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A four-tier anti-HCV / HCV-RNA interpretation grid for risk-selected clinical populations: a Bayesian sensitivity analysis defining its valid operating range

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Research Article

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Abstract

Background.

Combined anti-HCV antibody and HCV-RNA testing generates four result patterns, routinely interpreted as active infection (Ab+/RNA+), resolved or past infection (Ab+/RNA−), early acute window-period infection (Ab−/RNA+), and no infection (Ab−/RNA−). This four-tier grid is used as though its interpretation were prevalence-independent. Whether each tier is equally trustworthy across the prevalence range in which it is applied has not been quantified.

Objective.

To derive, from published assay performance characteristics, the posterior composition of each of the four result patterns as a function of population prevalence, and thereby define the prevalence range over which each tier's conventional interpretation holds.

Methods.

We performed a Bayesian decomposition of the 2 × 2 result grid across active-infection prevalence from 0.1% to 35%. Assay parameters were taken from published validation literature: anti-HCV antibody sensitivity 96.1% and specificity 99.2%; HCV-RNA sensitivity 97.8% and specificity 99.2% [1]. Window-period structure was modelled using published detection intervals (HCV RNA detectable approximately 1.5 weeks post-exposure; antibody approximately 9.5 weeks), giving an approximately 8-week interval in which a genuinely infected person is RNA-positive and antibody-negative [2]. For each prevalence we computed the marginal probability of each result pattern, the positive predictive value of the Ab+/RNA+ tier, and the fraction of Ab−/RNA+ results attributable to genuine window-period infection rather than assay error. No patient data were used.

Results.

The four tiers are not equally robust. The Ab+/RNA+ tier is reliable throughout: positive predictive value rises from 92.8% at 0.1% prevalence to 98.9% at 1% and exceeds 99.4% above 3%. The Ab−/RNA+ tier is strongly prevalence-dependent. At 0.1% prevalence only 1.0% of Ab−/RNA+ results represent genuine early acute infection, the remainder being assay artefact; the true-infection share reaches 8.7% at 1% prevalence, 43.1% at 10%, 55.2% at 20%, and 62.7% at 35%. The conventional "early acute infection" label is therefore correct for a minority of such results in screening populations and for a majority only in high-prevalence, risk-selected populations.

Conclusions.

The four-tier grid is diagnostically sound in risk-selected clinical populations but is not a general-purpose classification rule. Applied to low-prevalence screening, its Ab−/RNA+ tier would misclassify predominantly

artefactual results as acute infection. We propose that the grid be published and used with an explicit prevalence qualifier, and provide the operating-range table required to apply it correctly.

1. Introduction

Serological and molecular testing for hepatitis C virus are usually interpreted jointly. An anti-HCV antibody result and an HCV-RNA result together generate four patterns, and clinical guidance maps each to an interpretation: antibody-positive with detectable RNA indicates current infection; antibody-positive with undetectable RNA indicates resolved infection, successful treatment, or intermittent viraemia; antibody-negative with detectable RNA indicates early acute infection before seroconversion; antibody-negative with undetectable RNA indicates no infection [3,4]. The Ab−/RNA+ tier is recognised in national guidance as an entity that may represent acute infection during the window period or, in rare cases, a seronegative infection [5].

This mapping is sound as a statement about the biology of infection. It is not, however, a statement about the population of patients who generate each result pattern — and those are different claims. Any test-result interpretation is a posterior probability, and posteriors depend on the prior. The same Ab−/RNA+ result carries different information in a blood-donor screening programme than in a hepatology clinic seeing patients referred for documented risk factors, because the prior probability of infection differs by two orders of magnitude between them. Treating the four-tier grid as prevalence-independent implicitly assumes otherwise.

The Ab−/RNA+ tier is where this matters most. It is simultaneously the rarest pattern and the one whose conventional label — early acute infection — carries the most consequential clinical implications: urgent linkage to care, contact tracing, and potential early treatment. It is also the pattern most exposed to assay error, since it requires a false-negative antibody result or a false-positive RNA result to arise artefactually, and both occur at low but non-zero rates that do not scale with prevalence.

We therefore asked a quantitative question: across the prevalence range in which the four-tier grid is actually applied, what fraction of each result pattern arises from the biological state its label names, and what fraction from assay error? The answer defines the grid's valid operating range, and that derivation — rather than the grid itself — is the contribution of this paper.

2. Methods

2.1 Design

This is a Bayesian sensitivity analysis performed entirely from published assay performance parameters. No patient data, specimens, or individual-level records were used, and no ethical approval was required.

2.2 Assay performance parameters

Parameters were taken from published assay-validation literature and meta-analyses of anti-HCV and HCV-RNA assay performance.

Table 1
Model parameters.

Parameter	Value	Basis
Anti-HCV antibody sensitivity	96.1%	Dried-blood-spot meta-analysis, antibody arm [1]
Anti-HCV antibody specificity	99.2%	As above [1]
HCV-RNA sensitivity	97.8% (95% CI 96.8–98.5)	Same meta-analysis, RNA arm [1]
HCV-RNA specificity	99.2% (95% CI 98.3–99.7)	As above [1]
RNA detection onset post-exposure	~ 1.5 weeks	Midpoint of the 1–2-week CDC range [2,6]
Antibody detection onset post-exposure	~ 9.5 weeks	Midpoint of the 8–11-week CDC range [2]
Resulting pre-seroconversion interval	~ 8 weeks	Derived from the two preceding rows
Prevalence range evaluated	0.1% – 35%	Spans blood-donor screening to risk-selected referral cohorts
Note on provenance. The two assay-performance rows rest on a single meta-analysis of laboratory-based hepatitis C testing in dried blood spot samples, which reports the antibody and RNA arms side by side [1]. They should be read as one provenance, not two independent sources. The basis is restricted to dried blood spot because that is the specimen type the source evaluates; where serum or plasma performance is required, the independent meta-analyses cited below provide the appropriate comparators.		
Note on interval derivation. The CDC guidance on testing health care personnel potentially exposed to HCV states that HCV RNA becomes detectable on average within 1 week after exposure, with most infected persons detectable within 1–2 weeks, and that anti-HCV seroconversion occurs on average 8–11 weeks after exposure [2]. Taking the midpoints of those two ranges gives 1.5 and 9.5 weeks respectively, a difference of approximately 8 weeks. A separate CDC screening recommendation reports anti-HCV detectability at 4–10 weeks after infection (midpoint 7 weeks) while agreeing on RNA detectability as early as 1–2 weeks after exposure [6]. The 8–11-week range is used above; the resulting interval should consequently be read as the upper-bound estimate of the antibody-negative window.		

Cross-validation. Three independent meta-analyses bracket the Table 1 values. Pooled anti-HCV sensitivity in dried blood spot samples is reported as 98% (95% CI 95–99) with specificity 99% (95% CI 98–100) [7]; anti-HCV sensitivity 95% (95% CI 92–97) and specificity 99% (95% CI 98–99), with HCV-RNA sensitivity 95% (95% CI 93–97) and specificity 97% (95% CI 94–98) [8]; and overall pooled antibody sensitivity 97% (95% CI 97–98) with specificity 99% (95% CI 98–99) [9]. The Table 1 values sit within these ranges. Where these sources report interval estimates, they are noted here rather than propagated into the model.

2.3 Model structure

For each value of active-infection prevalence π we partitioned the population into three latent states: genuinely infected and past seroconversion; genuinely infected but within the pre-seroconversion interval; and uninfected. The pre-seroconversion fraction among incident infections was derived from the ratio of the approximately 8-week antibody-negative interval to the duration over which infections accumulate in the modelled population.

Each latent state generates the four observable result patterns with probabilities determined by the assay sensitivities and specificities in Table 1, with antibody and RNA results treated as conditionally independent given latent state. Marginal probabilities of each observable pattern were obtained by summing over latent states weighted by their prevalence.

Two quantities were then computed at each prevalence:

- **Positive predictive value of the Ab+/RNA+ tier**: the probability that a patient with this result is genuinely infected.
- **True early-acute share of the Ab−/RNA+ tier**: the probability that a patient with this result is genuinely infected and within the pre-seroconversion interval, as opposed to generating the pattern through assay error.

2.4 Computation

All computation was performed in Python 3. The full prevalence-stratified output is supplied as a supplementary table, and analysis code is available with it.

2.5 Assumptions not assessed

Conditional independence of the two assays given latent state is assumed rather than demonstrated; correlated failure modes — for example, a specimen-handling error affecting both assays — would alter the artefact fractions. Assay parameters are treated as point values, with one exception: the HCV-RNA arm now carries 95% interval estimates from its source (sensitivity 96.8–98.5, specificity 98.3–99.7) [1], but these were not propagated into the marginal frequencies, so the quantities reported here remain point estimates. The antibody arm is carried as a point estimate because no interval estimate is reported in the retrieved source. Genotype- and immunosuppression-related variation in assay sensitivity was not modelled. These are stated as bounds on interpretation, not assessed here.

3. Results

3.1 The Ab+/RNA+ tier is robust across the whole range

Positive predictive value for the Ab+/RNA+ pattern was 92.8% at 0.1% prevalence, 98.2% at 0.5%, 98.9% at 1%, and 99.4% at 3%, rising to 99.6% at 35% (Table 2, Fig. 1). Above approximately 1% prevalence the tier's conventional interpretation — current infection — is correct for more than 98 in 100 patients. This tier requires no qualifier and survives the analysis unchanged.

3.2 The Ab−/RNA+ tier is strongly prevalence-dependent

The fraction of Ab−/RNA+ results representing genuine pre-seroconversion infection varied by more than sixtyfold across the range examined: 1.0% at 0.1% prevalence, 4.6% at 0.5%, 8.7% at 1%, 21.3% at 3%, 30.0% at 5%, 43.1% at 10%, 55.2% at 20%, and 62.7% at 35%.

Two operating regimes follow. Below approximately 1% prevalence — the regime of blood-donor screening and general-population testing — more than 90% of Ab−/RNA+ results are artefactual, and the conventional "early

acute infection" label is wrong for the great majority of patients receiving it. Above approximately 10% prevalence — the regime of tertiary referral and risk-factor-selected clinical cohorts — the majority of such results are genuine window-period infections, and the label is appropriate, though still not certain.

Table 2
Prevalence-stratified composition of the result grid.

Active-infection prevalence	P(Ab+/RNA+)	P(Ab+/RNA−)	P(Ab−/RNA+)	P(Ab−/RNA−)	True early-acute share of Ab−/RNA+	PPV of Ab+/RNA+
0.1%	0.0009	0.0084	0.0080	0.9827	1.0%	92.8%
0.5%	0.0044	0.0100	0.0084	0.9771	4.6%	98.2%
1%	0.0087	0.0121	0.0090	0.9702	8.7%	98.9%
3%	0.0261	0.0204	0.0110	0.9425	21.3%	99.4%
5%	0.0435	0.0288	0.0130	0.9147	30.0%	99.5%
10%	0.0869	0.0496	0.0181	0.8454	43.1%	99.6%
20%	0.1736	0.0913	0.0284	0.7067	55.2%	99.6%
35%	0.3038	0.1538	0.0437	0.4987	62.7%	99.6%

P denotes the probability of each result pattern in the population at the stated active-infection prevalence. Full precision is provided in Supplementary Table S1.

3.3 Implied operating range

Combining the two results gives a usable rule. The grid may be applied as conventionally labelled when population active-infection prevalence exceeds approximately 10%, at which point every tier's conventional interpretation is correct for the majority of patients receiving it. Between approximately 1% and 10%, the Ab+/RNA+ tier remains reliable but the Ab−/RNA+ tier requires explicit acknowledgement that most such results will not be acute infection. Below approximately 1%, the Ab−/RNA+ label should not be used without confirmatory repeat testing, since it is wrong more than nine times in ten. Operational guidance on reflex-testing strategies is consistent with this sequencing, specifying that a reactive antibody result with detectable RNA determines current infection and that discordant patterns require resolution before a diagnosis is recorded [10].

4. Discussion

The four-tier grid is not uniformly trustworthy, and the variation is not distributed as intuition might suggest. The tier handling the commonest clinical question — is this patient currently infected? — proves robust across four orders of magnitude of prevalence. The tier handling the rarest and most urgent question — is this a pre-seroconversion acute infection? — proves almost entirely unreliable in the setting where population testing most often occurs, and reliable only where the prior probability of infection is already high.

This is a straightforward consequence of Bayes' theorem rather than a defect in any assay. False-positive and false-negative rates are properties of the test; the fraction of a given result pattern that they account for depends on how many true cases there are to compete with. In a population where one person in a thousand is infected, the artefact rate need only be a fraction of a percent to dominate the rarest result pattern entirely. Nothing about the assays needs to improve for the grid to work in a referral clinic; nothing about the assays can make it work unqualified in donor screening.

The practical recommendation follows directly. The grid should carry a prevalence qualifier wherever it is published or taught. In risk-selected clinical populations — which include the tertiary-care hepatology cohorts where this framework is most often applied — the conventional labels are appropriate and the grid is a useful summary of joint test interpretation. In screening populations, an Ab−/RNA+ result should trigger confirmatory repeat testing before any acute-infection diagnosis is recorded, and the framework should say so explicitly rather than leaving the inference to the reader.

We would emphasise that this analysis does not undermine the grid; it delimits it. The derivation of an operating range is what converts a clinically reasonable heuristic into a defensible decision rule, and the resulting table is directly usable at the point of interpretation given an estimate of local prevalence.

Relationship to existing predictive-value reporting. The meta-analysis from which the model parameters are drawn notes that both antibody-based and RNA-based approaches show a high negative predictive value but a low positive predictive value when prevalence falls below 10%, particularly for HCV-RNA testing [1]. Our results are concordant with that observation, and we state the concordance plainly: the direction of the effect is not a novel claim. What this analysis adds is the decomposition of the effect by result pattern. A single assay-level statement about positive predictive value does not tell a clinician what an Ab−/RNA+ result means, because that pattern arises from two distinct latent states whose relative contributions shift with prevalence. The tier-by-tier shares in Table 2, and the operating range derived from them, quantify that shift for the rarest and most clinically consequential pattern.

4.1 Limitations

The analysis rests on point estimates of assay performance drawn from published pooled figures; real-world sensitivity and specificity vary with assay platform, specimen type, genotype, and operator. Interval estimates are available for the HCV-RNA arm of the parameter source but have not been propagated into the reported quantities, and none are reported here for the antibody arm [1]. Conditional independence between the antibody and RNA assays given latent state is assumed; correlated failures would increase the artefact share above our estimates in the low-prevalence regime. The pre-seroconversion fraction depends on the incidence structure of the modelled population, which we treated in simplified form; where guidance documents give divergent seroconversion ranges, the widest range was adopted, and the window-period interval should be read accordingly [2,6]. Finally, the analysis is entirely theoretical: no empirical cohort was used to verify the predicted result-pattern frequencies, and such verification would strengthen the conclusions materially.

4.2 Verification that would strengthen this work

A cohort with paired antibody and RNA results, known local prevalence, and confirmatory resolution of discordant results would allow the predicted marginal frequencies in Table 2 to be checked directly against

observed pattern counts. Discordance between predicted and observed Ab−/RNA+ frequency would indicate either that assay performance in that setting differs from the pooled published values or that the conditional-independence assumption fails — both informative outcomes.

5. Conclusions

The four-tier anti-HCV / HCV-RNA interpretation grid should be used with an explicit prevalence qualifier. Its Ab+/RNA+ tier is reliable from 0.1% to 35% prevalence (positive predictive value 92.8% to 99.6%). Its Ab−/RNA+ tier is dominated by assay artefact below approximately 1% prevalence, where only about 1 in 100 such results represents genuine early acute infection, and becomes majority-genuine only above approximately 10–20% prevalence. The grid is therefore sound for risk-selected clinical populations and unsafe as an unqualified general-purpose rule. Table 2 provides the operating-range data needed to apply it correctly.

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Figures

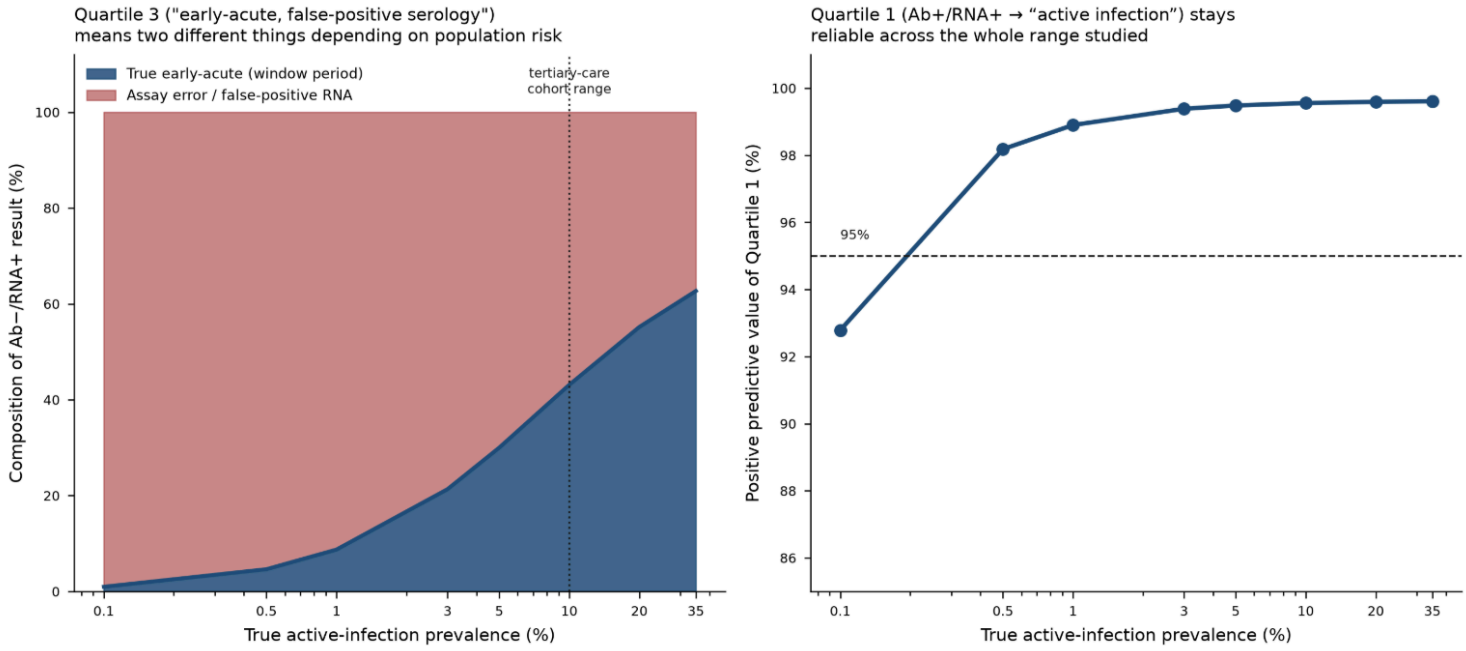


Figure 1

Bayesian decomposition of the four-tier anti-HCV / HCV-RNA grid across active-infection prevalence. The Ab+/RNA+ tier's positive predictive value is high throughout and effectively flat above approximately 1% prevalence. The true early-acute share of the Ab−/RNA+ tier rises monotonically with prevalence, crossing 50% between 10% and 20% prevalence: below that crossing, the tier is dominated by assay artefact rather than genuine window-period infection.

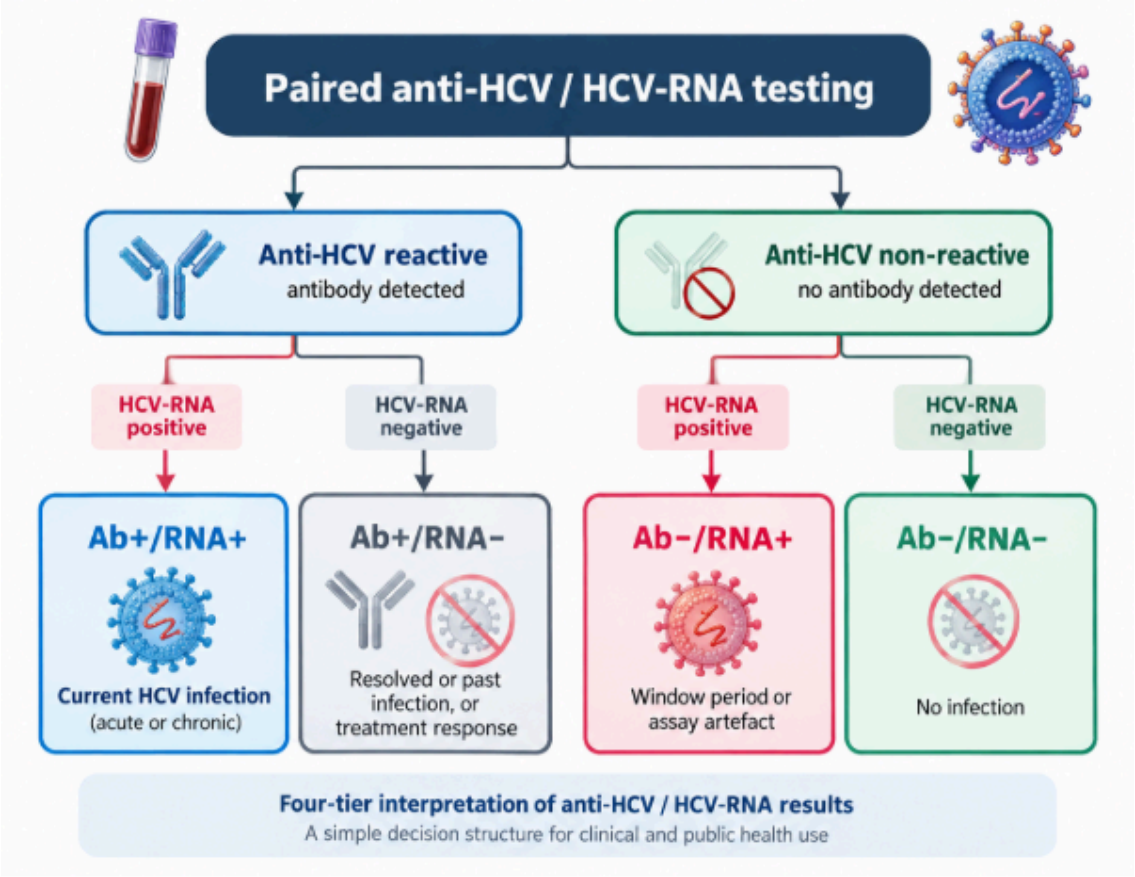


Figure 2

Decision structure of the four-tier anti-HCV / HCV-RNA interpretation grid. Paired antibody and RNA testing generates four result patterns. The two concordant patterns correspond to the Ab+/RNA+ and Ab+/RNA− tiers; the discordant Ab−/RNA+ pattern is labelled as window-period infection or assay artefact, since the prevalence-stratified decomposition shows that the two alternatives are not distinguishable from the result pattern alone.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.png