mechanical pipette is required to carry out the test. The test is claimed to have a sensitivity for HBsAg of 20 ng/ml, but according to the manufacturer is not designed for screening blood products. The reagents should be stored refrigerated.

***Dipstick-HBsAg test.*** The Dipstick-HBsAg test is a simple-to-perform dot immunoassay designed to detect HBsAg in serum or plasma. The test uses a monoclonal antibody coated on the end of each "tooth" of a "comb". HBsAg in the sample binds to the antibody and is detected using an antibody–colloidal gold signal reagent. A pink spot on the tooth signifies a reactive result. The test takes about 70 minutes to perform and requires no laboratory equipment except a pipette capable of delivering 200 µl of sample. Refrigeration of reagents is recommended, and the results can be stored as a permanent record. The test is intended to be a low-cost, blood screening tool for use in blood banks and transfusion centres in developing countries, and is claimed to be at least as sensitive as the reverse passive haemagglutination assay (RPHA) and the latex agglutination tests for HBsAg (it is probably 2–4 times more sensitive).

***INNO-LIA HCV Ab III test.*** The INNO-LIA HCV Ab III is a confirmatory assay that uses synthetic peptide antigens derived from the $E_2$/NS1, NS3, NS4, and NS5 regions of the HCV genome. The antigens are applied as discrete lines on a plastic-backed nylon strip. Positive reactions are visualized after reaction with a typical enzyme/substrate system.

## Results

### *Hepatitis C virus*

Of the 206 samples tested, 191 were negative in both the HCV-SPOT and the HCV EIA, while 10 were repeatedly reactive in both tests (these 10 samples had been frozen and had previously tested reactive for HCV by EIA). Of these 10 samples, 8 had absorbances in the range 4.3–7.3 (mean = 4.9) and their reactivity was confirmed by INNO-LIA; reactivity was exhibited to all antigens, except in three cases where reactivity was absent to either the NS1 or NS4 antigens. The remaining two of these 10 samples were reactive in both the EIA (absorbances = 2.0 and 1.0) and the HCV-SPOT, but were nonreactive to all antigens by the INNO-LIA (2 false-positives in both screening tests). Five freshly collected samples also produced divergent results in the EIA and the HCV-SPOT, four being reactive only in the latter. The one sample that was nonreactive in the HCV-SPOT but reactive by the EIA was only weakly so in the latter (absorbance = 2.2). Upon being retested, the five fresh serum samples produced results identical to these obtained initially; in the INNO-LIA they were unreactive to all antigens, and they were negative for HCV IgM antibodies (Table 1).

Table 1: **Results for the five sera that produced divergent outcomes in the rapid hepatitis assays**

| | Test for:[a] | | | | | |
|---|---|---|---|---|---|---|
| | Hepatitis C virus | | | | HBsAg[b] | |
| Sample | HCV-SPOT | EIA | INNO-LIA | IgM | Dipstick | SimpliRED |
| 03001071 | R | N | N | N | | |
| 03001083 | R | N | N | N | | |
| 42000549 | R | N | N | N | N | N |
| 42000554 | R | N | N | N | | |
| 42000556 | N | R(2.2)[c] | N | N | | |

[a] For details of the tests, see text. R = reactive; N = nonreactive.
[b] HBsAg: hepatitis B surface antigen.
[c] Figure in parentheses is the absorbance.

### *Hepatitis B surface antigen*

The results of the Dipstick-HBsAg test and the SimpliRED HBsAg autoagglutination test paralleled those of the EIA in all cases except one. A total of 15 samples were positive for HBsAg in the EIA but only 14 were positive in the HBsAg rapid test (previously, 8 of these samples had been found to be positive for HBsAg by EIA). The one sample that showed repeatedly negative results in both simple and rapid HBsAg tests was reactive (absorbance >2.0) by EIA in Harare and at the University of Maryland. Unfortunately, the small amount available of this sample precluded confirmatory HBsAg testing.

***Test performance.*** All three rapid/simple tests were easy to perform, required no (or little) laboratory equipment, and could be read visually and without difficulty. The HCV-SPOT and the SimpliRED tests were particularly rapid, producing results in less than 10 minutes. Although the Dipstick-HBsAg method required over 1 hour for completion, the time involved to set it up was about 30 minutes for 72 samples, which is comparable to the other tests when large numbers of samples are involved. The test performance characteristics, including estimated costs, are shown in Table 2.

## Discussion

Detection of HCV and HBV infections is most frequently accomplished using EIA methods. A number of studies have evaluated the performance and accuracy of serological screening and confirmatory tests for detecting antibodies to HCV (*9, 10*). Also, using African serum samples, two second-generation screening assays have been compared with

Table 2: **Operational characteristics of the tests used in the study**

| Characteristic | Test for:[a] | | | | |
|---|---|---|---|---|---|
| | Hepatitis C virus | | | HBsAg[b] | |
| | HCV-SPOT | EIA | Dipstick | SimpliRED | EIA |
| Completion time (min)[c] | 5 | 120 | 5[d] | 5 | 90 |
| Equipment needed | None | Yes | Minimal[e] | Minimal[e] | Yes |
| Storage temperature | RT | 4 °C | 4 °C | 4 °C | 4 °C |
| Cost (US$)[f] | 4 | 7 | 0.64 | 1.50 | 6 |

[a] For details of the tests, see text.
[b] HBsAg: hepatitis B surface antigen.
[c] Minutes per test, including controls.
[d] Set-up time; incubations require about 60 minutes
[e] A mechanical pipette is the only equipment needed.
[f] Manufacturer's estimate; may depend on number of tests purchased.

the results obtained using two confirmatory assays (*11*). The results indicated a high degree of discordance, with one of the screening tests producing a high number of false positives and a low positive predictive value, while the other lacked sensitivity. Since such a high degree of discordance has not been reported with sera from industrialized countries, the poor performance of the tests may have been due to the origin of the sera; consistent with this conclusion is the lower specificity and/or sensitivity that has been reported for tests of African sera for HIV antibodies (*12*, *13*). Therefore, it is perhaps not unreasonable that there may be some degree of discordance in the results of serological tests for hepatitis using African sera.

Our results show that a rapid HCV assay, requiring no equipment or refrigeration, can be performed easily and effectively in the field in a developing country setting. The test was extremely easy to carry out and interpret, and incorporated a quality control measure to ensure that it was performed properly; although its specificity was slightly lower than a routine EIA, its sensitivity was equivalent. Based on these findings, rapid HCV assays could be useful for screening blood for transfusion and in epidemiological studies. Since one or more of the discrepant sera could have been from individuals in the early stages of infection with HCV, we tested those sera for HCV IgM, but found no evidence for this. HCV IgM assays detect early infection in less than 50% of HCV seroconversion panels.[a] Two samples produced a false-positive result in both the HCV-SPOT and the EIA, fewer than the number of false positives for each of the assays individually. The assays performed in tandem have a higher combined specificity, and hence, a better positive predictive value than each has individually.

Both rapid/simple HBsAg tests had 100% specificity, but failed to detect one sample that was strongly reactive by EIA. This is surprising, since both rapid tests detected the remaining 14 reactive samples, some of which had absorbance values <2.0 (data not shown). This suggests that the rapid tests are not lacking in analytical sensitivity; however, the sample that they failed to detect could have been "problematic" since it gave discordant results with the HCV assays. Also, this sample may have been infected with a variant or mutant form of HBV that was not readily detected by the monoclonal antibody reagents used in either of the rapid tests; HBV mutants have been reported (*14*). Finally, this sample could have been from an individual not infected with HBV (confirmation by neutralization could not be performed), and was therefore an EIA false-positive for HBsAg (*15*). False-positive results with high absorbance values in the Auszyme EIA have previously been found to be negative in the Dipstick-HBsAg test (*16*). Further evaluation using a large number of positive samples will therefore be necessary before the ability of these two rapid/simple tests to detect all positive sera can be determined.

Although rapid tests for the detection of HIV antibodies are available and widely used, only recently has a rapid and simple HCV antibody assay been developed. Similar assays for detecting HBsAg have been available for some time, e.g., gel diffusion, latex agglutination, and reverse passive haemagglutination, but have not been widely used to screen blood for transfusion in developing countries, where their procedural characteristics should be attractive. In some developing countries hepatitis screening has only been adopted to a limited extent probably for the following reasons: lack of financial resources; the belief that hepatitis infections are highly prevalent; the view that hepatitis infection is less cause for concern than infection with HIV; or the general perception that simple-to-perform assays may not be as accurate as more sophisticated techniques. Now that rapid assays (for HIV) have been shown to have the potential to be accurate and effective (two such tests have been licensed by the U.S. Food and Drug Administration, and WHO recommends their use in certain situations), their use should increase. Also, as awareness extends about the importance of keeping blood supplies free of transmissible agents, appreciation of the need for screening and the search for appropriate testing methods will expand. New tests

[a] **Constantine, N. et al.** Dynamics of HCV seroconversion. Paper presented at: *Third International Meeting on Hepatitis C virus and related Viruses, Gold Coast, Australia, August 1995.* Abstract No. 112.

with novel procedural characteristics that have proven acceptable for HIV testing may therefore be more readily adopted to detect hepatitis viral infections. Rapid hepatitis assays are less expensive than corresponding enzyme-linked immunosorbent assays (ELISAs); however, the additional financial resources needed to include HCV and HBV screening for all blood units may be prohibitive for most developing countries. This problem could be addressed if manufacturers were to offer bulk purchase prices for assays to be used in developing countries, as is currently practised for HIV tests (*6*). Expenditures could also be reduced by developing cost- and time-saving HIV/hepatitis combination assays, producing test kits locally, and pooling blood prior to testing.

The use of alternative testing strategies for HIV infection has proved an effective and cost-saving measure (*6*). In developing countries or in situations where EIA technology cannot be supported, rapid and/or simple serological tests may be adopted. Also, in HIV testing the incorporation of such tests into a strategy as a second, supplemental assay has the potential to replace more sophisticated and expensive confirmatory tests (*2*, *7*, *8*). Similarly, rapid hepatitis assays could have important applications in a variety of situations. In particular, their use could be valuable for gathering epidemiological data in remote areas where more sophisticated testing methods cannot be employed, and they can be used in emergencies where a blood transfusion or organ transplantation is required urgently. At the National Blood Transfusion Service, Harare, it is not uncommon to need blood in emergency situations. In these instances, it may not be possible to screen all units for HCV and HBV (a rapid test to screen for HIV is used). Therefore, the addition of rapid hepatitis assays to the testing armamentarium may increase the potential to provide a safe blood supply. Finally, we have shown that the use of an HCV rapid test in combination with the results obtained by another screening test is a potential supplemental testing strategy, with the combined results having an increased specificity and positive predictive value. Use of two screening assays, rather than a more expensive confirmatory test, can provide an accurate indication of true infection at a much lower cost. At least one HCV confirmatory test costs over US$ 120 per test, considerably more expensive than the cost of two screening tests.

In the study our objective was to determine the use and accuracy of these novel rapid serological assays in an African population where the prevalence of hepatitis infection is low (prevalence of HCV and HBsAg: 0% and ca. 3% with fresh serum samples, resp.). Rapid HCV assays have been used effectively in a high-prevalence haemodialysis population from a developing country (*2*).

Although our findings were encouraging, the sample size was not large, thereby limiting the statistical significance of the conclusions. Further studies using larger populations from both Africa and other countries, should therefore be performed before final conclusions about the usefulness of these rapid hepatitis assays can be drawn.

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## Résumé

### Méthodes simples et rapides de dépistage de l'hépatite: résultats encourageants sur une population de donneurs de sang du Zimbabwe

Le service national de transfusion sanguine de Harare (Zimbabwe) a évalué une méthode rapide de détection des anticorps dirigés contre le virus de l'hépatite C (HCV) dans le sérum, et deux méthodes simples et rapides conçues pour détecter l'antigène de surface de l'hépatite B (HBsAg) dans le sang total ou le sérum. A cette fin, 206 échantillons de sérum (196 recueillis selon la méthode habituelle et 10 congelés) ont été testés par la méthode HCV-SPOT (Genelabs Diagnostics), et par les méthodes de recherche de l'HBsAg SimpliRED (AGEN) et Dipstick-HBsAg (PATH/Immuno-Chemical Laboratories). Les résultats ont été comparés à ceux obtenus par la méthode habituelle de dosage immuno-enzymatique de l'HbsAg (EIA) (Auszyme, Abbott), et avec un EIA de seconde génération fondé sur la recherche des IgG anti-HCV (HCV EIA, Abbott). Un test de recherche des IgM dirigées contre l'HCV (Abbott) a été appliqué aux échantillons donnant des résultats discordants. Tous les résultats positifs pour l'HCV ont été confirmés à l'aide du test INNO-LIA HCV Ab III utilisant un peptide de synthèse (Innogenetics).

Globalement, la concordance entre les tests HCV-SPOT et HCV EIA a été de 97,6% (201/206). Sur les 193 sérums qui étaient effectivement négatifs pour l'HCV, le nombre de faux positifs a été de six avec le test HCV-SPOT, et de trois avec le test HCV EIA (spécificité: 97,0% et 98,5%

respectivement). Parmi ces faux positifs, deux l'ont été dans les deux tests. Pour la recherche de l'HBsAg, la concordance entre les deux méthodes rapides et l'EIA a été de 99,5% (205/206). Un seul échantillon a donné un résultat positif avec la méthode EIA et négatif avec les deux méthodes rapides. Il n'a pas été possible de confirmer la véritable sérologie de cet échantillon, pour lequel la recherche de l'HCV a également donné des résultats discordants. Les trois tests rapides ont été faciles à exécuter et à interpréter, n'ont exigé qu'un minimum de matériel de laboratoire et ont pu être enseignés facilement et rapidement au personnel local.

Les trois tests évalués ont donné des résultats encourageants par comparaison avec les méthodes EIA habituelles pour la population étudiée, dans laquelle la prévalence de l'hépatite était faible. Ils représentent une solution intéressante pour les centres disposant de moyens d'analyse limités ou lorsque l'alimentation électrique est aléatoire. Ils peuvent aussi être utilisés lorsque les résultats doivent être connus rapidement. Leur coût est équivalent ou inférieur à celui des méthodes EIA classiques.

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