

# *Utility and future of immunoblot antibody assays*

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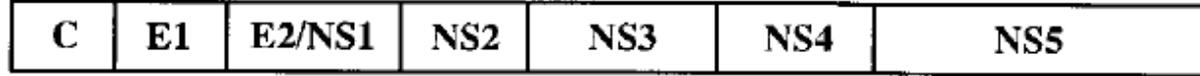
# HCV discovery minihistory

- Source: Chimp Rodney at the CDC (Dr D Bradley)
- Method: Text book procedure (Maniatis)
- Team: Chiron's (Drs Kuo, Choo, Houghton)
- Verification step 1: Dr Alters coded panel
- Verification step 2. Rapid confirmation by the global medical community – a severe disease was identified and “transaminitis” expired.
- (Other “new” viruses GBV-C, TTV, Senv, Parv 4, XRMV never made it)

# HCV antibody test developments

- Antigens: 5-1.1, C-100 for antibody ELISAs
- Soon 2-antigen immunoblots test launched to verify HCV antibody and suggest ongoing infection
- In 1991 2<sup>nd</sup> gen ELISA added antigens from HCV core and NS<sub>3</sub> helicase, in all 4 antigens
- Soon supplemented with 2<sup>nd</sup> gen immunoblot
- 1994 saw 3<sup>rd</sup> gen ELISAs and immunoblots, fine tuned NS<sub>3</sub> and adding NS<sub>5</sub>
- Other manufacturers produced their immunoblots
- And **PCR was there** almost from the very beginning

# Immunoblot (IB) antigens



**c22p**  
44 a.a.  
10-53

**5.1.1p**  
42 a.a.  
1694-1735

**c100p**  
16 a.a.  
1920-1935

**c33cr**  
SOD 266 a.a.  
1192 1457

**NS5r**  
SOD 942 a.a.  
2054 2995

RIBA-3.0

Pawlotsky et al

Transfusion 1996

The LiaTek III has five synthetic bands, C1 (core), C2 (core), E2/NS, NS4, and NS5, and one recombinant( NS3) antigenband.

# The aim of confirmatory testing

- To identify infected and hence treatable patients – which calls for detection of virus (PCR, HCV antigen)
- Epidemiological - to interrupt transmission (PCR, HCV antigen)
- Population monitoring of confirmed HCV exposure – IB used since 1990 in notification and statistics –
- But IB alone over-interprets current infection in those IB pos/HCV RNA negative

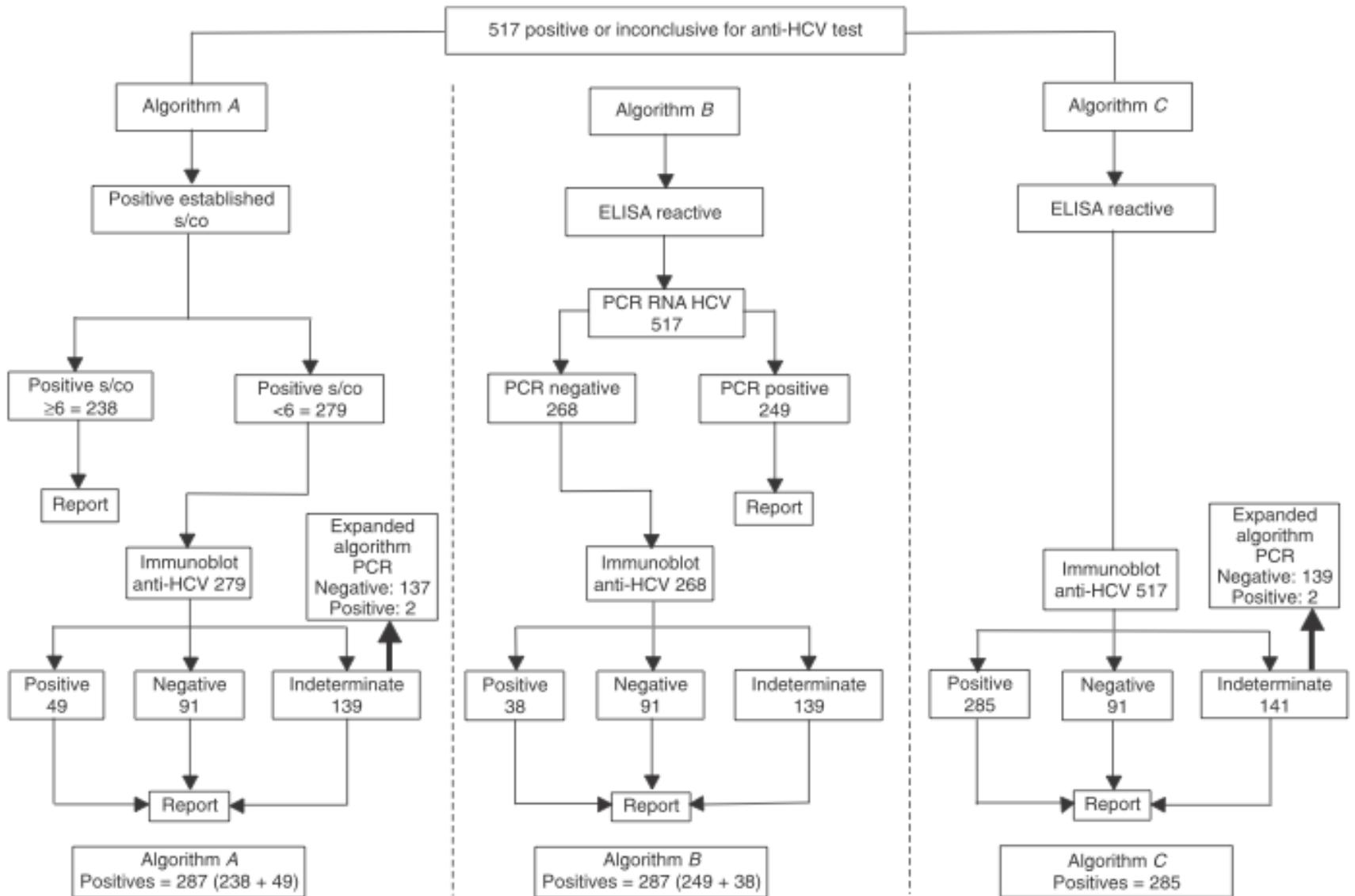
# What do HCV IBs do?

- Change of solid phase from plastic to nitrocellulose/nylon
- Four or more analyses in one (compare Luminex)
  - Two or more antibody specificities = confirmed anti-HCV
  - One antibody specificity = indeterminate, maybe anti-HCV
  - No antibody specificity = negative, (may-maybe anti-HCV)
- An analysis step done on the original sample – **are not degradation sensitive and wane slowly at clearance**
- **IBs defines HCV antibody status in PCR negative pats**
- IBs clarify HCV antibody status – also in persons **who do not return** for a requested PCR sample (e.g. IDUs)
- Costly and delaying – and an **interim step in diagnosis**

# Some test algorithms on anti-HCV screening positive patients

- Do IB and then ask for a PCR plasma (classical)
- Or test by PCR directly,
  - If PCR positive = confirmed ongoing HCV infection
  - If PCR negative then IB
- Or test for HCV antigen directly,
  - If HCVAg positive = confirmed ongoing HCV infection
  - If neg do PCR and if PCR is neg - do IB
- Or include **anti-HCV S/Co threshold** in your algorithm
- IB pos but RNA neg are resampled and retested for HCV RNA after 3-6 months in Sweden
- IB indeterminates should followed up by PCR and a second sample after 1 month

# Alternative flow algorithms from Brazil, Barreto et al 2008



Kit costs

21.300 USD

32.400 USD

37.675 USD

# Different risk levels – low risk blood and organ donors

- AIM: Stop HCV infected blood transfusions – ASAP
- Prevent transmission to organ recipients
- Handle and counsel deferred blood donors
- Screening for anti-HCV reactive donors – who may be:
  - Chronically HCV infected,
  - Recently infected (seroconverters),
  - Previously infected,
  - or
  - False positive by cross reacting to a non-HCV epitope,
  - False positive by stickiness to solid phase of the assay

# Screening of other low risk individuals

- Pre-operative screening
- Zero-sample in needle stick injuries
- Antenatal screening
  
- Here weak EIA and IB reactivity often is false, at least no ongoing HCV infection is present

# High risk settings

- Emergency wards, gastroenterology and dialysis units
- Prisons
- Needle exchange programs and other surveillance programs of injecting drug users
- Here a weak or indeterminate reaction often indicates incident HCV infection
- However, very often the blood sample is sent without clinical information – except ward allocation

The next slide shows that HCV RNA around seroconversion is not always easy.....



# Now back to antibody tests - Weaknesses of HCV immunoblot

- Interpretation:
  - Risk of longtime classification of an IB pos as “infected” if not followed up by PCR – a delay that may last up to 20 years
- Technical:
  - Antigens are derived around the patented Chiron Genotype 1a isolate
  - Anti-NS<sub>4</sub> in Gt 3 reacts less strongly in RIBA-3 (Dow et al, Transfusion 1996)
  - There was a Chiron serotyping IB, discontinued

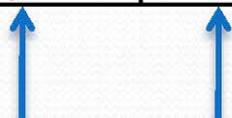
# What does an IB indeterminate result tell?

- Early infection – yes, but rarely , unless risk group
- Antibodies against an epitope from an unrelated source
- Last sign of a waning antibody e.g. "C22" = the Jolly Roger on the top mast of the sinking HCV ship.
- Additional support for earlier HCV infection by:
  - CMI assays (proliferation, ELISpot, tetramer staining)
  - Avidity?
  - Immune precipitation

# EIA, CLIA sensitivity slightly higher than RIBA 3.0

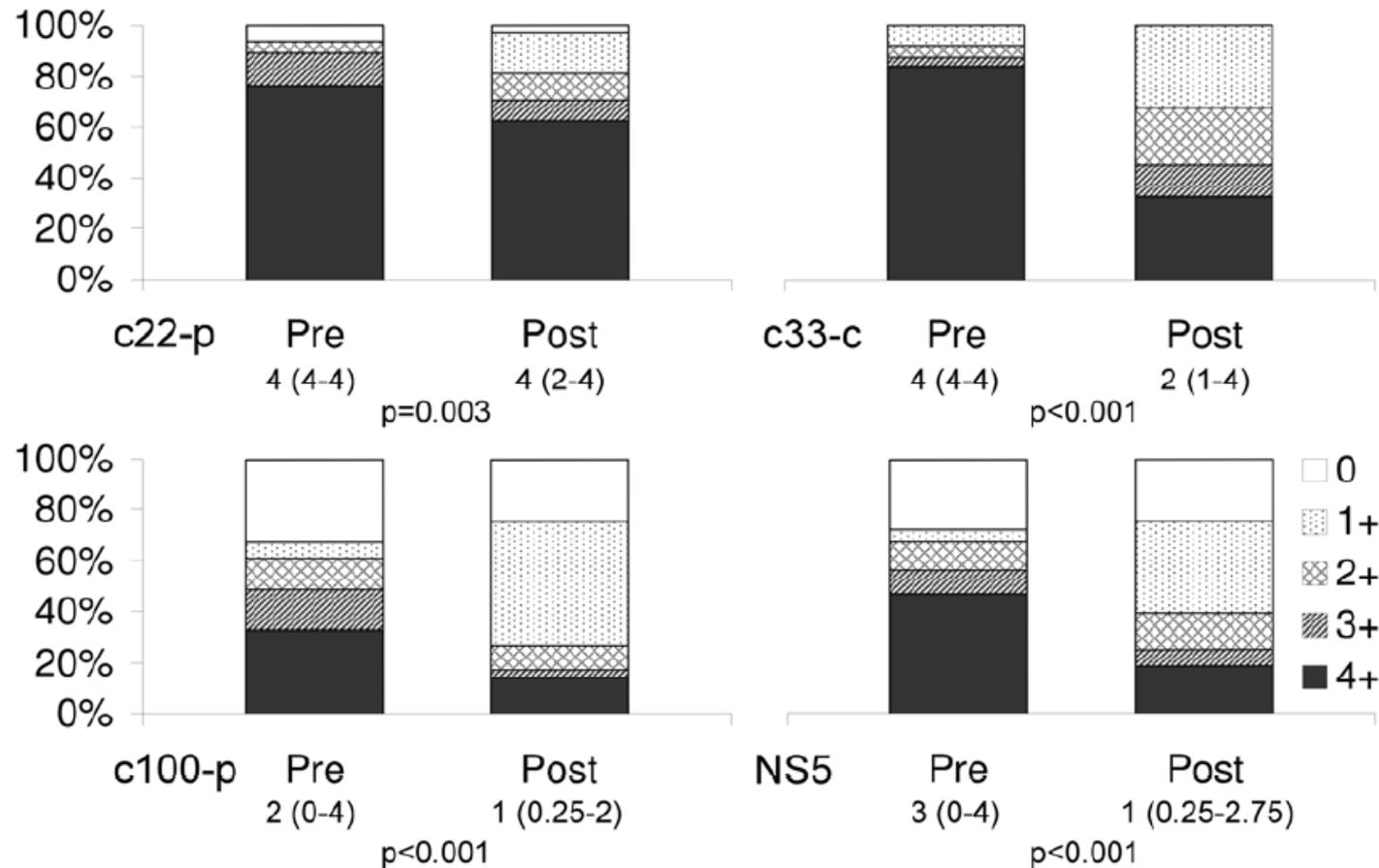
- EIA, CLIA cutoffs are set to detect the weakest anti-HCV positive donor (blood, organ)
- IBs: Cutoff levels “politically” set, to give a high yield of viremic (by PCR)
- Examples from HCV seroconverting IDUs in Malmö, pattern seen in 6/90 consecutive seroconversions

	Last anti-HCV neg		First lag	First anti-HCVpos			Next lag	Next sample		Follow up
	Anti-HCV Arch	HCV Ag		Anti-HCV Arch S/Co	First RIBA	HCV ag		Anti-HCV Arch S/Co	Second RIBA	
Pat1	Neg	50.44 fmol/L	2 m	5,8	0, 2, 0, 0	1879.57 fmol/L	8 m	15	4, 4, 4, 4	nd
Pat2	Neg	Nonreactive	5 m	1,7	0, 0, 0, 0	2698.58 fmol/L	6 m	9,2	1, 4, 0, 0	+++
Pat3	Neg	Nonreactive	6 m	1,5	0, 0, 0, 0	Nonreactive	12 m	6,4	?, 3, 3, 0	nd
Pat4	Neg	Nonreactive	3 m	2,9	0, 0, 0, 0	460.94 fmol/L	3,5 m	8,9	3, 2, 0, 0	++
Pat5	Neg	Nonreactive	3 m	8,3	0, 2, 0, ?	543.84 fmol/L	4.5 m	11,6	2, 4, 2, 0	- w4,5
Pat6	Neg	Exhausted	3 m	2,4	2, ?, 0, ?	88 / 5.59 fmol/L	8 m	10,1	4, 3, 0, 0	-



# Anti-HCV waning after 5 y of SVR following treatment (64 patients)

Figure 3.



# Will current a PCR, performed once, tell the final truth?

- **As shown, window phase viremia may drop to undetectable at seroconversion – to rebound later**
- Increased plasma input and TMA may increase sensitivity three to tenfold.
- But low viremia chronic patients probably are few
- Is there or not occult HCV infection (Michalak / Carreno and others?)
- Borderline viremia may be transmissible (studied in chimp challenge experiments)

# Cost aspects

- Immunoblots are expensive
- HCV in Malmö, Sweden customers prices
  - Anti-HCV screening 14 USD
  - Anti-HCV RIBA 118 USD
  - HCV RNA 136 USD
  - HCV Ag ?
- IB is run in batches – an average 1 week delay
- IB thus may add costs of worry and waiting
- HCV PCR is almost indispensable – unless HCVAg tests positive

# Summary

## Advantages of IBs

IB serology may distinguish true HCV exposure from false anti-HCV on available sample.

Of value in RNA negative patients/conselling

Pursues 20 years of easy notification routines

## Disadvantages of IBs

Many indeterminates

Costs in dollars and delay and worry

Only an interim step in patient handling versus PCR

Reflex PCR or HCVAg faster and reliable when positive

A second follow up sample is often necessary