Hepatitis C Antibody Testing: An Algorithm In Need of Review

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Douglass Hanly Moir Pathology
Post-transfusion hepatitis

**History of posttransfusion hepatitis**

- 25% Recipients Infected
- % Recipients Infected graph from 1965 to 2000
- Year of Transfusion

Key Events:
- All volunteer donors
- HBsAg test
- AIDS high-risk exclusions
- anti-HIV test
- ALT / HBcAb tests / anti-HCV test
- Improved HCV tests
Transfusion-associated hepatitis

- 1965: 20-25%
- 1970s: HBsAg screening, 10% “NANB” hepatitis
- 1980s: surrogate markers, 1 case per 400 units

- 1989: HCV identified, named, cloned, first commercial anti-HCV IgG assay released
  - Insensitive (80%, high prevalence)
  - Non-specific (70% FP low prevalence)
  - Long seroconversion window (16 weeks)

- Current risk TA-HCV: 1 per million transfusions
Hepatitis C Virus

- Positive sense, ss RNA virus
  - Genus Hepacivirus, family Flaviviridae
- 9.6 kb genome, single ORF
  - 10 structural/non-structural proteins
    - Structural: core, envelope (E1, E2), p7
    - Non-structural: NS2,3,4,4A,4B,5A, 5B
- 7 genotypes, multiple subtypes
  - Australia: genotypes 1a, 1b and 3a predominate
  - Genotype influences management and prognosis
HCV Genome

Hepatitis C virus RNA

9600 nt bases

Gene encoding precursor polyprotein

5’ NTR  |  Gene encoding precursor polyprotein  |  3’ NTR

Structural proteins
- p22
- gp35
- gp70
- p7

non-structural proteins
- p23
- p70
- p8
- p27
- p56/58
- p68

C  |  E1  |  E2  |  NS1  |  NS2  |  NS3  |  NS4A  |  NS4B  |  NS5A  |  NS5B

Envelope glycoproteins
- nucleocapsid

Proteases
- RNA helicase
- co-factors

RNA polymerase
- interferon resistant protein

Transmembrane protein
HCV Morphology

- Single-stranded RNA
- Nucleocapsid
- Envelope glycoproteins
- Envelope
Clinical Significance of HCV Infection

- Acute infection
  - usually subclinical (spontaneous clearance 10%)
  - symptomatic 10-15% (spontaneous clearance 25-50%)

- Chronic hepatitis in 55-85%
  - Chronic liver disease
  - Cirrhosis
  - Hepatocellular carcinoma
Disease Burden

- **Globally**
  - ~150 million chronic HCV infections
  - Asia, Africa: high prevalence (Egypt 10%)
  - North America, W. Europe, Australia: low prevalence (0.5-2.0%)

- **Australia**
  - 2012: ~230,000 chronic HCV infections, 10,114 newly diagnosed
  - 2013: 630 HCV-related deaths, 1000 HCC or ESLD
  - Commonest indication for liver transplant
Parenteral acquisition
  - Pre-1990: Blood transfusion
  - IVDU/unsafe injection practices
    - Nosocomial, sexual, tattooing, perinatal etc

Marginalised
  - IVDU, incarceration

Middle-class
  - Experimentation in teens /early adulthood
  - Baby-boomers: born 1945-1965
Prevalence of hepatitis C virus antibody, by age
Advances In Treatment

- Interferon/peg IFN
- pegIFN + Ribavirin
- Protease inhibitors
  - Boceprevir and telaprevir
- Polymerase inhibitors
  - Sofosbuvir
Advances in Treatment

- 48 weeks
- 8-12 weeks
Majority of diagnoses in chronic phase
  - Prevalent rather than incident
  - Follow-up of incidental LFT elevation
  - Screening of those from known risk groups
  - Donor screening (blood, tissue)
  - One-off, age-based screening

Serology remains cornerstone of diagnosis
Diagnosis of HCV Infection

Commercial HCV Assays

Indirect
Serological assays

Antibody assays
EIA, CMIA, CLIA, ELFA, RIBA

Direct
Virological assays

HCV RNA detection
- Qualitative
- Quantitative

Molecular HCV genotyping
Serological Diagnosis

- Identify immunodominant epitopes
- Produce recombinant proteins/synthetic peptides
- Incorporate into serological assays
  - Screening (EIA, CLIA, ICT)
  - Supplemental
    - Second immunoassay
    - Immunoblot
  - Is this nomenclature still relevant?

- **Serological diagnosis**
  - Indirect
  - Long seroconversion window
  - Cannot distinguish current and past infection
Screening Immunoassays

- **1989: First generation EIA**
  - Single recombinant antigen (C100-3, NS4)
  - Seroconversion window 16 weeks

- **1992: Second generation EIA**
  - Core, NS3, NS4
  - Improved sensitivity and specificity
  - Seroconversion window 10 weeks

- **1994: Third generation EIA**
  - Core, NS3, NS4, NS5
  - Seroconversion 56 days
  - False-positive issue unresolved

- **(Fourth generation: 3rd gen plus capsid Ag)**
Types of Screening Immunoassays

- **Enzyme Immunoassay (EIA)**

- **Chemiluminescence immunassay (CLIA)**
  - Architect (Abbott), Vitros (Ortho), Access (Bio-Rad), Elecsys (Roche)
  - Rapid, random access, fully automated
  - Improved specificity and PPV
  - False positives in low prevalence groups still an issue
  - **Initial positives require further testing/assessment before reporting**

- **Immunochromatography**
  - Rapid TAT (1 hour)
  - Point-of-care
Supplemental Immunoassays

- **Recombinant Immunoblot**
  - Obsolete: RIBA (Chiron) withdrawn
  - Immobilisation of core, NS3 and NS5 on nitrocellulose strip
    - Positive: 2 bands (RIBA), 1 band (MP Diagnostics)
    - Labour-intensive, complex, time-consuming, expensive
  - Specific but less sensitive than immunoassays
  - Cannot distinguish current from past infection
Signal to Cut-off Ratio (S/CO)

- Correlation between screening assay S/CO and confirmation of positive screen by immunoblot
  - CDC 2003:
    - 25,000 sera, high and low seroprevalence (0.8-25%)
    - S/CO values >95% predictive of RIBA confirmation
  - Architect anti-HCV CMIA (Abbott)
    - S/CO >/= 5 predicts RIBA positivity in 97%
    - S/CO < 5: Supplemental testing required
    - S/CO >/= 5: Further testing not required
  - Valid regardless of seroprevalence
  - Only valid for assays evaluated. Cannot generalise.
National Hepatitis C Testing Policy 2012

- Principles of testing
  - why, who, how
- Epidemiology
- Diagnostic strategies
- Transmission and Infection Control
- Quality Assurance
- Technology

- Testing Pathway
National HCV Testing Pathway

TESTING PATHWAY

Scren by HCV IA (a)

NEGATIVE

- Report to referring doctor who informs patient
- Repeat at 1-2 months if acute infection is suspected

REACTIVE

- Test by second alternative HCV IA

NEGATIVE

- Additional confirmatory tests may be performed at this stage e.g. HCV RNA, HCV Ag; Immunoblot

REACTIVE

- NAT for HCV RNA (b)

NEGATIVE

- NAT for HCV RNA

POSITIVE

- Active infection
- Report to referring doctor who informs patient
- Doctor refers for further evaluation genotype, viral load; Notifies relevant bodies

NEGATIVE

- Likely past infection with viral clearance OR if liver tests abnormal possible low V, Report HCV RNA in 6 months if infection still a concern

POSITIVE

- Possible very early seroconversion
- Likely false reactivity in first IA, Repeat HCV RNA in 6 months if infection is still a concern

NEGATIVE

- Repeat at 1-2 months if acute infection is suspected
- Report to referring doctor who informs patient

IA = Immunoassay. Encompasses all approved HCV IAs included on the ARTG for screening
(a) Screening IAs may include Antigen/antibody combination IAs
(b) HCV Antigen IA may be considered an alternative to NAT
Australian Anti-HCV Testing Algorithm

› **Primary screen**
  › Any TGA-licensed anti-HCV assay
    ‚ EIA, CLIA, MEIA
    ‚ Manual, semi-automated and automated assays available

› **Supplemental testing (1)**
  › To define serostatus:
    ‚ Another licensed screening assay
    ‚ Immunoblot

› **Supplemental testing (2)**
  › To define infection status
    ‚ NAT/HCV Capsid Ag
Primary screen: Negative

- “A sample non-reactive in the screening immunoassay can be generally regarded as anti-HCV negative.”

- Follow-up testing (repeat serology, NAT) may be necessary in some patients
  - Possible seroconversion
  - Immunocompromised
Primary screen: Positive

- “If reactive in two approved immunoassays can be reported as anti-HCV positive”

- “alternative immunoassay should include antigens different from the screening test in both specificity and method of preparation.”

- “Information available through pre-market evaluation reports of HCV immunoassays.”

- “Validity of selection must be demonstrated”

- Sounds easy
Is it That Easy?

- Information required to allow appropriate choice of supplemental immunoassay not always easy to find

- Proving the absence of common false-reactivity
  - **Proving a negative**

- Immunoblot interpretive criteria
  - One band? Two bands?
  - No longer relevant, should no longer be included in discussion
Role of NAT in Serological Diagnosis

- Demonstrates the presence or absence of infection
  - Negative result does not contribute to serostatus assessment

- Request for NAT always subsequent to anti-HCV
  - Specimen recollection always required
    - NAT result unavailable when anti-HCV requires reporting

- NAT crucial in HCV diagnostic algorithm

- NAT largely irrelevant in anti-HCV testing algorithm
DHM: Comparison of Anti-HCV Assays

- Concerns about current immunoassay combination (Architect/Murex)
  - Simultaneous non-specific reactivity leading to false positive reports

- Impression: Roche assay more specific than Murex
  - Would this be a better choice of supplemental assay?

- Assess workflow compatibility, performance characteristics
Immunoassays Included

- Architect Anti-HCV i2000 (Abbott)
- Murex anti-HCV v4.0
- Elecsys Anti-HCV II Cobas e411 (Roche)
- Liaison XL anti-HCV (DiaSorin)
- Vidas anti-HCV (bioMérieux)
- MP Diagnostics HCV Blot 3.0 (NRL, with thanks)
Description of Study

- December 2013 – February 2014
  - 102 sera reactive in Architect anti-HCV assay
  - Run in Murex and reported
  - Frozen @ -20° C

- May 2014
  - Specimens thawed, re-run on Architect
  - Murex, Elecsys, Liaison, Vidas 3.

- June, 2015
  - MP Diagnostics HCV Blot 3
Abbott Architect

- **Method**: Chemiluminescence (CLIA)
- **Antigens**: Hcr43 (NS3 and core) and c100-3 (NS4)
- **Amino Acid Regions**: 1192 to 1457 (33c) and 1 to 150 (Core)
- **Labelled substance**: Acridinium
- **Solid phase**: Paramagnetic particle
- **Sample volume**: 20 ul
- **Grey zone**: None
Murex anti-HCV v 4.0

- Method: Enzyme Immunoassay
- Antigen Source: Core, NS3, NS4, **NS5**
- Amino Acid Regions: Not stated
- Solid phase: Microtiter Well
- Sample volume: 20 ul
- Grey zone: None
DiaSorin Liaison XL

- Method: CLIA
- Antigen: HCV core, NS3, NS4
- Amino Acid Regions: Not Stated
- Solid phase: Magnetic particles
- Sample volume: 25 ul
- Grey zone: None
Elecsys Anti-HCV

- Method: Electrochemiluminescence (ECLIA)
- Antigen Source: Peptides/recombinant antigens Core, NS3, NS4
- Amino Acid Regions: Not Stated
- Solid phase: Magnetic particle
- Conjugate: Ruthenium
- Sample volume: 40 ul
- Grey zone: 0.9 – 1.0
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<tr>
<th>Method</th>
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<tr>
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<td>Core, NS3, NS4</td>
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<tr>
<td>Amino Acid Regions</td>
<td>Not Stated</td>
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<tr>
<td>Solid phase</td>
<td>SPR (Solid Phase Receptacle)</td>
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<tr>
<td>Sample volume</td>
<td>100 ul</td>
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<tr>
<td>Grey zone</td>
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# Results – Raw Data Ranges

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<thead>
<tr>
<th>Method</th>
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<tr>
<td>Abbott Architect G2-6275 (Old)</td>
<td>0.10 - 16.40</td>
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<td>Murex Version 4.0</td>
<td>0.325 - 13.970</td>
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<tr>
<td>Roche Version II</td>
<td>0.06 - 248.90</td>
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<tr>
<td>DiaSorin Liaison XL</td>
<td>0.11 - 12.00</td>
</tr>
<tr>
<td>BioMerieux Vidas 3</td>
<td>0.20 - 30.20</td>
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</table>
Results – Specimen Integrity Check

Architect, initial testing (y) vs Architect, post freeze-thaw (x)

Passing and Bablok Regression

\[ y = 0.9755x + 0.1813 \]

**R = 0.9911**  
**n = 102**

Mean of \( x \) = 10.50 S/CO  
Mean of \( y \) = 10.46 S/CO
Results – Specimen Integrity Check


Absolute Difference in S/CO

Repeated Analysis on Architect, Days after Initial Testing
Results – Specimen Integrity Check

Difference in S/CO Between Repeated and Original Analysis on Abbott Architect

Original S/CO Level of Anti-HCV

Absolute Difference in S/CO

1.24
Inter-assay Precision

Architect anti-HCV IFU G4-3043 (y) vs Architect anti-HCV IFU G2-62 75 (x)

Passing and Bablok Regression
\[ y = 0.9979x + 0.1142 \quad R = 0.9928 \quad n = 102 \]
Mean of x = 10.46 S/CO
Mean of y = 10.51 S/CO
<table>
<thead>
<tr>
<th>Method</th>
<th>Unit</th>
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<th>Borderline</th>
<th>Reactive/Positive</th>
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<tr>
<td>Abbott Architect</td>
<td>S/CO</td>
<td>&lt;1.00</td>
<td></td>
<td>&gt; OR = 1.00</td>
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<tr>
<td>Murex Version 4.0</td>
<td>S/CO</td>
<td>Varies depending on batch</td>
<td>Varies depending on batch</td>
<td></td>
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<tr>
<td>Roche Version II</td>
<td>COI</td>
<td>&lt;0.90</td>
<td>&gt; OR = 0.90 - &lt;1.00</td>
<td>&gt; OR = 1.00</td>
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<tr>
<td>DiaSorin Liaison XL</td>
<td>S/CO</td>
<td>&lt;1.00</td>
<td></td>
<td>&gt; OR = 1.00</td>
</tr>
<tr>
<td>BioMerieux Vidas 3</td>
<td>TV</td>
<td>&lt;1.00</td>
<td></td>
<td>&gt; OR = 1.00</td>
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</table>
## Results by Assay

<table>
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<th>Method</th>
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<th>Non-Reactive/Negative</th>
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<tr>
<td>Murex Version 4.0</td>
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<td>6</td>
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<td>DiaSorin Liaison XL</td>
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<td>7</td>
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<tr>
<td>BioMerieux Vidas 3</td>
<td>97</td>
<td>5</td>
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</table>
## Summary: Eight discordant sera

<table>
<thead>
<tr>
<th>Patients</th>
<th>Abbott Architect</th>
<th>Murex Version 4.0</th>
<th>Roche Version II</th>
<th>DiaSorin Liaison XL</th>
<th>BioMerieux Vidas 3</th>
<th>Immunoblot</th>
<th>HCV RNA</th>
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<tbody>
<tr>
<td>285127175</td>
<td>1.11/1.15</td>
<td>1.336</td>
<td>34.58</td>
<td>0.64</td>
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<td>Negative</td>
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<tr>
<td>285266779</td>
<td>0.14</td>
<td>1.512</td>
<td>0.43</td>
<td>0.17</td>
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<td>Negative</td>
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<tr>
<td>285208948</td>
<td>1.47/1.50</td>
<td>1.052</td>
<td>113.7</td>
<td>1.3</td>
<td>0.84</td>
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<td>Negative</td>
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<td>258249314</td>
<td>5.93</td>
<td>0.725</td>
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<td>285281661</td>
<td>4.71</td>
<td>0.325</td>
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<td>2.8</td>
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<tr>
<td>204515279</td>
<td>1.79/1.84</td>
<td>0.96</td>
<td>0.06</td>
<td>0.13</td>
<td>2.93</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>
285127175

- Architect (antenatal)
- Murex 4.0
- Roche v2
- Liaison XL
- Vidas 3
- RIBA
- HCV RNA

- HCV Infection status
  - Reactive (1.11/1.15)
  - Reactive (1.336)
  - Reactive (34.58)
  - Non-reactive
  - Non-reactive
  - Positive (core +++)
  - Negative
285266779

- Architect
- Murex 4.0
- Roche v2
- Liaison XL
- Vidas 3
- RIBA
- HCV RNA

- **HCV Infection Status**
  - Non-reactive
  - Reactive (1.512)
  - Non-reactive
  - Non-reactive
  - Non-reactive
  - Non-reactive
  - **Negative**
285208948

- Architect
- Murex 4.0
- Roche v2
- Liaison XL
- Vidas 3
- HCV RNA
- RIBA

- Reactive (1.47/1.50)
- Reactive (1.052)
- Reactive (113.7)
- Reactive (1.3)
- Non-reactive
- Negative
- Positive (core ++)

- HCV Infection Status
- Negative
258249314

- Architect
- Murex 4.0
- Rochev2
- Liaison XL
- Vidas 3
- RIBA
- HCV RNA

- Reactive (6.06)
- Non-reactive
- Non-reactive
- Reactive (3.8)
- Reactive (0.72)
- Negative
- Negative

- HCV Infection Status
- Negative
285281661

- Architect (preg)
- Murex 4.0
- Roche v2
- Liaison XL
- Vidas
- RIBA
- HCV RNA

- Reactive (4.78/4.72)
- Non-reactive
- Non-reactive
- Non-reactive
- Reactive (3.84)
- Positive (core ++)
- Negative

- HCV Infection Status
  - Negative
<table>
<thead>
<tr>
<th>Test Type</th>
<th>Result</th>
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<td>Architect</td>
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<td>Murex</td>
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<td>Roche v2</td>
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<td>Liaison XL</td>
<td>Non-reactive</td>
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<tr>
<td>Vidas 3</td>
<td>Reactive (4.39)</td>
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<tr>
<td>HCV RNA</td>
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<td>RIBA</td>
<td>Negative</td>
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<td>HCV Infection Status</td>
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</table>
285308958

- Architect
- Murex 4.0
- Roche v2
- Liaison XL
- Vidas
- HCV RNA
- RIBA

- **HCV Infection Status**
  - Reactive (0.93/1.01)
  - Non-reactive
  - Non-reactive
  - Non-reactive
  - Non-reactive
  - Negative
  - Indeterminate (NS5 ++)
  - Negative
<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
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<tr>
<td>Architect</td>
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<td>HCV Infection Status</td>
<td>Negative</td>
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</tbody>
</table>
MP Diagnostics HCV Blot 3.0

- **Positive**
  - 1+ or greater reactivity to 2 or more HCV antigens
  - OR
  - 2+ or greater reactivity to Core band only

- A sample which has been found non-reactive on another manufacturer’s HCV screening assay or confirmatory assay may be found reactive on MPD HCV BLOT 3.0 due to the presence of unique epitopes in this confirmatory test
Three patients anti-HCV positive
- One pregnant, none with risk factors for HCV
- All three HCV RNA negative
- All three based on a single band (core) of 2+ or 3+
- Each “missed” by 1,2 or 3 screening assays

Possible low-level antibody, resolved infection
- Probable non-specific reactivity

Immunoblot: adds no further information to that provided by screening immunoassay and HCV RNA
## Screening Combinations

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<tr>
<th>Patients</th>
<th>Arch/Murex</th>
<th>Arch/Roche</th>
<th>Arch/Liaison</th>
<th>Arch/Vidas</th>
<th>Blot</th>
<th>Serostatus</th>
<th>HCV RNA</th>
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</tr>
</tbody>
</table>

- Architect/Roche or Architect/Liaison combination superior to Architect/Murex
  - Fewer false-positive anti-HCV reports
- Architect/Roche: concordance with blot 2/3
- Architect/Liaison: concordance with blot 1/3
- Murex (which includes NS5) did not detect the only NS5-positive sample identified by immunoblot
Conclusions

- **Architect/Roche superior to Architect/Murex**
- **Immunoblot unhelpful, maybe misleading**
  - Should no longer be included in algorithm
- **NAT result irrelevant to anti-HCV algorithm**
  - Anti-HCV requires reporting before NAT is requested
  - anti-HCV testing algorithm vs HCV diagnostic algorithm
- **Selective supplemental testing rather than reflex**
  - Based on S/CO
- **Choice of supplemental immunoassay**
  - No gold-standard
  - Proving the absence of common false-reactivity
    - Absence of evidence or evidence of absence?
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