Hepatitis C (*Hepatitis C virus*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *hepatitis C virus*.

Authorisation: PHLN
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1 PHLN Summary Laboratory Definition

1.1 Condition:

Hepatitis C

1.1.1 Recent hepatitis C

1. Detection of confirmed anti-HCV antibody in a patient shown to be negative within the last 6 months
   OR
2. Detection of HCV RNA in a patient shown to be anti-HCV antibody negative in the last 6 months
   OR
3. Detection of HCV RNA from a child 1 to 6 months of age

1.1.2 Chronic hepatitis C

1. Detection of HCV RNA in a patient shown to be confirmed anti-HCV antibody or HCV RNA positive at least
   6 months previously

1.1.3 Unspecified hepatitis C

1. Detection of confirmed anti-HCV antibody from a patient >18 months of age
   OR
2. Detection of HCV RNA from a patient > 6 months of age
   AND

3. Does not meet the criteria for recent or chronic hepatitis C

1.1.4 Confirmed anti-HCV antibody

A repeatedly reactive anti-HCV antibody enzyme immunoassay verified by either:

1. supplemental enzyme immunoassay based on different antigens and different format
   OR
2. recombinant immunoblot assay

1.1.5 Links to related documents

- CDNA (clinical) case definitions: Hepatitis C (newly acquired) case definition (http://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_hepcnew.htm) and Hepatitis C (unspecified) case definition (http://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_hepcun.htm)
- Fact Sheet: (http://www1.health.gov.au/internet/main/publishing.nsf/Content/hepatitis+C-2)

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2 Introduction

Hepatitis C virus (HCV), a notifiable disease in Australia, infects approximately 170 million individuals worldwide. The virus is a single stranded RNA virus, and member of the family *Flaviviridae.* Six genotypes (1 to 6) and a series of subtypes of HCV have been identified. Genotypes 1 to 3 are distributed worldwide while genotypes 4 and 5 appear mostly in Africa and genotype 6 in Asia (1). HCV is transmitted predominantly by blood exposure, making intravenous drug users the highest risk group in Australia. Organ and blood transfusion transmission is extremely unlikely with the current standard of virological donor screening in Australia. Vertical transmission is uncommon (approximately 3% from carrier mothers) and vaginal intercourse is considered of low risk, compared to anal intercourse.

Approximately 25% of infected individuals spontaneously clear the infection, usually within 6 months, with the remainder developing chronic HCV infection. Chronic HCV infection has been estimated to cause approximately 250 000 to 350 000 deaths per year, essentially related to decompensated cirrhosis and hepatocellular carcinoma. Prevention of these HCV complications can be achieved by antiviral therapy based on a combination of pegylated interferon alfa and ribavirin, that yields a sustained eradication of infection in some cases. Since the introduction of diagnostic testing in 1990, the use of serological and nucleic acid tests has become essential in the management of HCV infection in order to diagnose infection, guide treatment decisions and assess the virological response to antiviral therapy.

3 Tests

3.1 Detection of HCV RNA

3.1.1 HCV qualitative RNA detection

Qualitative detection assays are based on the principle of target amplification using either conventional PCR, real-time PCR or transcription-mediated amplification (TMA). HCV RNA is extracted and reverse transcribed into a single-stranded DNA, which is subsequently cycled to generate a large number of detectable copies. Double-stranded DNA copies of the HCV genome are produced in PCR-based assays, whereas single-stranded RNA copies are generated in TMA. Detection of amplified products is achieved by hybridizing the amplicons with specific probes in conventional PCR or TMA techniques. In real-time PCR, each round of amplification leads to the emission of a fluorescent signal which is proportional to the amount of HCV RNA in the starting sample. Qualitative detection assays detect 50 HCV RNA IU/mL or less, and have equal sensitivity for the detection of the six HCV genotypes. This assay is used for the detection of active HCV infection, acute or chronic, and is used for the monitoring of therapy to decide on rapid, early and sustained virological responses. HCV RNA will become detectable one to two weeks after infection and will be detectable during the serological window period. It may be the only detectable marker of HCV infection in the immunosuppressed that mount a delayed or absent serological response. Transient RNA detection from neonatal blood may occur soon after birth from an infected mother which does not necessarily indicate infection of the neonate. Also, a negative HCV RNA test does not exclude a past infection and some cases of active HCV infection may have occasional negative HCV RNA results, presumably due to transient reduction in HCV RNA levels below the limit of detection of the test.

3.1.2 HCV quantitative RNA detection

HCV RNA can be quantified by means of target amplification techniques (competitive PCR or real-time PCR) or signal amplification techniques (branched DNA assay) which may also be coupled with an automated extraction platform. Originally, the HCV RNA quantitative units used in the various assays did not represent the same amount of HCV RNA in a clinical sample until the World Health Organization established an international standard of HCV RNA quantification called international units, which is currently used in all of the commercial HCV RNA quantitative assays. This allows recommendations and guidelines from clinical trials to be derived and applied in clinical practice with any of the HCV RNA assays. HCV viral load monitoring is useful for assessing virological response to therapy earlier than qualitative HCV RNA tests but the viral load does not predict the natural history of HCV infection. There have been some problems with under-quantifying some HCV genotypes and differences in calibration of the assays relative to the primary WHO HCV RNA standard may lead to slight differences between the results given for the same samples by different assays. This mandates that the monitoring of a patient over time should be performed with the same assay.

3.1.3 HCV genotype detection

The reference method for HCV genotype determination is direct sequencing of the NS5B or E1 regions of the HCV genome, followed by sequence alignment with prototype sequences and phylogenetic analysis. In clinical practice, the HCV genotype can be determined by various commercial kits, using direct sequence analysis of the 5’ noncoding region or reverse hybridization

analysis using genotype-specific probes located in the 5' noncoding region. Mistyping is rare with these techniques, but mis-subtyping may occur in 10% to 25% of cases when the 5' noncoding region is used. Mixed infections are not usually detectable by genotyping methods as one genotype tends to be dominant in an individual. Genotyping may also be performed serologically. The HCV genotype is the strongest predictor of treatment success currently with HCV genotype 2 and 3 responding in 80% and 75% of cases, respectively, compared to approximately 40% sustained virological response in non-genotype 2,3 cases.

3.2 Detection of anti-HCV antibody

3.2.1 EIA

The laboratory diagnosis of HCV infection is usually made by the detection of circulating antibodies. Qualitative determination of anti-HCV in blood is measured using solid-phase EIA employing recombinant HCV antigens. Recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtiter plates, microbeads, or specific holders adapted to closed automated machinery. The optical density (OD) ratio of the reaction (sample OD/internal control OD) is proportional to the amount of antibodies in the blood sample. With the discovery of HCV and the sequencing of its genome in 1989, the first generation anti-HCV EIAs was produced using recombinant NS4 antigen. However, they showed limited sensitivity and specificity. Second generation tests, which included HCV antigens from the core and non-structural regions NS3 and NS4 resulted in a marked improvement in sensitivity and specificity. The current third generation tests include antigens from the NS5 region of the genome. These tests have improved sensitivity, though this is more likely due to the improvements to the core and NS3 antigens rather than the inclusion of the NS5 antigen. The specificity of third-generation EIAs for anti-HCV is >99%. Their sensitivity is more difficult to determine, given the problems of using the RIBA as a gold standard method, but it is excellent (>99%) in HCV-infected immunocompetent patients. HCV EIAs can be fully automated, have the convenience of random access, and are well adapted to large volume testing. Anti-HCV antibody is usually detected a median of seven to eight weeks after infection but delayed seroconversion, up to 12 months, is described. Anti-HCV antibody may not be produced to detectable levels in immunosuppressed individuals. Maternal transfer of anti-HCV may be detectable in the child's blood until up to 18 months of age.

3.2.2 RIBA

The immunoblot assay is an in vitro qualitative enzyme immunoassay for the detection of antibody to HCV in human blood. Detection of anti-HCV antibody is based upon traditional blotting techniques, in which specific antigenic HCV polyproteins are immobilized onto a membrane support. With each individual recombinant or synthetic antigen applied as separate line to the solid phase the antibody reaction to the different antigens can be distinguished. Visualization of anti-HCV antibody reactivity in specimens to the individual HCV-encoded proteins is accomplished using standard EIA techniques. The application of established criteria to interpret the patterns of reactivity observed permits greater specificity. This assay is considered the gold standard for the detection of anti-HCV antibody. However, not all those infected with HCV produce a positive RIBA and many equivocal EIA screening results also produce indeterminate RIBA results, requiring a recommendation for repeat testing after at least one month.

3.2.3 Confirmatory serology testing

Serological tests for detecting antibodies to HCV are generally classified as screening tests or confirmatory tests. The most widely used anti-HCV antibody screening tests are EIAs as they are the most appropriate for screening large numbers of specimens on a daily basis (1). Screening tests provide the presumptive identification of antibody-reactive specimens, whilst supplemental tests are used as confirmatory assays to ensure that specimens found reactive with a particular screening test contain antibodies specific to HCV. A second EIA or an immunoblot assay may be used as the confirmatory test. The second EIA strategy was advocated by the Public Health Laboratory Service Virus Reference laboratory, UK in 1992 (2), soon after introduction of the commercial EIA tests, and is currently recommended by the UK Health Protection Agency (3) and the Australian National Hepatitis C Testing Policy (4). However, if choosing a second EIA confirmatory strategy, the screening and supplemental tests must be selected carefully to ensure that common false reactivity between these assays does not occur. Also, it has become clear that many low-level reactive EIA results (signal to cut-off [S/CO] ratios 1.0-3.0) although considered positive according to the manufacturer's instructions, do not subsequently confirm on supplemental testing with a more specific test. This has prompted some laboratories to report high S/CO EIA results as reactive but low S/CO results as equivocal but this will have the regulatory consequences of converting the commercial assay into an in-house IVD. The Centres for Disease Control and Prevention has recently introduced another reporting option that uses S/CO ratios of the screening test positive result (5-8). Analysis of individual assays has identified specific S/CO ratios for each commercial assay that is >95% predictive of a true antibody-positive result, regardless of the anti-HCV prevalence or characteristics of the population being tested. This option is currently absent from the Australian National Hepatitis C Testing Policy. HCV RNA detection may be used to confirm active infection in screening EIA reactive individuals but, if negative, supplemental serology testing is still required.

3.2.3 Serological detection of genotype

The HCV genotype can be determined by targeting antibodies directed to genotype-specific HCV epitopes with a competitive EIA. The currently available assay identifies the type (1 to 6), but does not discriminate among the subtypes, and provides interpretable results in approximately 90% of chronically infected patients. Mixed serological reactivities are sometimes found that could be related to either mixed infection, cross-reactivity or recovery from one genotype infection and persistence of viremia with another genotype.

3.3 Detection of HCV antigen

3.3.1 EIA

The HCV core antigen (HCV Ag) is present in the blood of infected individuals, probably in both complete virions and RNA-free core protein structures. Various HCV Ag assays indicate that the kinetics of HCV Ag are similar to those of HCV RNA in all phases of infection, and that the concentrations of HCV Ag and HCV RNA roughly correlate. It has been estimated that 1 pg of HCV core Ag is equivalent to approximately 40,000 IU using the currently available commercial test (9). HCV Ag testing can reduce the serological window and may also be used to monitor antiviral therapy in chronically infected patients. All HCV genotypes and subtypes can be detected with a lower detection limit of approximately 0.06 pg/mL, inferior to the HCV RNA assay of approximately 10

IU/mL. The HCV Ag test is appropriate for screening for acute HCV infections in blood or organ donors, and high risk groups and could also be used to monitor treatment, except for the end point, where a very sensitive technique is needed to show a sustained response. This test is not routinely available in Australia at present.

3.4 Quality Assurance

Suitable and Unsuitable specimens
HCV EIA testing can be performed on individual patient serum and plasma and HCV RNA testing can be performed on plasma. Samples for EIA may be stored on or off the clot or RBCs for up to 7 days at 2-8^{0}C but samples for HCV RNA need to be separated from the clot within hours. The tests are not validated for cadaveric samples or for pooled specimens.

Internal QC
As the HCV EIA and HCV RNA tests are available commercially the manufacturer's instructions should be followed. Positive and negative controls are run every 24 hr on automated EIA machines and with each batch for plate EIAs and HCV RNA tests

External QC Programmes
NRL – qualitative HCV RNA and genotyping
RCPA QAP – HCV antibody, HCV RNA qualitative and quantitative

4 agreed Typing & Subtyping Methods

- The international nomenclature for the organism is *Flaviviridae, hepatitis C virus*.

4.1 Laboratory Nomenclature for National Database Dictionary

Finding - SNOMED ConceptID

Hepatitis C virus - 62944002
Antibody to hepatitis C - 72165005
Hepatitis C antibody test - 313612007
Hepatitis C antibody test positive - 314706002
Hepatitis C antibody test negative - 314707006
Hepatitis C enzyme immunoassay test positive - 406104003
Hepatitis C enzyme immunoassay test negative - 406105002
Hepatitis C antibody, confirmatory test - 104375008
Hepatitis C recombinant immunoblot assay - 398463007
Hepatitis C RNA - 121204002
Hepatitis C RNA assay - 122366001
Hepatitis C nucleic acid assay - 398513000
PCR test for hepatitis C virus - 399117003
Hepatitis C genotype determination - 397662006

5 References

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