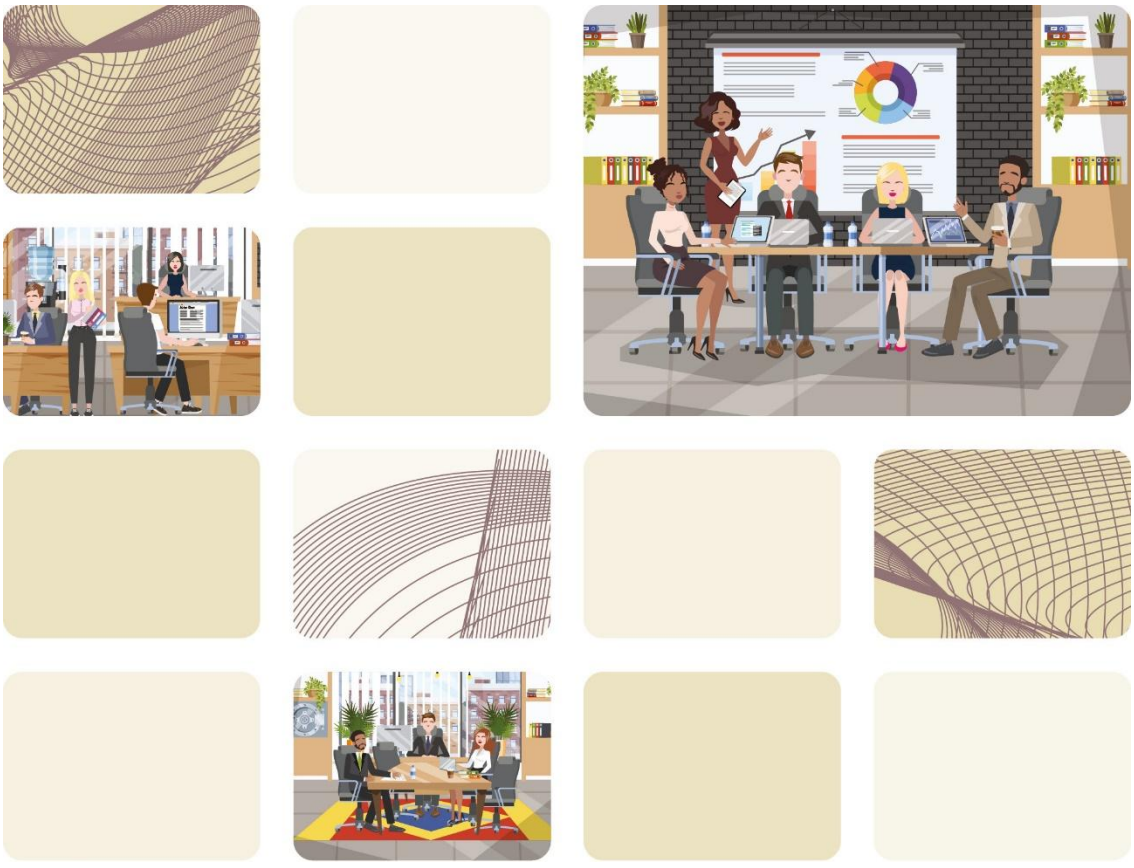
UK Health Security Agency

# UK Standards for Microbiology Investigations

## Review of users' comments received by

## Working group for microbiology standards in clinical virology/serology

V 5 Laboratory diagnosis of hepatitis C virus



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Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING



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# 1st Consultation: 04/04/2025 – 25/04/2025

Version of document consulted on: V 5 dj+

# Comments on Section 3 - Scope of Document

### Manchester Medical Microbiology Partnership

Comment number: 1

Date received: 07/04/25

1. "CE-marked" not "CE marked". Should UKCA be mentioned?
2. Should be "hepatitis C virus" not capitalised.
3. Quantitative "log10" - the 10 should be subscript.

Recommended action

1. ACCEPT. Text amended to clarify that CE- or UKCA-marked specimen containers should be used.
2. ACCEPT. This has been corrected.
3. ACCEPT. This has been corrected.

### Severn Infection Sciences, North Bristol NHS Trust

Comment number: 2

Date received: 14/04/25

1. Page 5: CE marked assays should be validated and verified prior to use.

   Did you mean do both. CE marked assay may only need verification.
2. Page 6, Reporting stage for serology: The recommendations for reporting results to end users (which include the person being tested) needs further thought. For example, reactive is a technical term with no clear meaning to many service end users. Positive and negative are much more widely understood and would be better. Similarly, detected and not detected are not very distinct when viewing reports.

Recommended action

1. ACCEPT. This has been changed.
2. ACCEPT. Serology reporting terminology has been revised to use "positive" and "negative" results. A caveat has also been added that reporting terminology should be determined through local laboratory clinical governance arrangements.

### Chair, National Strategy Group for Viral Hepatitis

Comment number: 3

Date received: 07/04/25

1. Top of page 6 - "For blood-borne virus (BBV) testing following dialysis away from base (DAFB), refer to DHSC guidelines (6)":

   The DHSC guidelines are from 2010. I suggest referring to the UK Kidney Association guidelines - https://www.ukkidney.org/sites/default/files/FINAL-BBV-Guideline-June-2019.pdf - these were published in 2019 and a revised guideline will be published in 2025.

Recommended action

1. ACCEPT. The DHSC guideline was replaced by the UK Kidney Association guideline

### West of Scotland Specialist Virology

Comment number: 4

Date received: 24/04/25

1. Page 7: scenario 2 result should read: Detected at low level.

Recommended action

1. PARTIAL ACCEPT. The quantitative result has been amended to indicate "below the lower limit of quantitation of the assay". No change has been made to the qualitative result, as the low-level nature of the result is captured by the quantitative reporting.

## Comments on Section 4 - Introduction

### Chair, National Strategy Group for Viral Hepatitis

Comment number: 5

Date received: 07/04/25

1. Section 4.3, page 9, end of para 2.  "and now fourth generation (also with HCV capsid antigen) (35,40)": HCV core protein (already included in 2nd generation assays) is the same as HCV capsid antigen - so the explanation for how the 4th generation assays have improved things is false.
2. Section 4.4, Fig 1(b). Why is the HCV RNA line interrupted? Doesn't make any sense unless you are introducing treatment to the figure (which I don't think is correct). If fig 1(b) is intended to show the pattern of results in someone with chronic infection, then HCV RNA will be positive throughout.

Recommended action

1. ACCEPT. It has been clarified that 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ generation assays only detect antibodies to the viral antigens while 4$^{th}$ generation assays detect both the antibody to the antigen and the core antigen itself.
2. NONE. Figure 1(b) shows persistence of chronic HCV RNA for over 6 months (26 weeks). The line indicated is for ALT levels.

### Manchester Medical Microbiology Partnership

Comment number: 6

Date received: 07/04/25

1. "Flaviviridae" should not be italicised.
2. Should be "single-stranded, positive-sense".
3. There are eight "known" subtypes of HCV.
4. 4.2 - no need to capitalise "chronic".
5. "Molecular methods are recommended over HCV core antigen testing for confirmation due to the higher rates of false negatives and lower sensitivity compared to HCV RNA NAAT (44-46). Therefore, hepatitis C core antigen testing will not be discussed further within this document. " -> "Molecular methods are recommended over HCV core antigen testing for confirmation as they have lower rates of false negatives and higher sensitivity (44-46). Therefore, we do not recommend hepatitis C core antigen testing."
6. 4.4 - IgG persists beyond the course of natural infection - often lifelong.

Recommended action

1. ACCEPT. *Flaviviridae* formatting has been corrected.
2. ACCEPT. Terminology has been amended to "single-stranded, positive-sense".
3. ACCEPT. This has been corrected.
4. ACCEPT. This has been corrected.
5. ACCEPT. The sentence was rephrased as appropriate.
6. ACCEPT. Sentence amended.

**Severn Infection Sciences, North Bristol NHS Trust**

Comment number: 7

Date received: 14/04/25

1. Pages 9 and 10 - In one paragraph the time to detectable IgG is given as 8-12 weeks and later 5-12 weeks- please pick one. Unless you meant that was the difference between production of antibody and ability to detect it.
2. Page 11 - “In addition, when samples with low viral load are diluted to bring them within the assay’s quantifiable range, there is a risk that viral concentration could either fall below the detection threshold or be at the lower limit of detection. For example, a negative HCV RNA after a 2-fold dilution may suggest that the original viral load was too low to be detected after dilution, making current infection extremely unlikely. Reporting the dilution factor helps clinicians make a more informed decision, particularly in low-level positive results or low viral load”.

   I don't know what you are trying to say here- it implies diluting out a low-level detection to undetectable is a strategy useful to hide possible false positive results. Why would you dilute a sample to bring it into quantifiable range unless it was already tested and shown to be above upper limit of quantification?

Recommended action

1. ACCEPT. Section 5.2 Immunoassays has been reviewed and the window period of 5 – 12 weeks has been removed.

2. ACCEPT. This section has been revised and updated as appropriate.

### Microbiology, University Hospitals Sussex NHS Foundation Trust/LabMedUK

Comment number: 8

Date received: 23/04/25

1. One line in section 4.4 could be worded more accurately: "Immunoglobulin G (IgG) antibodies emerge after IgM antibodies and persist throughout the course of HCV infection." The IgG antibodies persist for longer than the course of infection as they are still present after infection has resolved/ RNA has been cleared.

Recommended action

1. ACCEPT. The sentence “throughout the course of HCV infection” was replaced by “beyond the course of natural infection – often lifelong”.

### West of Scotland Specialist Virology

Comment number: 9

Date received: 24/04/25

1. Page 9 (final paragraph of 4.3): lower sensitivity/higher rate of false negatives is the same thing. Maybe you don’t need to use both terms?

Recommended action

1. ACCEPT. This has been changed.

## Comments on Section 5 - Technical Information and Limitations

### Chair, National Strategy Group for Viral Hepatitis

Comment number: 10

Date received: 07/04/25

1. Section 5.2 " In addition, antibody tests cannot distinguish between historical HCV infection with sustained viral clearance and reinfection with a different HCV genotype." This statement is incomplete. Antibody tests cannot distinguish between historical infection with sustained viral clearance, OR CHRONIC HCV infection or reinfection. This is important, as for patients who are anti-HCV positive, the clinician will want to know whether or not the patient has chronic/current infection - whether this is "primary" or a reinfection.
2. Section 5.3, 1st sentence. Intermittent viraemia is mentioned here (and elsewhere in the document). This is not a concept I am familiar with, other than perhaps in the first few weeks following infection and must surely be an extremely rare event compared to persistent viraemia. By mentioning it without qualification or primary reference, you are effectively saying that any patient who is Ab +ve RNA -ve may nevertheless still have chronic infection and therefore needs repeat testing ..... forever ... in case they have intermittent viraemia. I would be extremely cautious about raising this possibility, as current dogma has it that an SVR equates to non-infection and patients are discharged

from clinic. If you have evidence of intermittent infection, the specific circumstances (eg shortly after exposure) must be stated and referenced.

Recommended action

1. ACCEPT. Chronic HCV infection has been added to the statement.
2. ACCEPT. Intermittent viraemia has been removed from the document.

### Chester and Wirral Microbiology Service

Comment number: 11

Date received: 07/04/25

1. 5.1 Standard sample types ie serum, plasma, EDTA-blood should be included here before DBS etc. (as in 7.1.1)

Recommended action

1. PARTIAL ACCEPT. The standard sample types were included. Whole blood was added instead of EDTA-blood

### Manchester Medical Microbiology Partnership

Comment number: 12

Date received: 07/04/25

1. 5.2 - individuals with recent exposure or acute hepatitis should be tested with HCV RNA PCR in addition to serological testing.
2. 5.3 - consider recommending a repeat sample with a low-level viraemia

Recommended action

1. ACCEPT. This has been added.
2. ACCEPT. This has been added.

### West of Scotland Specialist Virology

Comment number: 13

Date received: 24/04/25

1. Section 5.3, Page 10: low level PCR result can also represent false positive.
2. When would you dilute a sample? – this practice should be discouraged in clinical practice unless for a neonate.
3. The section on internal controls and inhibition seems generic. Most assays are commercial assays and therefore won't be multiplexed and won't have cellular internal controls. Do you really need this section? If so, make it more specific to the HCV assays in use today.
4. In relation to contamination - maybe say that labs should have "app controls and procedures in place to reduce/detect contamination".

Recommended action

1. ACCEPT. Additional wording has been included regarding cautious interpretation of low-level HCV RNA results.

2. NONE. The addition of a section on dilution has been discussed and agreed by the Virology Working Group. This section has since been reviewed and updated to consider relevant comments.
3. ACCEPT. This section has been removed. *UK SMI Q 4: Good practices when performing molecular amplification assays* is referenced for general information.
4. NONE. This section has been removed. *UK SMI Q 4: Good practices when performing molecular amplification assays* is referenced for general information.

### UKHSA regional laboratory, Bristol

Comment number: 14

Date received: 24/04/25

1. Page 10 “HCV RNA is a marker of active viral replication, but low-level results should be interpreted with caution as they may have various causes including intermittent viraemia, assay variability and antiviral treatment response. Laboratories should provide detailed quantification for more informed clinical interpretation. When low-level positivity is observed, careful evaluation is required with further testing or clinical correlation as appropriate. In addition, when samples with low viral load are diluted to bring them within the assay’s quantifiable range, there is a risk that viral concentration could either fall below the detection threshold or be at the lower limit of detection. For example, a negative HCV RNA after a 2-fold dilution may suggest that the original viral load was too low to be detected after dilution, making current infection extremely unlikely. Reporting the dilution factor helps clinicians make a more informed decision, particularly with low viral loads.”

   Not sure if the bit saying “making current infection extremely unlikely” makes sense in that sentence?

Recommended action

1. ACCEPT. This section has been removed.

## Comments on Section 6 - Safety Considerations

### Manchester Medical Microbiology Partnership

Comment number: 15

Date received: 07/04/25

1. 6.1 - CE-marked, leak-proof
2. 6.2 - mention the ACDP hazard groups? Group 3 but derogated

Recommended action

1. ACCEPT. This has been changed.
2. ACCEPT. This has been added.

# Comments on Section 7 - Methodology

## Chair, National Strategy Group for Viral Hepatitis

Comment number: 16

Date received: 07/04/25

Section 7.1

1. 1st paragraph - "Additionally, in settings where the general population has an HCV antibody seroprevalence of ≥ 2% or ≥ 5% (21)." This is not a sentence despite beginning with a capital letter and ending with a full-stop. Is something missing?
2. 3rd paragraph - You mention groups who are routinely screened for HCV - you should also mention HCWs who are about to start training in a speciality where they will be required to perform exposure-prone procedures.
3. 4th paragraph - "Routine testing of pregnant women for HCV infection is currently not recommended." It would be more accurate to say that routine testing of pregnant women for HCV infection is not currently approved by the National Screening Committee. It is recommended by the National Strategy Group for Viral Hepatitis, and by NHS England, and we have been trying (unsuccessfully) to get the NSC to approve it. A number of centres do screen antenates routinely and it would be a major step backwards if centres were discouraged from doing so because of your statement above.
4. Sections 7.1.1 and 7.1.2 contain whole sentences repeated, less than half a page apart. Is this really necessary?
5. Section 7.1.2, page 13, 3rd para. "If a sub-optimal volume is provided (perhaps due to dilution)" I don't think you mean this. The sub-optimal volume will be due to an inadequate amount of blood being sent to the laboratory, and has nothing to do with dilution. Suggest rephrasing to "If a sub-optimal volume is provided, which requires dilution within the laboratory prior to testing, .....

Section 7.1.4

6. "to avoid missing cases of HCV infections" should be "to avoid missing cases of HCV infection."
7. Immunocompetent people. "The initial screening assay should be a 3rd or 4th generation antibody assay which if positive should be followed by a confirmatory HCV RNA NAAT." This is inaccurate and misleading. The NAAT assay does NOT confirm the antibody result and this is NOT its purpose. The NAAT assay is to determine whether or not the patient is currently infected with HCV or not, given that the Ab +ve result indicates that the patient has been infected at some time. Suggest rephrasing to ".... which if positive should be followed by an HCV RNA NAAT in order to determine whether or not the patient is currently infected with HCV or not."
8. Pregnant women and infected babies. Page 14, 3rd para. "consideration should be given to investigate previous pregnancies". This implies digging out lab samples from previous pregnancies - why not simply say "consideration should be given to test children from previous pregnancies"? That would be the plain English version of what it is you would like clinicians to do.

### Recommended action

1. ACCEPT. This has been corrected.
2. ACCEPT. This has been added.
3. ACCEPT. This section has been amended as appropriate.
4. ACCEPT. This has been corrected.
5. ACCEPT. This has been changed.
6. ACCEPT. This has been corrected.
7. ACCEPT. This has been changed.
8. ACCEPT. This has been changed.

## Chester and Wirral Microbiology Service

### Comment number: 17

Date received: 07/04/25

Section 7.1

1. Is the reference 21 given in this section correct for this content?
2. Sentence "Additionally.....(21). is incomplete. Reference 21 does mention 2%-5% but for special groups, not for the general population.
3. 7.1.4 Pregnant women, second sentence beginning "If the initial results....are negative, this should be repeated later..." suggest should be "`If the initial results ...are negative, the tests should be repeated later, in the third trimester".

### Recommended action

1. ACCEPT. The section has been reviewed and amended as appropriate.
2. ACCEPT. The wording has been revised and corrected.
3. ACCEPT. This has been changed.

## Manchester UKHSA

### Comment number: 18

Date received: 07/04/25

1. In Table 1 under the heading "Following a negative HCV RNA result at 2-3mths of age, testing for HCV antibodies using HCV antibody test at 12-18 months" line 4 scenario is if HCV antibody is detected at 12-18 months the Interpretive comment is "No evidence of active infection". This interpretation can't be made from the antibody result alone and a sample for HCV PCR should be requested to determine if there is active HCV infection.

### Recommended action

1. NONE. This table has been removed.

## Manchester Medical Microbiology Partnership

Comment number: 19

Date received: 07/04/25

1. 7.2 - We comment to recommend referral to hepatitis services if RNA is detected as in DAAs are usually initiated without a repeat sample.
2. 7.4 - We comment equivocal antibody with negative RNA to explain this may represent false reactivity or waning of antibody.
3. We are usually asked to use the genotyping assay on the existing HCV RNA positive sample - this might be suggested rather than asking for a new sample.
4. Table 2 - 4. This does not look right.
5. Mention electronic reporting methods?

Recommended action

1. None. This has already been included.
2. ACCEPT. This has been added.
3. ACCEPT. Reflex genotyping is recommended in the document.
4. NONE. This table has been removed.
5. NONE. Laboratories to report according to local procedures.

### Italian translator

Comment number: 20

Date received: 12/04/25

1. Could it be useful to add some comments about the HCV Antigene test? It is still in the market, but it is not useful for the management of the diagnosis.
2. Confirmatory test could be still useful when there are patients with antibodies test positive and negative molecular test

Recommended action

1. NONE. Hepatitis C core antigen testing is not discussed in the document.
2. NONE. This is covered in the ‘Notes’ section of the table.

### Severn Infection Sciences, North Bristol NHS Trust

Comment number: 21

Date received: 14/04/25

1. P12- pre-testing considerations - Which groups should be offered testing is out of place here, and unnecessary- better in introduction. Would be of more use to discuss predictive values of tests for given populations.
2. P13- definition of immunosuppression better taken from immunisation handbook (green book) as that is likely to be more regularly reviewed and be regarded as a more relevant text (unless there are differences in definitions the writing group specifically wish to use).

### Recommended action

1. PARTIAL ACCEPT. The relevant section has been moved to the introduction for consistency with other UK SMI Virology documents. Information on predictive values is locally determined based on the data available.
2. ACCEPT. Reference to the Green book has been included.

## West of Scotland Specialist Virology

### Comment number: 22

Date received: 24/04/25

1. Page 13 - Approach to dilution is inconsistent. On this page it says low volume samples should be reported and repeat requested. Whereas earlier there is a section on diluting and what to do. Needs a more consistent approach.
2. Page 17 – remove the comment about antigen testing – it has been stated above that antigen testing less sensitive and will not be in this document.
3. Page 19 – remove the equivocal from box 4 – equivocal should be in own box when RNA is not detected – wrong to state people are HCV antibody positive indicating past infection with an equivocal result. While an equivocal antibody in the absence of RNA may indicate waning past infection it can equally indicate a false positive result, therefore we should not label those with equivocal antibodies as past infection. It would be clearer at this point to suppress equivocal antibody result and report on the negative RNA result.

### Recommended action

1. ACCEPT. This has been reviewed and changed as appropriate.
2. ACCEPT. This has been removed.
3. ACCEPT. Separate rows have been created for ‘Equivocal’ results as appropriate.

## UKHSA regional laboratory, Bristol

### Comment number: 23

Date received: 24/04/25

1. Page 14 - The references for the section saying that RNA negative mothers are highly unlikely to transmit HCV ultimately refer to an old paper in the Lancet that I couldn’t get hold of – the paper quotes a small percentage of transmission in non-viraemic patients. I’d be interested to know whether these were tested using an assay with a relatively high lower limit of detection back in 1995 or whether they’d ruled out reinfection – if not it just calls into question the management of HCV antibody positive, PCR negative patients in general regarding transmission as, and implies really that all patients (not just pregnant) should have a repeat test to confirm RNA negative status. Which we’ve just removed from the UK SMI – I was very keen for this as requesting a follow up sample on all patients causes a lot of issues particularly for low-risk groups, given the high prevalence of antibody positivity, and the fact that the EASL guidelines suggest it’s only necessary if recent infection is suspected.

2. Reporting table - Should it not be positive rather than reactive?
3. Table 2 - don’t think the notes seem relevant to infants born to infected mothers.

Recommended action

1. ACCEPT. This section has been reviewed and updated as appropriate.
2. ACCEPT. Terminology has been revised to use positive and negative results.
3. NONE. Table 2 has been removed from the document.

### Chair, National Strategy Group for Viral Hepatitis

Comment number: 24

Date received: 07/04/25

1. Section 7.4, page 22 interpretive comments. "No evidence of active HCV infection. Please send EDTA plasma for a repeat HCV NAAT to confirm evidence of infection." These 2 statements are diametrically opposite to each other. Surely the second statement should be "please send EDTA plasma to confirm absence of infection"?
2. Final comment - it should be mandatory (or at least strongly recommended) for laboratories to reflexly perform genotyping on any sample found to be HCV RNA positive. Genotyping is essential to ensure the patient receives the correct therapy. Putting that responsibility back on to the clinician, to send a repeat sample or to make a specific request for genotyping, is detrimental to patient care and runs the significant risk that treatment will be delayed and the patient will be lost to follow up. The lab should play an active role in minimising the chances of this happening by providing the clinician with all necessary data on a single sample. I am extremely disappointed that all that this document says is "Consider genotyping". To me, this is a complete abrogation of the laboratory's responsibility towards patient care, and suggests labs exist in some sort of ivory tower remote from clinical management.

Recommended action

1. NONE. The following table has been removed from the document - *Table 2: Investigation of hepatitis C virus infection in babies of women confirmed to have HCV infection during pregnancy using both NAAT and HCV antibody test*.
2. ACCEPT. This has been added.

### Manchester Medical Microbiology Partnership

Comment number: 25

Date received: 07/04/25

1. I think where possible genotyping should be performed on the initial RNA positive sample and reported out as quickly as possible

Recommended action

1. ACCEPT. Reflex genotyping is recommended in the document.

### Gian Sagar Medical College & Hospital

Comment number: 26

Date received: 12/04/25

1. In Table 1; point 4: HCV antibody (Reactive) & HCV RNA (Not detected)

   In the note can we append the possibility that negative HCV RNA after 2-fold dilution may suggest that the original viral load was too low to be detected after dilution. As its been discussed in section 5.3 (Molecular method)

Recommended action

1. NONE. The dilution guidance has been revised, and this interpretation is no longer included.

## Comments on the Algorithms

### Chester and Wirral Microbiology Service

Comment number: 27

Date received: 07/04/25

1. 7.2 HCV antibody test lozenge - add serum etc to sample types

Recommended action

1. ACCEPT. This has been added.

### Severn Infection Sciences, North Bristol NHS Trust

Comment number: 28

Date received: 14/04/25

1. Algorithm 7.2 - Qualitative positive follow up sample if not detected reports as '<lower limit'. This needs further narrative as interpretation depends on relative sensitivity of the tests, and whether there is sample error if different samples, and the need for another sample etc.
2. Algorithms general - If a quantitative RNA test is done the report is never 'not detected' but only ever refers to limit of detection. I don't think this is helpful to the end user. those who understand it will interpret it as negative, those who do not will need a further comment. The use of limits of detection on reports is helpful but is also somewhat misleading in the overall context of diagnostics- all tests have limits of detection, including antibody tests, but it is correctly not common practice to report 'below limit of detection' for qualitative assays, but technically that is the result.

Recommended action

1. ACCEPT. Additional clarification has been included in the reporting guidance.
2. ACCEPT. This has been changed in the algorithm.

### Department of microbiology, Royal Oldham Hospital

Comment number: 29

Date received: 09/04/25

1. The terminology of HCV Antibody Reactive / NOT Reactive should be reported as POSITIVE / NEGATIVE but it's a wider discussion for the expert group in the UK SMI to standardise serological terminologies.

Recommended action

1. ACCEPT. This has been discussed with the Virology Working Group and the terminology has been changed for HCV serology to ‘Positive/Negative’.

### Consultant in paediatric infectious diseases, Royal Hospital for Children, Glasgow

Comment number: 30

Date received: 10/04/25

1. Algorithm for testing for mother-to-child transmission for Hep C. I see the revised algorithm now advocates for a single RNA PCR at 2-3 months followed by an antibody test at 12-18months. Given that the transmission rate is approx 4% this commits 96% of infants to 2 blood tests (associated inconvenience for often vulnerable families and expense for NHS) where the first PCR test is arguably meaningless as the second antibody test is required to rule out transmission. Of the 4% who are positive at first RNA test none would require or be offered treatment before 18 months of age.

   Early RNA testing may well be driven by the US experience of poor follow-up for testing (quoted as 30%) but in Glasgow we have 89% follow-up over 1 year of age for antibody testing

   Doing arguably unnecessary blood tests on children is ethically questionable and I would suggest caveating your recommendation to say that a single antibody test over 12 months of age is acceptable.

   Of note CDC (2023) moved to recommending a single PCR at 2-6months and as this would mean just one blood test then this would be a welcome recommendation if viewed as robust

Recommended action

1. NONE. This algorithm has been removed. A statement has been added for users to refer to local or national guidelines for paediatric HCV testing and management.

### Microbiology, University Hospitals Sussex NHS Foundation Trust/LabMedUK

Comment number: 31

Date received: 23/04/25

1. Under the report comments for NAAT testing it says report '<lower limit' for both detected and undetected. Would '<lower limit detected' and '<lower limit undetected' be clearer?

Recommended action

1. ACCEPT. This has been corrected.

### West of Scotland Specialist Virology

Comment number: 32

Date received: 24/04/25

1. Page 15/16: A negative result would not go out as <lower limit of detection – should be reported as Not detected
2. The dotted line from the DBS/capillary blood detected to the Quantitative box does not make sense. It looks like advocating DBS quantitative testing.

Recommended action

1. ACCEPT. This has been changed.
2. ACCEPT. The algorithm has been revised to clarify the specimens suitable for quantitative testing.

### Department of microbiology, Royal Oldham Hospital

Comment number: 33

Date received: 09/04/25

1. The flow chart and the interpretation of results in the new UK SMI document under consultation (Laboratory diagnosis of hepatitis C virus) refers to an 'EQUIVOCAL' result but does not define how an 'EQUIVOCAL' result is inferred. Some HCV antibody kits do not have an 'EQUIVOCAL' range but in routine serology laboratory practice, many low level HCV antibody results with index values close to the cut-off is noticed and is reported as 'POSITIVE'. Some companies suggest repeating all positives as a triplicate and use the best of 3 option to report as POSITIVE or NEGATIVE. In my opinion, it is safer to have a different methodology /alternative hepatitis C antibody assay to confirm the HCV antibody positive result. It would be similar to the principle used to confirm HBsAg positivity using an alternative assay (UK SMI Investigation of hepatitis B infection - Virology | V 4 | Issue no: 6.1 | Issue date: 20.07.21 | Page: 12 of 22). A POSITIVE HCV antibody result on the alternative assay will confirm the HCV antibody result irrespective of the index vale of the result. But, if the alternative assay gives a NEGATIVE result, a repeat test on 1st assay could be done and it will determine if the final result is NEGATIVE or 'EQUIVOCAL'.

Recommended action

1. PARTIAL ACCEPT. It has been noted in the document that some assays may not have an equivocal range, which may be established using local data. In addition, second-line antibodies are not recommended but it has been acknowledged in the document that laboratory protocols may differ based on manufacturer's instructions and resource availability.

### Chair, National Strategy Group for Viral Hepatitis

Comment number: 34

Date received: 07/04/25

1. Section 7.2 Flow diagram. The comment underneath Qualitative PCR detected "Evidence of active infection with HCV" Please send repeat sample to confirm." Please please please could you recommend adding some sort of comment to indicate that the patient should be appropriately referred? In Nottingham we say "Please refer to the hepatitis clinic for further assessment and management". This arose because of our study (a long time ago but still relevant) which indicated that an alarming percentage of HCV RNA positive results were not acted upon, for a variety of reasons (Irving WL et al. Clinical pathways for patients with newly diagnosed hepatitis C – what actually happens. Journal of Viral Hepatitis 2006; 13: 264-271). In the same flow diagram, the comments under Quantitative PCR - detected - are "Report: <lower limit, Report: actual value and Report: >upper limit" These don't even mention that the patient is currently infected with HCV, never mind that they need appropriate referral. There is absolutely no point in diagnosing HCV infection if nothing is going to be done. Again, please, please, please add "Indicates current infection with HCV. Please refer to a specialist clinic for further assessment and management" to all of these reports.

Recommended action

1. NONE. This is already included in the document.

## General comments

### Department of microbiology, Royal Oldham Hospital

Comment number: 35

Date received: 09/04/25

1. The introduction of NHS ED blood borne virus opt out testing programme in UK has augmented the issue of low level HCV antibody POSITIVEs in a big way and hence a reasonable solution to confirm the HCV antibody POSITIVE results (especially the low level positives) is crucial.

Recommended action

1. ACCEPT. Guidance on the interpretation of low-level HCV antibody-positive results has been included.

### University Hospitals Plymouth NHS Trust

Comment number: 36

Date received: 15/04/25

1. It would be helpful to have some guidance/comments for likely false Positives results and the role of RIBA testing

Recommended action

1. NONE. RIBA testing discontinued and replaced by HCV RNA PCR or secondary antibody testing as appropriate.

### Microbiology, University Hospitals Sussex NHS Foundation Trust/LabMedUK

Comment number: 37

Date received: 23/04/25

1. Generally, a very clear, easy to understand comprehensive document on HCV testing.

Recommended action

1. NONE. No action required.

### West of Scotland Specialist Virology

Comment number: 38

Date received: 24/04/25

1. I'm wondering whether this SMI should include an algorithm on maternal diagnosis. There are different recommendations in numerous guidelines. Is this really in the scope of this document? How does this differ to other guidelines?

Recommended action

1. ACCEPT. The algorithm on maternal diagnosis has been removed and referral to local guidelines is recommended.

## Respondents indicating they were happy with the contents of the document

| **Overall number of comments: 5** | | | |
|---|---|---|---|
| **Date received** | 09/04/2025 | **Lab name** | Virology Laboratory, Scarborough, Hull, and York Pathology Service |
| **Date received** | 22/04/2025 | **Professional body** | Keith Shuttleworth and Associates Limited |
| **Date received** | 24/04/2025 | **Professional body** | RCGP |
| **Date received** | 24/04/2025 | **Professional body** | Institute of Biomedical Science |
| **Date received** | 24/04/2025 | **Lab name** | HCA Laboratories |

## 2nd Consultation: 14/05/2026 – 28/05/2026

Version of document consulted on: V 5 dq+

## Comments on Section 3 - Scope of Document

### Sheffield Teaching Hospitals

Comment number: 1

Date received: 15/05/2026

1. Perhaps refer to UK SMI 'Vertical and perinatal transmission of hepatitis C'?

Recommended Action

1. NONE. UK SMI V 8 has been merged with this document and will be withdrawn once this document is published.

### Newcastle upon Tyne Hospitals NHS Foundation Trust (Virology Laboratory)

Comment number: 2

Date received: 28/05/2026

1. 3 (p5) introduction - says "This document covers the different types of samples - whole blood, plasma and serum and the various methods of collection including venous and capillary blood sampling and dried blood spot (DBS) collection." However, it then says it is not intended to cover "testing of specimens other than blood, plasma and serum obtained by venepuncture, such as oral fluid and saliva." DBS samples are also not collected by venepuncture.

Recommended Action

1. ACCEPT. The wording has been reviewed and modified to include DBS samples.

## Comments on Section 4 - Introduction

### University of Nottingham

Comment number: 3

Date received: 26/05/2026

1. 4.4 "HCV RNA is a marker of replicating virus in peripheral blood." This is ambiguous, as it implies HCV is replicating in peripheral blood. Better phrasing would be "HCV RNA in peripheral blood is a marker of replicating virus."
2. 4.4 "Most assays are designed to detect IgG which become detectable ..." "Become" should be "becomes"

Recommended Action

1. ACCEPT. This sentence has been rephrased.
2. ACCEPT. This has been corrected.

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 4

Date received: 26/05/2026

1. Generally useful information but it would be acceptable to shorten it a lot= it's a BBV, there are elimination targets, cleared or chronic, antibody unreliable in immunocompromised, DBS and cap blood for outreach.
2. p9- 'HCW common in PWID.....This is especially the case within PWID in prison....' In what way, higher prevalence?
3. 'However, laboratory protocols may differ base don local practice....' This is self-evident, can delete that line.
4. The lines about generations of tests would read better as second generation assays incorporate.....third incorporate....'
5. p10 4.4- 'HCV RNA is a marker of replicating virus in peripheral blood'. I know what you mean, but it's a marker mostly of replicating in the liver. Suggest rephrase.
6. If using an expansive approach, it would be valuable to state why IgM is not used- specificity, duration, reinfection issues.

Recommended Action

1. NONE. The key points highlighted are already addressed within the relevant sections of the document. The current level of detail has been retained to ensure clarity and completeness.
2. ACCEPT. The text has been reviewed and updated.
3. NONE. This statement reflects discussions with the working group, which represents laboratories with varying practices.
4. ACCEPT. Text reviewed and modified as appropriate.
5. ACCEPT. Text reviewed and modified as appropriate.
6. PARTIAL ACCEPT. The document now clarifies that IgM testing is not routinely available.

## Comments on Section 5 - Safety Considerations

### University of Nottingham

Comment number: 6

Date received: 26/05/2026

1. Section 5.1 The sentence "Refer to current guidance on the safe handling of all organisms documented in this UK SMI." is repeated word for word 3 lines later, in section 5.2

Recommended Action

1. ACCEPT. Duplication removed.

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 7

Date received: 26/05/2026

1. There are several instances of repetition between sections 5 and 6 relating to sampling handling.
2. 5.1- why mention use aseptic technique?
3. Top of p11 '.....of all organisms covered in this .....' Rather than 'documented'

Recommended Action

1. ACCEPT. Both sections have been reviewed and any duplications removed.
2. NONE. This reflects standard practice for specimen collection and is included for completeness.
3. ACCEPT. This has been changed as requested.

## Comments on Section 6 - Pre-laboratory processes

### Sheffield Teaching Hospitals

Comment number: 8

Date received: 15/05/2026

1. Section 6.5.3 - perhaps refer to UK SMI 'Vertical and perinatal transmission of hepatitis C'?

Recommended Action

1. NONE. UK SMI V 8 has been merged with this document and will be withdrawn once this document is published.

### Scarborough, Hull and York Pathology Service

Comment number: 9

Date received: 19/05/2026

1. Section 6.5.1 includes the statement: There is no need for a second antibody assay because it is of very little clinical utility.

   While I agree that past hepatitis C viral infection is of little ongoing clinical consequence, the test does not have 100% sensitivity. We therefore perform a second test in antibody-reactive, NAAT negative samples to distinguish true from false-positive results, with the caveat that waning antibody from a distant past infection may also produce discordant results. This is largely to reassure patients with false-positive results, and to inform those with naturally cleared infections.

Recommended Action

1. ACCEPT. The wording has been revised to allow confirmation according to local protocols.

### University of Nottingham

Comment number: 10

Date received: 26/05/2026

1. Section 6.4.2 "or that are immunocompromise" should be "or who are immunocompromised"
2. Section 6.5.3 "current HCV positive mother." should be " current HCV positive mothers."
3. Section 6.5.3 "Infants infected with HCV should be monitored and assessed clinically every 6 - 12 months to identify any risks of progressive liver fibrosis during childhood." I think it would be more appropriate (and correct) to say that such children should be referred to the paediatric Operational Delivery Node (based in Birmingham Children's Hospital, I can supply details if needed) for follow-up and treatment with DAAs. I don't know if your SMIs are UK-wide or just for England - if the former, you might need to add "or an appropriate consultant paediatric hepatologist/infectious diseases specialist". This is important, as a study by the pODN (manuscript almost ready for submission, I can supply if needed) has shown that such babies are often lost to follow-up. In other words, these children need to be "captured" by the specialist paediatric teams as soon as the diagnosis is made.

Recommended Action

1. ACCEPT. This has been changed.
2. ACCEPT. This has been corrected.
3. ACCEPT. This section has been reviewed and updated.

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 11

Date received: 26/05/2026

1. Needs commas between specimen types on first line.
2. Why is venous blood preferred? Evidence?
3. 6.3- storage for NAAT. suggest at -20 or lower, unless evidence that anything between -20 and -70 causes problems, or less that -70 is a problem.
4. 6.4.2- dialysis patients are suboptimal example for immunocompromised.
5. In low prevalence populations weakly reactive results are more likely to be false, so it doesn't mean in high prevalence they are always true.
6. 6.4.3- '...a single low level result cannot determine virological responses' Do you mean cannot determine host response with respect to clearance?
7. There are some repetitions about diluting samples and reporting.
8. I suggest an example dilution calculation is never placed on a final report- it would be confusing.
9. 6.5.3- I wouldn't exceptionalise pregnancy at all in this document- anyone with ongoing risk factors should be screened regularly.

10. Using 'consider' in guidance should be avoided unless with an explanation and a steer. So should we test babies born to current HCV positive mothers? And what is HCV positive in this context? Antibody or RNA?
11. Add a statement about legality of testing a baby without parental or guardian consent.
12. I prefer laboratory to 'lab' in official documents.
13. Suggest avoiding clinical care guidance in a testing guideline- monitoring and assessing infected infants.

Recommended Action

1. ACCEPT. Specimen wording has been revised and punctuation corrected.
2. ACCEPT. Additional explanation and supporting evidence for the use of venous blood have been included.
3. ACCEPT. Storage requirements have been revised to "-20°C or lower".
4. ACCEPT. The example has been revised and dialysis is no longer used as the principal example of immunocompromise.
5. ACCEPT. Additional wording has been included regarding false-positive antibody results in low-prevalence settings.
6. ACCEPT. The wording has been revised to refer to virological status.
7. ACCEPT. Repetitive text has been consolidated and revised.
8. ACCEPT. The example calculation is now identified as being for information only and not for inclusion in the final report.
9. PARTIAL ACCEPT. The section has been revised to address ongoing risk during pregnancy, although pregnancy-specific guidance has been retained.
10. PARTIAL ACCEPT. The section has been revised to clarify maternal HCV status and infant follow-up.
11. ACCEPT. The relevant wording has been removed.
12. ACCEPT. Terminology has been amended to use "laboratory".
13. ACCEPT. Infant monitoring and clinical management guidance have been reduced, and users are directed to relevant specialist guidance.

**Newcastle upon Tyne Hospitals NHS Foundation Trust (Virology Laboratory)**

Comment number: 12

Date received: 28/05/2026

1. 6.1 (p11) and/or Section 6.4.1 (p12) under technical limitations. DBS samples. Suggest add statement. ‘HCV PCR on DBS samples lacks the sensitivity required to confirm sustained virological response (SVR) in treated patients.’ and suggest an additional comment that DBS should mainly be used for asymptomatic screening, and different comments may be required to those suggested for use on venous blood samples.

Recommended Action

1. PARTIAL ACCEPT. The limitations of DBS for quantification and monitoring have been clarified.

### Italian Translator

Comment number: 13

Date received: 28/05/2026

1. Would it be more practical to include the final result, calculated with the dilution factor, in the report? Eliminating the suggested notes, which the clinician may not necessarily evaluate appropriately.

Recommended Action

1. ACCEPT. This section has been reviewed and clarified to reflect that reporting should focus on the dilution factor; the example calculation is included for information only and is not intended for final reporting.

## Comments on Section 7 - Laboratory processes

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 14

Date received: 26/05/2026

1. If an assay does not permit indeterminate as a result, then 'low level' would be preferable, with an associated comment.
2. Footnote d- follow up with a VL test only if qualitative is positive.

Recommended Action

1. PARTIAL ACCEPT. Reporting terminology may be determined using locally validated data.
2. ACCEPT. This footnote has been edited.

## Comments on Section 8 - Post-laboratory processes

### Sheffield Teaching Hospitals

Comment number: 16

Date received: 15/05/2026

1. table 1, scenario 2 reflex genotyping has now ceased in many labs due to pan-genotypic drug use - perhaps refer to UKHSA guidelines on when to refer samples for genotyping based on country of origin? also relevant to scenario 3

Recommended Action

1. NONE. It was agreed to retain the current wording as previously approved.

### University of Nottingham

Comment number: 17

Date received: 26/05/2026

1. 8.1 "the diluted factor" should be "the dilution factor"

Recommended Action

1. ACCEPT. This has been corrected.

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 18

Date received: 26/05/2026

I'm loving the permission to use wording based on professional judgement etc- thanks.

1. Table 1- second profile - suggest mentioning possible acute infection in the comment.
2. Also, additional BBV testing must have been requested, it cannot be added by the laboratory team just because someone is HCV antibody or RNA positive.
3. Profile 5- I think there is some value in stating past HCV infection is likely to have occurred.

Recommended Action

1. NONE. This is already included.
2. NONE. This wording has been retained.
3. NONE. This is already included.

### West of Scotland Specialist Virology Centre

Comment number: 19

Date received: 26/05/2026

1. Our laboratory does not report equivocal HCV Ab - and this something which was done in the past but not anymore.  Please add a footnote with a disclaimer relating to local verification data to allow approaches within laboratories to be based on audited performance data which that laboratory can then discuss during UKAS visits.

Recommended Action

1. ACCEPT. Reporting terminology may be determined through local clinical governance and validated data.

## Comments on Section 10 - Public health responsibilities of diagnostic laboratories

### University of Nottingham

Comment number: 20

Date received: 26/05/2026

1. "It is recognised that additional testing and referral of samples may entail some costs that has to be borne ...." Plural noun (costs), singular verb (has).

Recommended Action

1. ACCEPT. This has been corrected.

## Comments on the Algorithms

### Newcastle upon Tyne Hospitals NHS Foundation Trust (Virology Laboratory)

Comment number: 22

Date received: 28/05/2026

1. Table 1 (p18) - Only a single HCV antibody assay is now recommended. Confirmation of HCV antibody status may be helpful in some patients where clarification of past infection is required. Further details as to how antibody status may be confirmed (e.g. referral laboratories) may be helpful.

Recommended Action

1. PARTIAL ACCEPT. Confirmation may be undertaken according to local protocols where clinically indicated.

## General comments

### UKHSA regional laboratory SW and Severn Infection Sciences

Comment number: 23

Date received: 28/05/2026

1. Great to see this refreshed.

Recommended Action

1. NONE. Thank you for your feedback. No action required.

## Respondents indicating they were happy with the contents of the document

| **Overall number of comments: 2** | | | |
|---|---|---|---|
| **Date received** | 14/05/2026 | **Lab name** | Central Laboratory Coltea Clinic Hospital |
| **Date received** | 19/05/2026 | **Professional body** | Keith Shuttleworth and Associates Ltd |