Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus*

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Summary

Testing for the presence of antibody to hepatitis C virus (anti-HCV) is recommended for initially identifying persons with hepatitis C virus (HCV) infection (CDC. Recommendations for prevention and control of hepatitis C virus [HCV] infection and HCV-related chronic disease. MMWR 1998;47[No. RR-19]:1--33). *Testing for anti-HCV should include use of an antibody screening assay, and for screening test-positive results, a more specific supplemental assay. Verifying the presence of anti-HCV minimizes unnecessary medical visits and psychological harm for persons who test falsely positive by screening assays and ensures that counseling, medical referral, and evaluation are targeted for patients serologically confirmed as having been infected with HCV. However, substantial variation in reflex supplemental testing practices exists among laboratories, and an anti-HCV--positive laboratory report does not uniformly represent a confirmed positive result. These guidelines expand recommendations for anti-HCV testing to include an option for reflex supplemental testing based on screening-test--positive signal-to-cut--off (s/co) ratios. Use of s/co ratios minimizes the amount of supplemental testing that needs to be performed while improving the reliability of reported test results. These guidelines were developed on the basis of available knowledge of CDC staff in consultation with representatives from the Food and Drug Administration and public health, hospital, and independent laboratories. Adoption of these guidelines by all public and private laboratories that perform in vitro diagnostic anti-HCV testing will improve the accuracy and utility of reported anti-HCV test results for counseling and medical evaluation of patients by health-care professionals and for surveillance by public health departments.*

Introduction

Tests to detect antibody to hepatitis C virus (anti-HCV) were first licensed by the Food and Drug Administration (FDA) in 1990 (*1*). Since that time, new versions

of these and other FDA-approved anti-HCV tests have been used widely for clinical diagnosis and screening of asymptomatic persons.

Persons being tested for anti-HCV are entitled to accurate and correctly interpreted test results. CDC has recommended that a person be considered to have serologic evidence of HCV infection only after an anti-HCV screening-test--positive result has been verified by a more specific serologic test (e.g., the recombinant immunoblot assay [RIBA®; Chiron Corporation, Emeryville, California]) or a nucleic acid test (NAT) (*2*). This recommendation is consistent with testing practices for hepatitis B surface antigen and antibody to human immunodeficiency virus (HIV), for which laboratories routinely conduct more specific reflex testing before reporting a result as positive (*1,3*). However, for anti-HCV, the majority of laboratories report a positive result based on a positive screening test result only, and do not verify these results with more specific serologic or nucleic acid testing unless ordered by the requesting physician. Unfortunately, certain health-care professionals lack an understanding of the interpretation of anti-HCV screening test results, when more specific testing should be performed, and which tests should be considered for this purpose.

In certain clinical settings, false-positive anti-HCV results are rare because the majority of persons being tested have evidence of liver disease and the sensitivity and specificity of the screening assays are high. However, among populations with a low (<10%) prevalence of HCV infection, false-positive results do occur (*4--11*). This is of concern when testing is performed on asymptomatic persons for whom no clinical information is available, when persons are being tested for HCV infection for the first time, and when testing is being used to determine the need for postexposure follow-up. Without knowledge of the origin of the test sample or clinical information concerning the person being tested, the accuracy of a screening-test--positive result for any given specimen cannot be determined.

Multiple reasons exist regarding why laboratories do not perform reflex supplemental testing for anti-HCV, including lack of an established laboratory standard for such testing, lack of understanding regarding the performance and interpretation of the screening and supplemental HCV tests, and the high cost of the supplemental HCV tests. To facilitate practice of reflex supplemental testing, the recommended anti-HCV testing algorithm has been expanded to include an option that uses the signal-to-cut--off (s/co) ratios of screening-test--positive results to minimize the number of specimens that require supplemental testing and provide a result that has a high probability of reflecting the person's true antibody status.

Background

Available Anti-HCV Screening Assays

FDA-licensed or approved anti-HCV screening test kits being used in the United States comprise three immunoassays; two enzyme immunoassays (EIA) (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Illinois, and ORTHO® HCV Version 3.0 ELISA, Ortho-Clinical Diagnostics, Raritan, New Jersey) and one enhanced chemiluminescence immunoassay (CIA) (VITROS® Anti-HCV assay, Ortho-Clinical Diagnostics, Raritan, New Jersey). All of these immunoassays use HCV-encoded recombinant antigens.

Available Supplemental Tests

Supplemental tests include a serologic anti-HCV assay and NATs for HCV RNA. In the United States, the only FDA-licensed supplemental anti-HCV test is the strip immunoblot assay (Chiron RIBA® HCV 3.0 SIA, Chiron Corp., Emeryville, California). RIBA 3.0 uses both HCV-encoded recombinant antigens and synthetic peptides. Because it is a serologic assay, it can be performed on the same serum or plasma sample collected for the screening anti-HCV assay.

FDA-approved diagnostic NATs for qualitative detection of HCV RNA using reverse transcriptase polymerase chain reaction (RT-PCR) amplification include AMPLICOR® Hepatitis C Virus (HCV) Test, version 2.0 and COBAS AMPLICOR® Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems, Branchburg, New Jersey), which have a lower limit of detection of approximately 50 IU/mL (*12*). Detection of HCV RNA by these tests requires that the serum or plasma

sample be collected and handled in a manner suitable for NAT and that testing be performed in a laboratory with facilities established for this purpose (see Recommendations). Other NATs for HCV RNA, both qualitative and quantitative, are available on a research-use basis from multiple manufacturers of diagnostic reagents, and certain laboratories perform NATs by using in-house laboratory methods and reagents (*12,13*).

Interpreting Anti-HCV Test Results

Screening Immunoassay Test Results

Anti-HCV testing includes initial screening with an immunoassay. Criteria for interpretation of a reactive[†] anti-HCV immunoassay result are based on data from clinical studies performed under the auspices of each manufacturer. For EIAs (e.g., HCV EIA 2.0 and HCV Version 3.0 ELISA), specimens with a reactive result are retested in duplicate. If the result of either duplicate test is reactive, the specimen is defined as repeatedly reactive and is interpreted as screening-test--positive. For CIAs, (e.g., VITROS Anti-HCV assay), specimens with a single reactive result are considered screening-test--positive and do not require retesting.

The specificity of the HCV EIA 2.0 and HCV Version 3.0 ELISA is $\geq$99%. However, among a population with a low prevalence of infection, even a specificity of 99% does not provide the desired predictive value for a positive test. Among immunocompetent populations with anti-HCV prevalences <10% (e.g., volunteer blood donors, active duty and retired military personnel, persons in the general population, health-care workers, or clients attending sexually transmitted disease [STD] clinics), the proportion of false-positive results with HCV EIA 2.0 or HCV Version 3.0 ELISA averages approximately 35% (range: 15%--60%) (*4--11*; CDC, unpublished data, 2002). Among immunocompromised populations (e.g., hemodialysis patients), the proportion of false-positive results averages approximately 15% (*14*; CDC, unpublished data, 2002). For this reason, not relying exclusively on anti-HCV screening-test--positive results to determine whether a person has been infected with HCV is critical. Rather, screening-test--positive results should be verified with an independent supplemental test with high specificity.

Supplemental Serologic Test Results

The strip immunoblot assay (RIBA), a supplemental anti-HCV test with high specificity, is performed on screening-test--positive samples and provides results that are interpreted as positive, negative, or indeterminate. A positive RIBA result is interpreted as anti-HCV--positive (Box). Although the presence of anti-HCV does not distinguish between current or past infection, a confirmed anti-HCV--positive result indicates the need for counseling and medical evaluation for HCV infection, including additional testing for the presence of virus (NAT for HCV RNA) and liver disease (e.g., alanine aminotransferase [ALT]) (*2,15*). Anti-HCV testing usually does not need to be repeated after the anti-HCV--positive result has been confirmed.

A negative RIBA result is interpreted as anti-HCV--negative and indicates a false-positive screening test result. In this situation, the additional testing with RIBA minimizes unnecessary medical visits and psychological harm from reporting a false-positive screening test result. Typically, persons whose anti-HCV test results are negative (screening-test--negative or RIBA-negative) are considered uninfected (Box). However, false-negative anti-HCV test results can occur during the first weeks after infection (i.e., before antibody is detectable or during seroconversion), although HCV RNA can be detected as early as 1--2 weeks after exposure to the virus (*16,17*). Rarely, antibody seroconversion might be delayed for months after exposure (*18,19*). In certain persons whose HCV infection has resolved, anti-HCV declines below detectable levels (*20*). Occasionally, persons with chronic HCV infection, including those who are immunocompromised, are persistently anti-HCV--negative, and detection of HCV RNA might be the only evidence of infection (*14,21*).

An indeterminate RIBA result indicates that the anti-HCV result cannot be determined (Box). Indeterminate anti-HCV supplemental test results have been observed in recently infected persons who are in the process of seroconversion, and occasionally in persons chronically infected with HCV (*22*). Indeterminate results also might indicate a false-positive screening test result, which is the most common interpretation for these results among those at low risk for HCV

infection (*23,24*). Another sample should be collected for repeat anti-HCV testing (>1 month later) or for HCV RNA testing.

Supplemental NAT Results

NATs that detect HCV RNA also can be used as supplemental tests for anti-HCV. They are used commonly in clinical practice for diagnosis of acute and chronic HCV infection and for evaluating and managing patients with chronic hepatitis C.

If the NAT result is positive in persons with a positive screening test result, NAT has the advantage of detecting the presence of active HCV infection as well as verifying the presence of anti-HCV (Box). If the NAT result is negative in persons with a positive screening test result, the HCV antibody or infection status cannot be determined. Among persons with these results, additional testing with RIBA is necessary to verify the anti-HCV result and determine the need for counseling and medical evaluation (Box); if the anti-HCV screening test results are judged falsely positive (i.e., RIBA-negative), no further evaluation of the person is needed; whereas if the anti-HCV screening test results are verified as positive by RIBA, the person should undergo medical evaluation, including serial determinations of HCV RNA and ALT activity.

Certain situations exist in which the HCV RNA result can be negative in persons with active HCV infection. As the titer of anti-HCV increases during acute infection, the titer of HCV RNA declines (*17*). Thus, HCV RNA is not detectable in certain persons during the acute phase of their hepatitis C, but this finding can be transient and chronic infection can develop (*25*). In addition, intermittent HCV RNA positivity has been observed among persons with chronic HCV infection (*21,26,27*). Therefore, in the absence of additional clinical information, the significance of a single negative HCV RNA result is unknown, and the need for further medical evaluation is determined by verifying anti-HCV status.

A negative HCV RNA result also can indicate resolved infection. Among anti-HCV--positive persons who acquired their HCV infection as older adults (aged >45 years), 15%--25% apparently resolve their infection; this proportion is higher (40%--45%) among anti-HCV--positive persons who acquired their infection as children or younger adults (*20*). To determine if HCV infection has resolved, a negative HCV RNA result should be demonstrated on multiple occasions; however, such follow-up testing is indicated only in persons with serologically confirmed anti-HCV positive results.

Anti-HCV Testing Practices

Multiple commercial, hospital-based, and public health laboratories that perform anti-HCV testing routinely report screening test results only. More specific testing (i.e., RIBA or NAT) is performed only when ordered by a physician. Moreover, in certain laboratories, more specific tests are not available.

During 2002, two surveys regarding anti-HCV testing practices were performed, one by the Association of Public Health Laboratories (Barbara Werner, Ph.D., Association of Public Health Laboratories, personal communication, September 2002) and one by a Veterans Affairs (VA) Medical Center (D. Robert Dufour, M. D., VA Medical Center, Washington, D.C., personal communication, October 2002). Forty-three (80%) of 54 U.S. state and territorial public health laboratories and 67 (39%) of 172 VA medical center laboratories responded (Table 1).

Of the respondents, the public health laboratories were less likely to offer screening or supplemental tests for HCV than were the hospital-based VA laboratories. However, the public health laboratories that did offer both types of testing were more likely to perform reflex supplemental testing than were the hospital-based laboratories, 75% of which performed supplemental testing only by physician request. Regarding the type of supplemental testing performed, the majority of hospital-based laboratories performed only NATs, whereas the public health laboratories most commonly performed either RIBA alone or NAT followed by RIBA if the NAT result was negative.

Although substantial differences existed in testing practices between and among these two types of laboratories, the majority of public and private sector laboratories depend on the requesting physician to be knowledgeable concerning the appropriate tests to order and the correct interpretation of their results. However, a general lack of understanding exists among health-care professionals regarding the interpretation of screening test results, when more specific testing should be performed, and which tests should be considered for this purpose.

Using Screening-Test--Positive S/Co Ratios To Determine Need for Reflex Supplemental Testing

Analysis of early versions of anti-HCV EIA results from volunteer blood donors indicated that average repeatedly reactive s/co ratios could be used to predict supplemental test-positive results (*28*). Similar data from volunteer blood donors were generated by using HCV Version 3.0 ELISA, for which the average s/co ratios of 24,700 samples repeatedly reactive for anti-HCV were compared with their RIBA 3.0 results (Susan Stramer, Ph.D., American Red Cross, personal communication, March 1999). Overall, 64.0% were RIBA-positive. The proportion that tested RIBA-positive was 5.8% for samples with an average s/co ratio 1.0--2.9; 37.1% for those with average s/co ratio 3.0--3.4; 67% for those with average s/co ratio 3.5--3.7; 88.1% for those with average s/co ratio 3.8--3.9; and 94.1% for those with average s/co ratio ≥4.0.

Additional data from other populations were generated by CDC to determine if a specific s/co ratio could be identified that would predict a true antibody-positive result ≥95% of the time, regardless of the anti-HCV prevalence or characteristics of the population being tested. The anti-HCV screening tests evaluated were the two FDA-licensed EIAs, HCV EIA 2.0 and HCV Version 3.0 ELISA, and the one FDA-approved CIA, VITROS Anti-HCV assay.

EIAs

All specimens with EIA screening-test--positive results were tested by RIBA 3.0, and a sample of screening-test--positive specimens were tested for HCV RNA by ≥2 of the following NAT methods: transcription-mediated amplification (TMA) (Procleix™, Chiron Corporation, Emeryville, California); AMPLICOR; and nested RT-PCR (*13*). Test results were used from serum samples that had been collected as part of CDC-sponsored anti-HCV seroprevalence studies that were conducted among different groups of asymptomatic persons (Robert Gunn, M.D., San Diego County Department of Health and Human Services Agency; Steven Harris, M.D., Travis County, Texas Department of Health; Lu-Yu Hwang, M.D., University of Texas --- Houston School of Public Health; Leslie Tobler, Ph.D., Blood Centers of the Pacific, San Francisco; Gayle Shimokura, University of North Carolina at Chapel Hill School of Public Health; Isaac Weisfuse, M.D., New York City Department of Health and Mental Hygiene, personal communications, 2001--2002; CDC, unpublished data, 2002). Anti-HCV prevalences ranged from 0.8% to 25% (Table 2).

The proportion of screening-test--positive results that were serologically confirmed as anti-HCV--positive (i.e., RIBA-positive) increased as the anti-HCV prevalence in the population increased (Table 2). Conversely, the proportion of screening-test--positive results that were falsely antibody-positive (RIBA-negative) or RIBA-indeterminate was inversely related to prevalence (Table 2).

For each study group, the proportion of screening-test--positive results that were RIBA-positive increased as the screening-test--positive average s/co ratios increased (Figure 1). On the basis of these data, screening-test--positive average s/co ratios ≥3.8 were highly predictive of RIBA positivity (≥95%), with limited variability (95%--97%) between groups with different prevalences (Table 2). Screening-test--positive average s/co ratios ≥3.8 also were highly predictive of HCV RNA positivity, although the proportions that were HCV RNA-positive were slightly lower than those for RIBA (Table 2).

These results indicate that for licensed EIAs, reporting anti-HCV screening-test--positive results as anti-HCV positive for samples with average s/co ratios ≥3.8 would be highly predictive of the true anti-HCV status. Reflex supplemental testing before reporting the anti-HCV results could be limited to screening-test--positive samples with average s/co ratios <3.8. The feasibility of this approach is supported further by the limited proportion (2.4%) of samples from persons at

high risk that have s/co ratios below this cut-off value. When testing for anti-HCV is performed on persons at increased risk for infection as recommended ([2](#)), a limited number of samples will require additional testing.

CIA

The relation between s/co ratios and RIBA 3.0 results also was evaluated for specimens that were screening-test--positive by CIA (i.e., reactive by VITROS Anti-HCV) from four groups. These included a group of 162 volunteer blood donors with substantially low anti-HCV prevalence (Leslie Tobler, Ph.D., Blood Centers of the Pacific, San Francisco, personal communication, September 2002), a group of 163 persons with low anti-HCV prevalence (college students, persons in the general population, and health-care workers as described previously), a group of 219 hemodialysis patients with intermediate anti-HCV prevalence (as described previously), and a group of 689 hospital-based patients with high anti-HCV prevalence (signs or symptoms of liver disease or risk factors for HCV infection) (D. Robert Dufour, M.D., VA Medical Center, Washington, D.C., and Michael De Lucia, Ortho-Clinical Diagnostics, personal communications, September 2002).

Overall, the proportion of CIA screening-test--positive samples that tested RIBA-positive was 77.8% among the blood donors, 74.2% among the low prevalence group, 86.3% among the hemodialysis patients, and 94.5% among the high prevalence group. The direct relation between increasing s/co ratios and RIBA positivity that was observed among samples tested with the two EIAs evaluated by CDC also was observed among the samples tested with the CIA ([Figure 2](#)). However, the range of screening-test--positive s/co ratios obtained with VITROS Anti-HCV was greater than that obtained with HCV EIA 2.0 or HCV Version 3.0 ELISA; thus, s/co ratios that were highly predictive of RIBA positivity also were higher. Using VITROS Anti-HCV, an s/co ratio of ≥ 8 predicted RIBA positivity in 95%--98% of the screening-test--positive samples ([Figure 2](#)). The proportion of CIA samples with low s/co ratios was inversely related to anti-HCV prevalence (i.e., 4.9% in the high prevalence group, 8.7% in the intermediate prevalence group, and 21.5% in the low prevalence group). These results indicate that for the FDA-approved CIA, reflex supplemental testing of screening-test--positive samples also could be limited to those with a low (<8) s/co ratio; and among persons at increased risk for infection, $\leq 5\%$ will have s/co ratios below the cut-off value.

Estimated Costs of Implementing Reflex Supplemental Testing Based on Screening-Test--Positive S/Co Ratios

To assist laboratories in assessing the potential financial impact of implementing reflex supplemental testing for screening-test--positive samples with low s/co ratios, the incremental costs associated with such testing were estimated for three hypothetical populations of 10,000 persons each, representing anti-HCV prevalences of 2%, 10%, and 25%, respectively (similar to those of the groups evaluated previously). For each population, the costs of performing the screening test (by using EIAs as the example) and each of two different supplemental testing schemes (schemes 1 and 2) were compared with the cost of performing only the screening test (base scheme).

All schemes included performing a screening EIA on each sample and repeating initially reactive specimens in duplicate. Scheme 1 also included RIBA testing on all screening-test--positive samples with average s/co ratios <3.8, and scheme 2 included NAT testing on all screening-test--positive samples with average s/co ratios <3.8, followed by RIBA on those that were NAT-negative.

The increased costs for schemes 1 and 2 were calculated per sample tested compared with the base scheme. For RIBA and NAT, minimum and maximum costs were estimated; minimum costs were defined as costs for reagents only, and maximum costs were defined as costs incurred for tests performed by a referral laboratory. The following assumptions were made:

- The percentage of initially reactive samples that were repeatedly reactive (screening-test--positive) was assumed to be 90% in the groups with anti-HCV prevalences of 2% and 10%, and 95% in the group with anti-HCV prevalence of 25%.
- The proportion of screening-test--positive samples with average s/co ratios <3.8 and the proportion of such samples that tested RIBA-positive for each population was derived ([Table 2](#)).

- The proportion of screening-test--positive samples with average s/co ratios <3.8 that were NAT-positive was derived ([Table 2](#)) for the populations with anti-HCV prevalences of 2% and 10%. For the population with a prevalence of 25%, this proportion was assumed to be zero (on the basis of data from high-prevalence hospital-based patients) (D. Robert Dufour, M.D., VA Medical Center, Washington, D.C., personal communication, September 2002).

Costs were estimated as follows and do not include personnel time or additional equipment:

- $5/sample for initial screening test;
- $15/sample for those testing initially reactive and repeated in duplicate;
- $65--$158/sample tested with RIBA; and
- $50--$295/sample tested with a NAT.

Compared with performing only the screening test, performing reflex RIBA testing on all screening-test--positive samples with average s/co ratios <3.8 (scheme 1) increases the cost of testing per sample for immunocompetent populations from a minimum of 5%--12% ($0.41--$0.66) to a maximum of 13%--30% ($1.00--$1.60), depending on the anti-HCV prevalence of the population being tested ([Figure 3](#)). For hemodialysis patients, the cost increases from a minimum of 16% ($1.00) to a maximum of 38% ($2.44). Performing reflex NATs on all screening-test--positive samples with average s/co ratios <3.8, followed by RIBA on those that are NAT-negative (scheme 2), increases the cost of testing per sample for immunocompetent populations from a minimum of 9%--21% ($0.73--$1.14) to a maximum of 37%--85% ($2.88--$4.54), compared with performing only the screening test. For hemodialysis patients, the cost increases from a minimum of 27% ($1.73) to a maximum of 109% ($6.88). The higher incremental costs of scheme 2 compared with scheme 1 are because virtually all the screening-test--positive samples with s/co ratios <3.8 test HCV RNA-negative and require follow-up testing with RIBA to verify anti-HCV status.

Recommendations

Rationale

Testing for HCV infection by using anti-HCV is performed for 1) clinical diagnosis of patients with signs or symptoms of liver disease; 2) management of occupational and perinatal exposures; and 3) screening asymptomatic persons to identify HCV-infected persons who should receive counseling and medical evaluation. Anti-HCV test results also are used for public health surveillance to monitor incidence and prevalence and to target and evaluate HCV prevention efforts.

Anti-HCV testing is performed in multiple settings, including hospitals and other health-care facilities, physicians' offices, health department clinics, HIV or other freestanding counseling and testing sites, employment sites, and health fairs. The interpretation of anti-HCV screening-test--positive results in these settings can be problematic. Clinical information related to the persons tested often is lacking, and even persons with risk factors for HCV infection might be at sufficiently low enough risk for infection that their screening test results could be falsely positive (e.g., health-care professionals are at occupational risk for HCV infection, but their overall prevalence of infection is low) (*29*). Without knowledge of the origin of the test sample or clinical information related to the person being tested, the accuracy of a screening-test--positive result for any given specimen cannot be determined.

However, despite previous recommendations for reflex supplemental testing of all anti-HCV screening-test--positive results (*2*), the majority of laboratories report positive anti-HCV results based only on a positive screening assay. To facilitate and improve the practice of reflex supplemental testing, the recommended anti-HCV testing algorithm has been expanded to include an option for more specific testing based on the s/co ratios of screening-test--positive results that can be implemented without substantial increases in testing costs.

Implementation of these recommendations will provide more reliable results for physicians and their patients, so that further counseling and clinical evaluation are limited to those confirmed to have been infected with HCV. This is critical for persons being tested for HCV infection for the first time, for persons being tested in nonclinical settings, and for those being tested to determine the need for postexposure follow-up. Implementation of these recommendations also will improve public health surveillance systems for monitoring the effect of HCV prevention and control activities.

Laboratory Algorithm for Anti-HCV Testing and Result Reporting

All laboratories that provide anti-HCV testing should perform initial screening with an FDA-licensed or approved anti-HCV test according to the manufacturer's labeling.

- Screening-test--negative (i.e., nonreactive) samples require no further testing and can be reported as anti-HCV--negative (Figure 4).
- Screening-test--positive samples require reflex serologic or nucleic acid supplemental testing according to the testing algorithm (Figure 4). Laboratorians can choose to perform reflex supplemental testing 1) based on screening-test--positive s/co ratios, or 2) on all specimens with screening-test--positive results.
  --- For screening-test--positive samples that require reflex supplemental testing (according to the testing option chosen), the anti-HCV result should not be reported until the results from the additional tests are available.

Reflex Supplemental Testing Based on Screening-Test--Positive S/Co Ratios

- Laboratories should use only screening tests that have been evaluated for this purpose[§] and for which high s/co ratios have been demonstrated to predict a supplemental-test--positive $\geq$95% of the time among all populations tested.
- Screening-test--positive samples with high s/co ratios can be reported as anti-HCV--positive without supplemental testing (Figure 4).
- A comment should accompany the report indicating that supplemental serologic testing was not performed, and it should include a statement that samples with high s/co ratios usually ($\geq$95%) confirm positive, but <5 of every 100 samples with these results might be false-positives. The ordering physician also should be informed that more specific testing can be requested, if indicated.
- Screening-test--positive samples with low s/co ratios should have reflex supplemental testing performed, preferably RIBA (Figure 4).

Reflex Supplemental Testing on All Specimens with Screening-Test--Positive Results

- RIBA only; or
- NAT, followed by RIBA for specimens with NAT-negative results (Figure 4).

Considerations When Choosing a Reflex Supplemental Testing Option

Serologic Supplemental Testing.

- RIBA can be performed on the same sample collected for the screening test.
- RIBA is the most cost-effective supplemental test for verifying anti-HCV status for screening-test--positive samples with low s/co ratios.
- The RIBA result is used to report the anti-HCV result.

Nucleic Acid Supplemental Testing.

- NATs can be performed in laboratories that have facilities specifically designed for that purpose.
- Serum or plasma samples must be collected, processed, and stored in a manner suitable for NATs to minimize false-negative results (*30*).
  --- Blood should be collected in sterile collection tubes with no additives or in sterile tubes by using ethylenediaminetetraacetic acid (EDTA).
  --- Serum or EDTA plasma must be separated from cellular components within 2--6 hours after collection.
  --- Storage of serum or EDTA plasma at 2°C--5°C is limited to 72 hours; for longer storage, freezing at --20°C or --70°C is recommended.
     If shipping is required, frozen samples should be protected from thawing.
  --- Samples collected for serologic testing can be used only if the previous conditions are met.
- Because of assay variability, rigorous quality assurance and control should be standards of practice in clinical laboratories performing this assay; proficiency testing is recommended, including monitoring for false-positive results.
  --- Technician proficiency can vary and increases in direct relation to experience.
  --- Intra-assay contamination can occur, including aerosolization, splashing, and carry-over.
- If the HCV RNA result is positive, the presence of active HCV infection can be reported as well as a positive anti-HCV result.
- An HCV RNA-negative result requires that RIBA be performed and the RIBA result used to report the anti-HCV result.

Other Reflex Supplemental Testing Options

Certain laboratories might choose to modify the recommended supplemental testing options to provide additional information before reporting results. One such modification might include reflex NAT of screening-test--positive results with high s/co ratios, which might be of interest to hospital-based laboratories that usually test specimens from patients being evaluated for liver disease. If the NAT result is positive, the presence of active HCV infection can be reported as well as a positive anti-HCV result. However, if the NAT result is negative, reflex RIBA testing still is required before reporting the results to verify the anti-HCV status. Certain specimens will test RIBA-positive, indicating that the person should receive further evaluation, including repeat testing for HCV RNA (see Interpretation of Anti-HCV Test Results).

Implementation

To implement these recommendations for anti-HCV testing and result reporting, laboratories should review their present testing and reporting methods and determine how those should be modified. This process should include

- determining which reflex supplemental testing option will be implemented;
- revising standard operating procedures to include the reflex testing option selected (Figure 4), the procedure for reporting results, and the interpretation of those results (Table 3);
- educating the laboratory staff, physicians, and other end-users; and
- modifying the laboratory requisition form, if necessary. For purposes of reimbursement, the circumstances under which reflex supplemental testing will be performed might need to be included on the form to serve as documentation that the additional tests were ordered.

Laboratories that select a reflex supplemental testing option based on screening-test--positive s/co ratios need to ensure that their analyzers generate optical density (OD) values in a range sufficient to calculate s/co ratios at or above the value defined as a high s/co ratio for the screening test being used. The s/co ratio is calculated by dividing the OD value of the sample being tested by the OD value of the assay cut-off for that run. Depending on the type of equipment in the laboratory, the calculation of s/co ratios might be automatically performed by the analyzer or require that the technician manually perform the calculation.

For screening tests that require only one reactive result to indicate a screening-test--positive result (e.g., VITROS Anti-HCV), the s/co ratio of the reactive result is

used to determine the next step in the algorithm (i.e., reporting the result or reflex supplemental testing). For screening tests that require repeating initially reactive results in duplicate (e.g, HCV EIA 2.0 and HCV Version 3.0 ELISA), the s/co ratio of each of the duplicate results is calculated. The average of the s/co ratios of the reactive results is used to determine the next step in the algorithm. If all three results are reactive for the sample, the average s/co ratio can be determined either by averaging the ratios of all three or by averaging only the ratios of the two duplicate reactive results. If only one of the duplicate results is reactive, the average s/co ratio is determined by averaging the ratios from the initial reactive result and the one duplicate reactive result.

For those screening-test--positive samples that undergo reflex supplemental testing (according to the testing option chosen), the screening test anti-HCV results should not be reported before the results from the additional testing are available. If necessary, an interim report can be issued indicating that the result is pending. This procedure should be followed even if the laboratory does not perform the supplemental testing in-house, but sends the sample to another reference laboratory for such testing. After the results are received from the reference laboratory, the final results can be reported on the basis of the testing performed by both laboratories.

The reported results should be accompanied by interpretive comments as determined by each laboratory (Table 3). The content of these comments will vary on the basis of type of supplemental testing option selected by the laboratory. These comments are critical if screening-test--positive results are reported as anti-HCV--positive on the basis of high s/co ratios, because the health-care professional or other person interpreting the results needs to understand the limitations of the testing option used.

Before implementation, the laboratory staff should be educated regarding new methods of testing, calculating, and reporting final results for the selected testing option. Laboratories also should inform and educate all customers regarding the planned changes and what effects they will have on test results generated. This information should be disseminated as widely as possible (e.g., by laboratory bulletins, letters, Internet, or continuing education programs).

Depending on the setting, reimbursement of clinical laboratory tests used for reflex supplemental testing might depend on documentation that the physician ordered the tests. This documentation can be achieved through a printed requisition form that clearly identifies for anti-HCV the specified level of results of the screening test that will trigger additional supplemental testing and what type(s) of supplemental testing will be performed. In addition, each of the supplemental tests (e.g., RIBA or NAT) that are offered by the laboratory should be listed separately, because physicians should be able to order these as they deem necessary for further medical evaluation.

Future Considerations

As new anti-HCV screening assays are approved or licensed for use, each will need to be evaluated for its specificity among populations with different anti-HCV prevalences. In addition, before using a new assay to perform reflex supplemental testing based on screening-test--positive s/co ratios, the s/co ratio value at or above which supplemental test results are positive $\geq$95% of the time in populations in which the test will be used, should be determined. Such documentation also should be required for approved screening assays if any modifications are made to the testing procedures that might affect the s/co ratio values. Similarly, the relation between screening-test--positive results and the results of newly available supplemental tests will need to be evaluated.

Acknowledgments

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Terms and Abbreviations Used in This Report

ALT	Alanine aminotransferase
Anti-HCV	Antibody to hepatitis C virus
CIA	Chemiluminescence immunoassay, a screening test format for anti-HCV (e.g., VITROS® Anti-HCV assay)
EIA	Enzyme immunoassay, a screening test format for anti-HCV (e.g., Abbott HCV EIA 2.0 and ORTHO® HCV Version 3.0 ELISA)
FDA	Food and Drug Administration
FDA-approved	Assays intended only for in vitro diagnostic use; approved by the Center for Devices and Radiological Health, FDA
FDA-licensed	Assays intended primarily for donor screening; licensed by the Center for Biologics Evaluation and Research, FDA
HCV	Hepatitis C virus
HCV	RNA Hepatitis C virus ribonucleic acid
NAT	Nucleic acid test, detects HCV RNA by amplification of viral genetic sequences
OD	Optical density
Reflex testing	Additional testing automatically performed in response to a screening-test--positive result
RIBA®	Recombinant immunoblot assay, a more specific serological anti-HCV assay
RT-PCR	Reverse transcriptase-polymerase chain reaction amplification, a nucleic acid testing method for detection of HCV RNA
S/Co ratio	Signal to cut-off ratio, calculated by dividing the OD value of the sample being tested by the OD value of the assay cut-off for that run

Screening tests	Serologic immunoassays for detection of anti-HCV (e.g., Abbott HCV EIA 2.0, ORTHO HCV Version 3.0 ELISA, and VITROS Anti-HCV)
Screening-test--negative	Screening immunoassay test result interpreted as negative on the basis of criteria provided by the manufacturer
Screening-test--positive	Screening immunoassay test result interpreted as positive on the basis of criteria provided by the manufacturer
STD	Sexually transmitted disease
Supplemental test	More specific test (e.g., RIBA or NAT) used to verify a positive anti-HCV screening test result
TMA	Transcription-mediated amplification, a nucleic acid testing method for detection of HCV RNA
VA	Veterans Affairs

* These guidelines are not intended to be used for blood, plasma, organ, tissue, or other donor screening or notification as provided for under FDA guidance or applicable regulations. They also are not intended to change the manufacturer's labeling for performing a specific test.

† The terms *reactive* or *nonreactive* are used to describe serum or plasma specimen test results from anti-HCV screening immunoassays before final interpretation. The terms *positive* and *negative* are used to describe the final interpretation of screening immunoassay test results (e.g., screening-test--positive indicates that the specimen tested is repeatedly reactive by EIA or reactive by CIA, or screening-test--negative indicates that the specimen tested is nonreactive or not repeatedly reactive). The terms *positive*, *indeterminate*, and *negative* are used to describe the interpretation of RIBA results based on reactivity with a specific pattern of bands.

§ Data are available from three screening assays. For the two EIAs (HCV EIA 2.0 or HCV Version 3.0 ELISA), high s/co ratios are defined as screening-test--positive results with average s/co ratios ≥3.8, and low s/co ratios as screening-test--positive results with average s/co ratios <3.8. For CIA (VITROS Anti-HCV), high s/co ratios are defined as screening-test--positive results with s/co ratios ≥8, and low s/co ratios as screening-test--positive results with s/co ratios <8.

Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus

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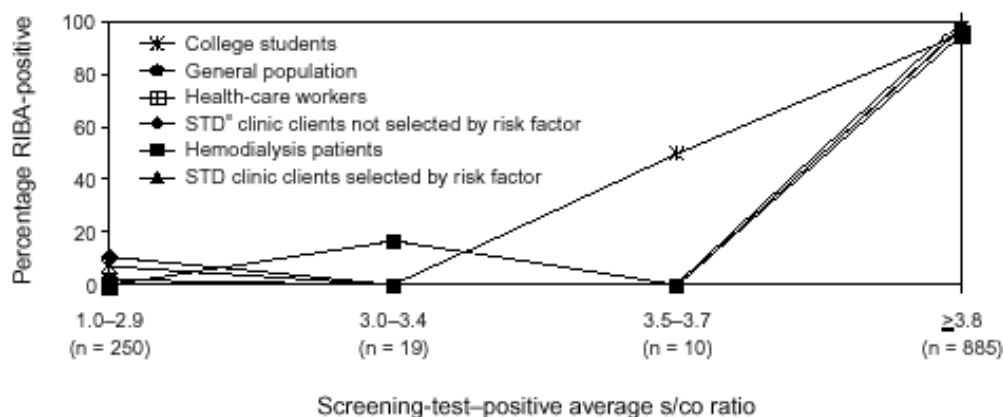
Table 1

TABLE 1. Antibody to hepatitis C virus testing practices of state and territorial public health laboratories and Veterans Affairs Medical Center (VAMC) laboratories, 2002

Testing practices	Public health laboratories	VAMC laboratories
Tests offered	n = 43	n = 67
Screening test	65%	100%
Recombinant immunoblot assay (RIBA®)	38%	21%
Qualitative nucleic acid test (NAT)	29%	75%
Quantitative NAT	13%	98%
Supplemental testing performed	n = 29	n = 67
All screening-test–positive results	35%	22%
Low-positive* screening-test results	10%	3%
Only by physician request	17%	75%
None offered	38%	0%
Type of supplemental test performed[†]	n = 13	n = 16
RIBA only	31%	31%
NAT only	15%	63%
NAT followed by RIBA, if NAT-negative	39%	6%
RIBA followed by NAT, if RIBA-positive	15%	0%

* Signal-to-cut–off ratio below a specified value.
[†] Includes only laboratories that routinely performed supplemental testing on all specimens with screening-test–positive results or with low-positive results.

Figure 1

FIGURE 1. Proportion of antibody to hepatitis C virus enzyme immunoassay* screening-test–positive results that tested recombinant immunoblot assay (RIBA®) 3.0-positive by average signal-to-cut–off (s/co) ratios and group tested

* HCV EIA 2.0 or HCV Version 3.0 ELISA.
[†] Sexually transmitted diseases.

Table 2

TABLE 2. Antibody to hepatitis C virus (anti-HCV) screening-test–positive average signal-to-cut–off (s/co) ratios by recombinant immunoblot assay (RIBA®) 3.0 and nucleic acid test (NAT) results in groups with different anti-HCV prevalences

Anti-HCV prevalence	Study group	Total tested*	Average s/co ratio	No. (%) screening-test–positive		RIBA results (%)			HCV RNA-positive (%)
						Negative	Ind[†]	Positive	
2% (average)	Multiple groups[§] with prevalences ranging from 0.8% to 4.4%	24,012	Total	689	(100.0)	26.9	7.3	65.9	41.6[¶]
			<3.8	231	(33.5)	78.8	16.9	4.3	3.7
			>3.8	458	(66.5)	0.7	2.4	96.9	80.2
9.5%	Hemodialysis patients	2,936	Total	351	(100.0)	9.7	7.3	83.0	80.3
			<3.8	45	(12.8)	64.4	33.3	2.2	4.4
			>3.8	306	(87.2)	1.6	3.4	95.0	91.5
24.9%	STD clinic clients selected based on risk**	498	Total	124	(100.0)	1.6	4.0	94.4	NA[††]
			<3.8	3	(2.4)	66.7	33.3	0	NA
			>3.8	121	(97.6)	0	3.3	96.7	NA

* HCV EIA 2.0 or HCV Version 3.0 ELISA.
[†] Indeterminate.
[§] College students, general population, health-care workers, and sexually transmitted disease (STD) clinic clients not selected by risk factor.
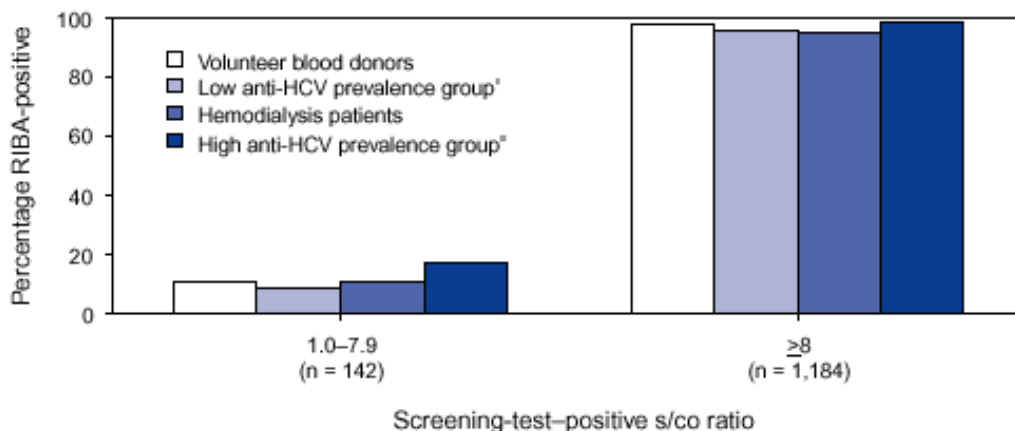[¶] In the low (2%) prevalence group, only a sample of 214 were tested for HCV RNA.
** History of injection-drug use, blood transfusion before 1992, or incarceration.
[††] Not available.

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Figure 2

FIGURE 2. Proportion of antibody to hepatitis C virus (anti-HCV) enhanced chemiluminescence immunoassay* screening-test–positive results that tested recombinant immunoblot assay (RIBA®) 3.0-positive by signal-to-cut–off (s/co) ratios and group tested

* VITROS® Anti-HCV assay.
[†] College students, general population, and health-care workers.
[§] Hospital-based patients.

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Table 3

TABLE 3. Recommendations for reporting results of testing for antibody to hepatitis C virus (anti-HCV) by type of reflex supplemental testing performed
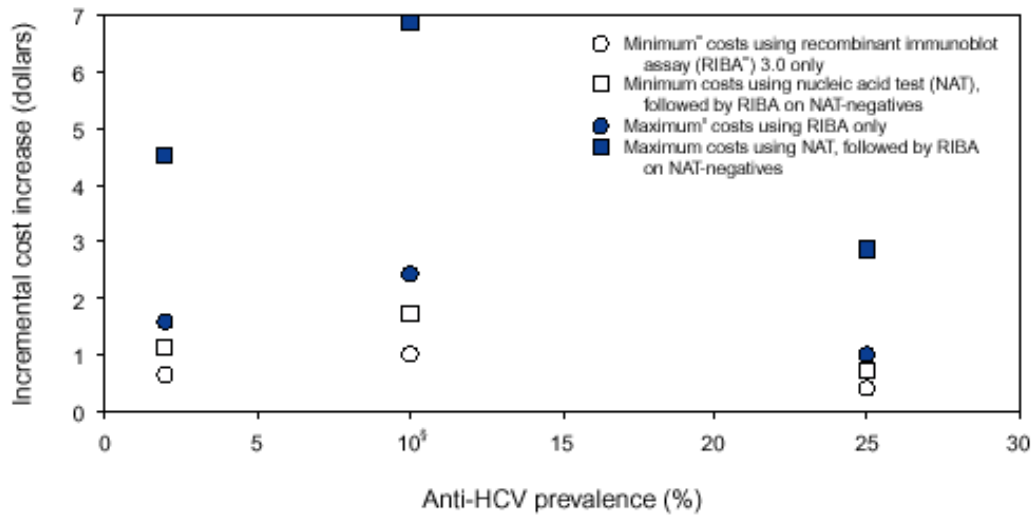
Anti-HCV screening test results	Supplemental test results	Interpretation	Comments
Screening-test–negative*	Not applicable	Anti-HCV–negative	Not infected with HCV, unless recent infection is suspected or other evidence exists to indicate HCV infection
Screening-test–positive* with high signal-to-cut–off (s/co) ratio	Not done	Anti-HCV–positive	Probably indicates past or present HCV infection; supplemental serologic testing not performed. Samples with high s/co ratios usually (≥95%) confirm positive, but <5 of every 100 might represent false-positives; more specific testing can be requested, if indicated
Screening-test–positive	Recombinant immunoblot assay (RIBA®)-positive	Anti-HCV–positive	Indicates past or present HCV infection
Screening-test–positive	RIBA-negative	Anti-HCV–negative	Not infected with HCV, unless recent infection is suspected or other evidence exists to indicate HCV infection
Screening-test–positive	RIBA-indeterminate	Anti-HCV–indeterminate	HCV antibody and infection status cannot be determined; another sample should be collected for repeat anti-HCV testing (>1 month) or for HCV RNA testing
Screening-test–positive	Nucleic acid test (NAT)-positive	Anti-HCV–positive, HCV RNA-positive	Indicates active HCV infection
Screening-test–positive	NAT-negative RIBA-positive	Anti-HCV–positive, HCV RNA-negative	The presence of anti-HCV indicates past or present HCV infection; a single negative HCV RNA result does not rule out active infection
Screening-test–positive	NAT-negative RIBA-negative	Anti-HCV–negative, HCV RNA-negative	Not infected with HCV
Screening-test–positive	NAT-negative RIBA-indeterminate	Anti-HCV-indeterminate HCV RNA-negative	Screening test anti-HCV result probably a false-positive, which indicates no HCV infection

* Screening immunoassay test results interpreted as negative or positive on the basis of criteria provided by the manufacturer.

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Figure 3

FIGURE 3. Estimated increased cost per sample for reflex supplemental testing of antibody to hepatitis C virus (anti-HCV) screening-test–positive results with low* average signal-to-cut–off ratios compared with performing only the screening test among populations with different anti-HCV prevalences
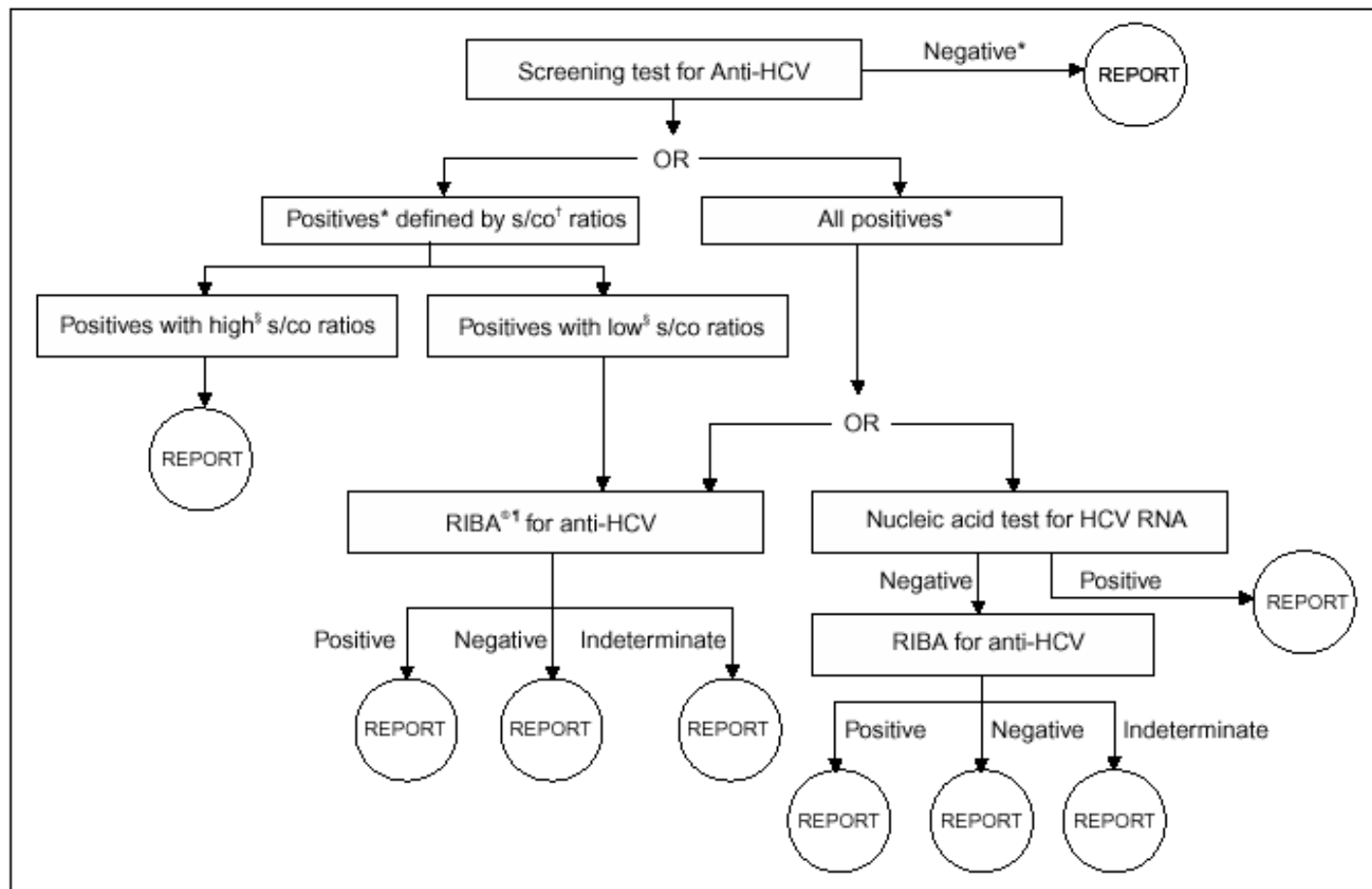

* <3.8 by HCV EIA 2.0 or HCV Version 3.0 ELISA.
† Minimum costs based on reagents only; maximum costs based on testing performed by referral laboratory.
§ Includes only hemodialysis patients.

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Figure 4

FIGURE 4. Laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting

* Interpretation of screening immunoassay test results based on criteria provided by the manufacturer.
† Signal-to-cut–off.
§ Screening-test–positive results are classified as having high s/co ratios if their ratios are at or above a predetermined value that predicts a supplemental-test–positive result ≥95% of the time among all populations tested; screening-test–positive results are classified as having low s/co ratios if their ratios are below this value.
¶ Recombinant immunoblot assay.

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Box

BOX. Interpreting antibody to hepatitis C virus (anti-HCV) test results

Anti-HCV–Positive

An anti-HCV–positive result is defined as 1) anti-HCV screening-test-positive* and recombinant immunoblot assay (RIBA®)- or nucleic acid test (NAT)-positive; or 2) anti-HCV screening-test–positive, NAT-negative, RIBA-positive.

- An anti-HCV–positive result indicates past or current HCV infection.
  — An HCV RNA-positive result indicates current (active) infection, but the significance of a single HCV RNA-negative result is unknown; it does not differentiate intermittent viremia from resolved infection.
- All anti-HCV–positive persons should receive counseling and undergo medical evaluation, including additional testing for the presence of virus and liver disease.
  — Anti-HCV testing usually does not need to be repeated after a positive anti-HCV result has been confirmed.

Anti-HCV–Negative

An anti-HCV–negative result is defined as 1) anti-HCV screening-test–negative*; or 2) anti-HCV screening-test–positive, RIBA-negative; or 3) anti-HCV screening-test–positive, NAT-negative, RIBA-negative.

- An anti-HCV–negative person is considered uninfected.
- No further evaluation or follow-up for HCV is required, unless recent infection is suspected or other evidence exists to indicate HCV infection (e.g., abnormal liver enzyme levels in an immuno-compromised person or a person with no other etiology for their liver disease).

Anti-HCV–Indeterminate

An indeterminate anti-HCV result is defined as anti-HCV screening-test–positive, RIBA-indeterminate.

- An indeterminate anti-HCV result indicates that the HCV antibody status cannot be determined.
  — Can indicate a false-positive anti-HCV screening test result, the most likely interpretation among those at low risk for HCV infection; such persons are HCV RNA-negative.
  — Can occur as a transient finding in a recently infected person who is in the process of seroconversion; such persons usually are HCV RNA-positive.
  — Can be a persistent finding among persons chronically infected with HCV; such persons usually are HCV RNA-positive.
- If NAT is not performed, another sample should be collected for repeat anti-HCV testing (≥1 month later).

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* Interpretation of screening immunoassay test results based on criteria provided by the manufacturer.

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