Development and Comparison of Hepatitis C Cross-Sectional Incidence Testing Methods

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Background

- The ability to accurately estimate HCV incidence from a cross-sectional survey using biomarker-based tests for recent infection would reduce the need for longitudinal data.
- HCV avidity assays have been shown to detect recently-infected individuals [1]; however, the relevant test properties needed for their applicability in measuring HCV incidence are unknown.
- We developed two HCV avidity-based tests for recent infection using sera from two longitudinal cohorts of people who inject drugs (PWID), and comprehensively evaluated the performance of these tests for potential use in HCV incidence estimation.

Methodology

Study Population

- The Baltimore Before-and-After Acute Study of Hepatitis (BBAASH) and the AIDS Linked to the Intravenous Experience (ALIVE) cohorts of PWIDs provided 1857 samples from 1151 individuals with known duration of HCV infection (Table 1).
- Of the total 1857 samples, 97.3% had known HCV RNA quantities obtained by either Cobas Amplicor HCV Monitor v2.0 or Cobas Taqman HCV Test v2.0.

Development of Avidity Assays

- *Avidity* – measured by running a sample in duplicate (intra-assay ELISA) and adding a dissociating step after sample incubation by treating one of the wells with a chaotropic agent and the other well with wash buffer.
- *Avidity Index* – the ratio of absorbance of the well treated with the chaotropic agent compared to wash buffer.
- *Ortho 3.0 HCV Avidity Assay* – the Ortho 3.0 HCV ELISA test system was modified by an additional 30 minute incubation with 0.25M diethylamine at 37°C [1].
- *Genedia 3.0 HCV Avidity Assay* – the Genedia 3.0 HCV ELISA test system was modified by an overnight incubation of samples at room temp. and with three five minute washes using 11M urea.

Evaluating Optimality of Incidence Assays

- *Mean Duration of Recent Infection (MDRI)* – the average time spent recently-infected less than a specified time cut-off (T = 2 years). Please refer to [2] for statistical methodology.
- *False-Recent Rate (FRR)* – proportion of long-term infected individuals (>T) being misclassified as 'recent'.
- *Optimality* – incidence assays should have a large MDRI (~360 days) and a low FRR (<2% is acceptable but ideally, 0%) [3].

Results

Table 1. Distribution of Assayed Samples by T = 2 years.

Cohort	< 2 years (T) Post-Seroconversion		> 2 years (T) Post-Seroconversion	
	Ortho 3.0	Genedia 3.0	Ortho 3.0	Genedia 3.0
BBAASH				
No. of Samples	308	308	88	88
No. of Individuals	72	72	44	44
ALIVE				
No. of Samples	0	0	692	1441
No. of Individuals	0	0	512	1068

Figure 1. HCV Avidity as a Function of Time Since Seroconversion by Infection Status (N=72 Individuals).

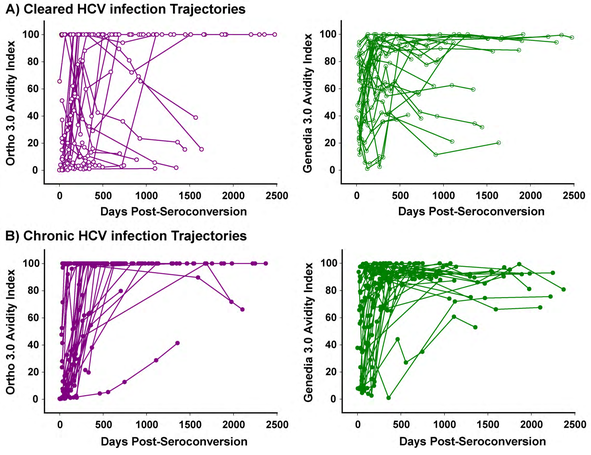


Figure 2. Comparison of Avidity Index Values Between Ortho 3.0 and Genedia 3.0 Before and After T = 2 years.

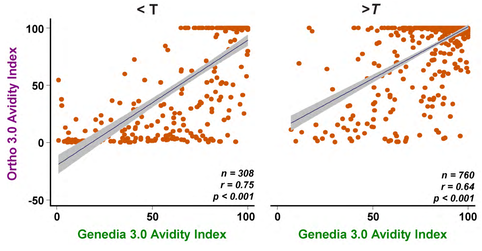


Figure 3. Distribution of Avidity Index Values from PWIDs Infected Longer than T = 2 years by HCV RNA Status.

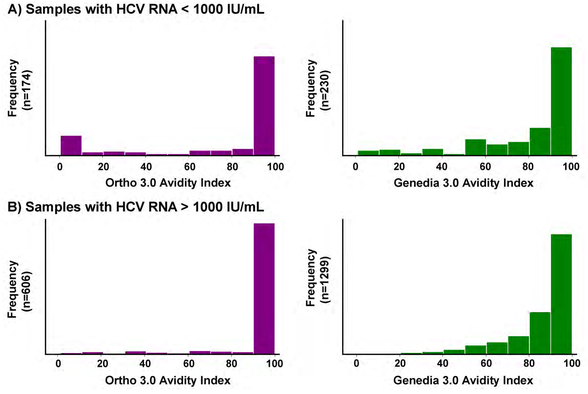


Table 2. Comparison of Incidence Test Properties for Each Assay at Various Avidity Index Cut-offs with and without HCV RNA Confirmation (VL > 1000 IU/mL).

Avidity Index	Ortho 3.0 Avidity Assay		Ortho 3.0 Avidity Assay + HCV RNA Confirmation	
	MDRI, days (95% CI)	FRR, % (95% CI)	MDRI, days (95% CI)	FRR, % (95% CI)
20	235 (202, 265)	5.1 (3.7, 6.9)	102 (83, 125)	1.3 (0.7, 2.7)
30	277 (244, 308)	6.5 (4.9, 8.5)	126 (103, 150)	2.1 (1.2, 3.3)
40	312 (276, 347)	8.6 (6.7, 10.8)	148 (123, 175)	3.6 (2.5, 5.1)
50	327 (292, 361)	10.0 (8.0, 12.3)	158 (133, 187)	4.5 (3.2, 6.1)
60	352 (318, 385)	10.9 (8.8, 13.3)	176 (150, 204)	5.1 (3.7, 6.8)
70	380 (344, 412)	13.3 (11.0, 15.9)	191 (162, 220)	6.5 (4.9, 8.4)
80	392 (357, 428)	15.8 (13.2-18.5)	195 (167, 225)	8.1 (6.3, 10.1)

Avidity Index	Genedia 3.0 Avidity Assay		Genedia 3.0 Avidity Assay + HCV RNA Confirmation	
	MDRI, days (95% CI)	FRR, % (95% CI)	MDRI, days (95% CI)	FRR, % (95% CI)
20	75 (54, 98)	1.2 (0.7, 1.9)	46 (31, 65)	0.3 (0.1, 0.8)
30	105 (81, 131)	2.0 (1.4, 2.9)	66 (48, 89)	0.9 (0.5, 1.5)
40	146 (120, 177)	3.5 (2.7, 4.6)	83 (63, 106)	1.8 (1.2, 2.6)
50	184 (152, 215)	5.8 (4.6, 7.0)	99 (76, 125)	3.9 (3.0, 5.0)
60	223 (188, 256)	10.5 (9.0, 12.1)	115 (91, 140)	7.4 (6.2, 8.9)
70	287 (252, 324)	16.2 (14.3, 18.1)	148 (122, 176)	12.2 (10.6, 13.9)
80	354 (320, 388)	24.8 (22.6, 27.0)	193 (162, 223)	19.5 (17.6, 21.2)

Note: *Please refer to Table 1 for sample sizes.*

Results

- At various avidity index cutoffs, the MDRI varied between 235–393 days for the Ortho 3.0 avidity assay alone and 75–354 days for the Genedia 3.0 avidity assay alone.
- However, both avidity assays independently produced high FRRs (1.2% to 24.8%), primarily due to individuals who spontaneously cleared the virus.
- The high FRRs for the avidity assays decreased when evaluated in combination with HCV RNA confirmation, but remained above the ideal FRR of 0%. Inclusion of HCV RNA compromised the range in MDRI.
- The Ortho 3.0 avidity assay consistently outperformed the Genedia 3.0 avidity assay.

Conclusions

- This is the first study to evaluate hepatitis C avidity tests for recent infection for potential use in cross-sectional incidence estimation using MDRI and FRR.
- HCV avidity and viral load testing algorithms were able to identify recently infected individuals, however, further work is needed to determine additional biomarkers that will reduce the FRRs of these testing algorithms.
  This will require investigation of factors associated with misclassification beyond viremia.
- Performance of these assays are similar to predicate assays for HIV incidence testing, which provides promise for the future of HCV incidence estimation.
- Efforts to optimize cross-sectional HCV incidence testing will contribute to a more practical and timely method to determine rates of new infection, yielding more rapid assessment of the epidemic and of the impact of intervention efforts.

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