Antibody Testing and NAT in Oral Fluid and Dried Blood Spots

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Outline

Oral fluid testing

- Background biology
- Development
- Examples of applications

Dried blood spot testing

- Comparison with oral fluids
- Sensitivity
- HCV RNA detection & characterisation
- Possible applications

Why Oral Fluid ?

BENEFITS

More acceptable

- non-invasive
- painless

More convenient

- self-collected (cheaper)
- rapid, simple
- no sterile precautions

Less hazardous

Easier access

- community studies
- outbreaks

POTENTIAL DISADVANTAGES

- Lower IgG/IgM levels
  - Lower sensitivity ?
- Less well characterised
- Subject-to-subject variability
- Limited markers available

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

Salivary antibody testing: Secretory IgA?

Archibald et al, Blood, 1986

	IgA Anti-HIV (RIPA)	
	serum	saliva
AIDS (20)	20	14
ARC (15)	14	14
Partners of AIDS/ARC (20)	9	9
Healthy homosexual men (18)	4	4
Healthy HIV +ve blood rec. (1)	1	1
Controls (21)	0	0

Salivary antibody testing:
Preliminary experiments suggest class-specific IgG capture may be optimal

PHLS Virus Reference Division (1985/6)

Patient *(status unknown)*	IgA Capture	indirect EIA (IgG)	Competitive Assay (RIA)	IgG capture
1.	*neg*	*neg*	*neg*	*neg*
2.	*neg*	*neg*	*neg*	9.1
3.	*neg*	*neg*	*neg*	1.1
4.	*neg*	*neg*	*neg*	*neg*
5.	*neg*	*neg*	equivocal	15.9
6.	*neg*	*neg*	*neg*	*neg*
7.	1.5	*neg*	equivocal	8.2

Salivary testing:
Reaching an understanding…
IgG capture: serum ≈ saliva S/CO

PHLS Virus Reference Division (1985/6)

Patient	IgG capture (S/CO)		Competitive (%inhibn.)	
	serum	saliva	serum	saliva
1.	0.4	0.3	5	-10
2.	9.5	7.5	81	26
3.	11.5	11.5	82	57
4.	15.5	10.0	91	10
5.	14.5	13.0	83	36

Another piece of the jigsaw: oral immunology

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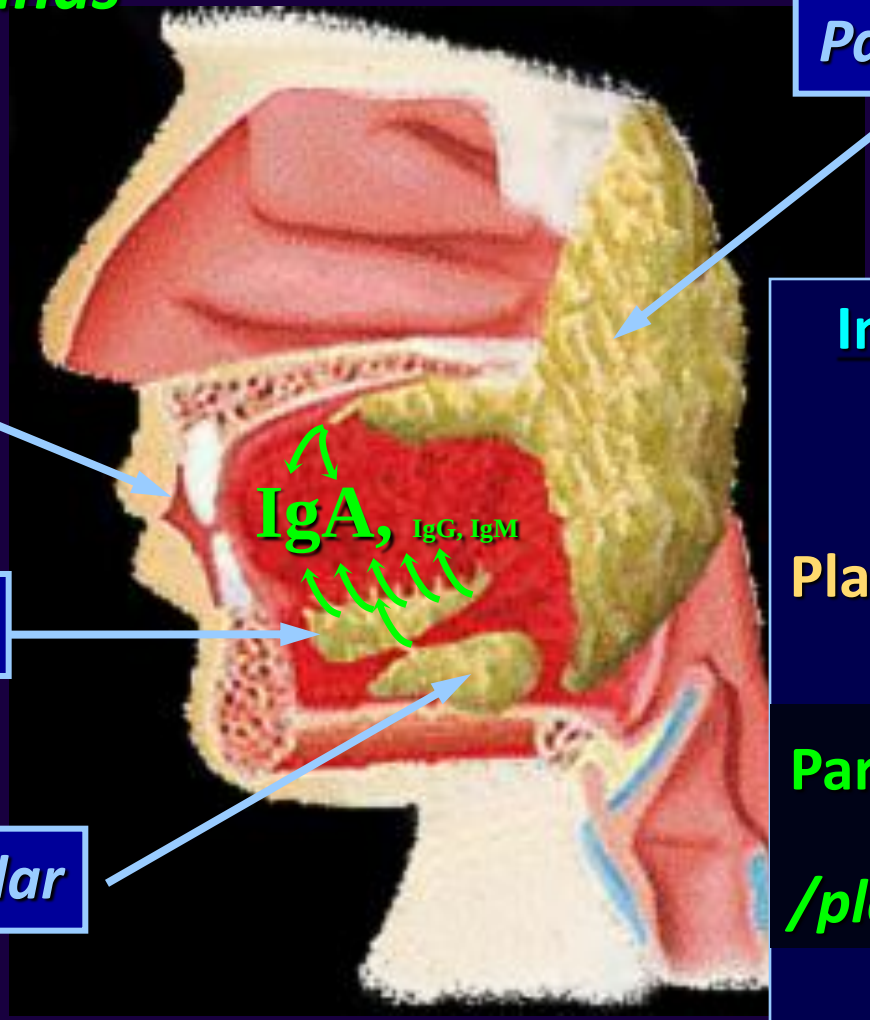
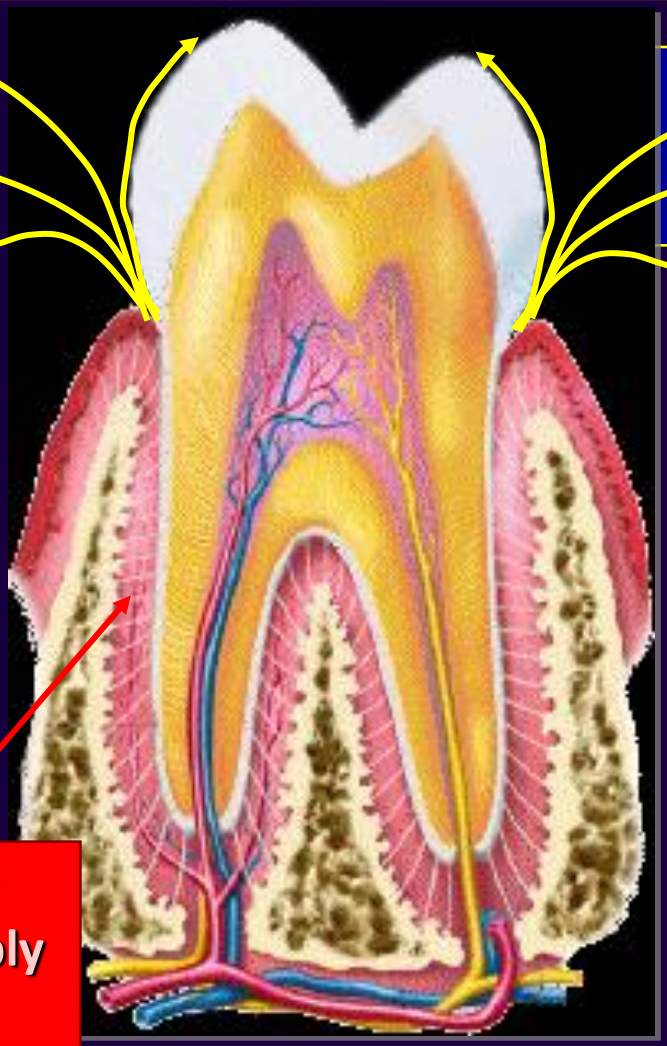
Salivary Glands



Immunoglobulin Levels (mg/L)	IgA	IgG
Plasma	2,200	12,500
Parotid	40	0.4
/plasma	1/55	1/30,000

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

IgG (and IgM) in oral fluid is plasma-derived



Capillary blood supply to gums

Gingival crevicular fluid → *Whole saliva*

Immunoglobulin Levels (mg/L)

	IgA	IgG
Plasma	2,200	12,500
Gingival crevicular	1,100	3,500
/plasma	*2*	*3.5*
Whole saliva	190	14
/plasma	*1/12*	*1/900*

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

Initial proof of principle – Lancet, 1987

SENSITIVE ASSAYS FOR VIRAL ANTIBODIES IN SALIVA: AN ALTERNATIVE TO TESTS ON SERUM

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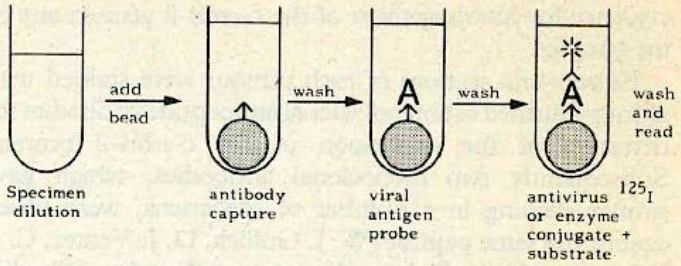
Summary Paired serum and saliva specimens were tested by conventional assays and by IgG-capture radioimmunoassays (GACRIA) and enzyme-linked immunosorbent assays (GACELISA) for antibody to hepatitis A virus (HAV, 100 pairs), human immunodeficiency virus (HIV, 53), l (HBc, 62), and rubella virus (30). Con to detect viral antibodies in the s seropositive subjects. However, GA antibodies in both serum and sa seropositive by conventional tests, exc false-negative for anti-HBc and 1 fa rubella-virus. For anti-HIV and anti-l



Procedure for detection of viral antibody in serum and saliva by class-specific antibody-capture assay.

phases are saturated by a wide range of serum dilutions and the test signals therefore remain more or less constant.[2,4] It

TABLE I—RESULTS IN CONVENTIONAL TESTS AND BY CAPTURE ASSAYS ON PAIRED SERUM AND SALIVA SPECIMENS

—	Conventional test: serum	GACRIA*		GACELISA:* saliva†
		Serum	Saliva†	
Anti-HAV				
Positive	34	34 (20–63; 40)	34 (6–56; 19)	32/32 (0·47–>2·00; >2·00)
Negative	66	66 (0·5–1·8; 0·9)	66 (0·6–1·4; 0·9)	65/65 (0·05–0·13; 0·08)
Anti-HIV				
Positive	43	43 (11–33; 18)	41/41 (4–28; 18)	43 (0·37–>2·00; 1·37)
Negative	10	10 (0·6–1·2; 1·0)	10 (0·8–1·0; 0·9)	10 (0·08–0·12; 0·09)
Anti-HBc				
Positive	25	25 (2–141; 20)	23 (1–184; 13)	18/22 (0·04–>2·00; 0·17)
Negative	37	37 (0·7–1·5; 1·0)	37 (0·7–1·3; 1·0)	36/36 (0·01–0·05; 0·02)
Anti-rubella				
Positive	27	27 (5–49; 16)	26 (2–43; 12)	17 (0·14–0·97; 0·33)
Negative	3	3 (0·8–1·1; 1·0)	3 (0·7–0·9; 0·9)	3 (0·08–0·10; 0·09)

*Figures in parentheses = range (including both positive and negative findings); for GACRIA median test/negative ratio or for GACELISA median optical-density value.
†Not all saliva specimens could be tested because volumes collected were inadequate.

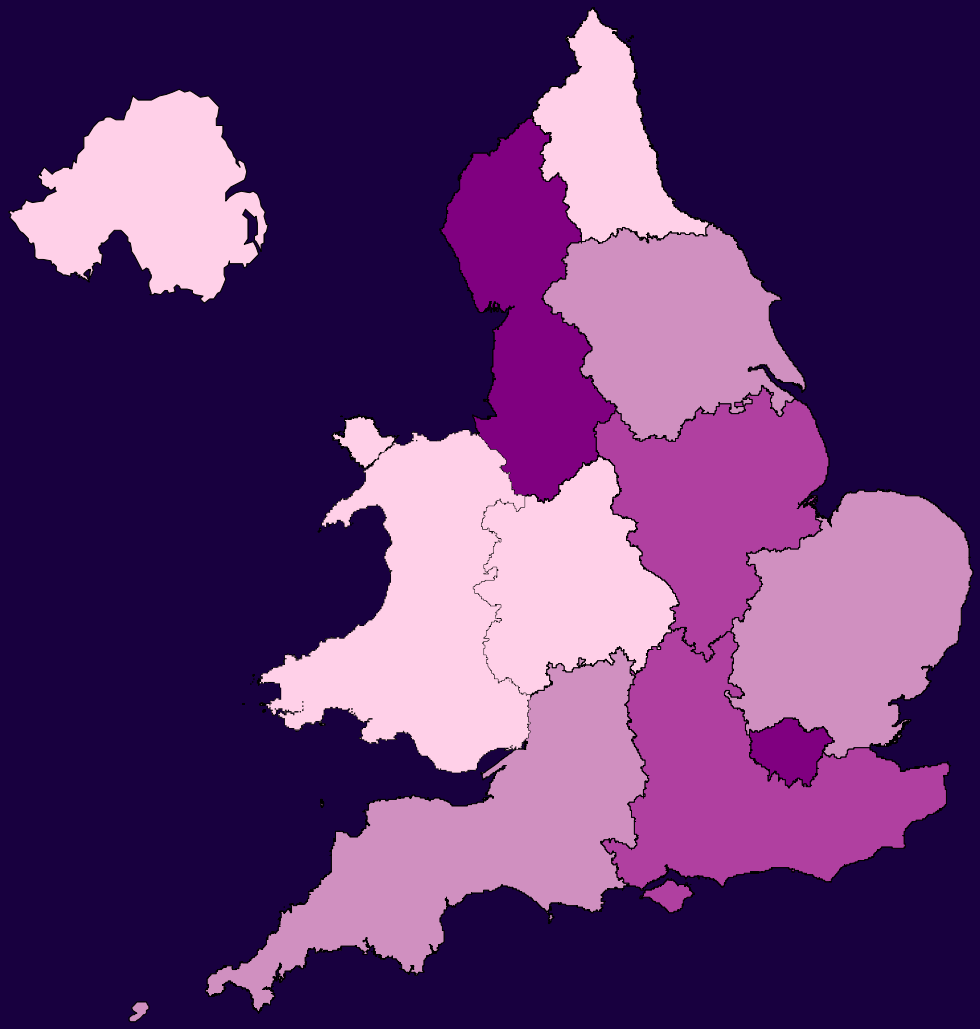
Collection of oral fluids - which method ?

Oral Fluid Anti-HCV: Modified Ortho Assay

NEG mean + SD	Orasure Sens (n=252)	Orasure Spec (n= 392)	Salivette Sens (n=251)	Salivette Spec (n=391)
+2 SD	91.7	99.2	74.1	99.0
+3 SD	88.1	99.5	69.3	99.7
+5 SD	84.1	100	64.5	99.7

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

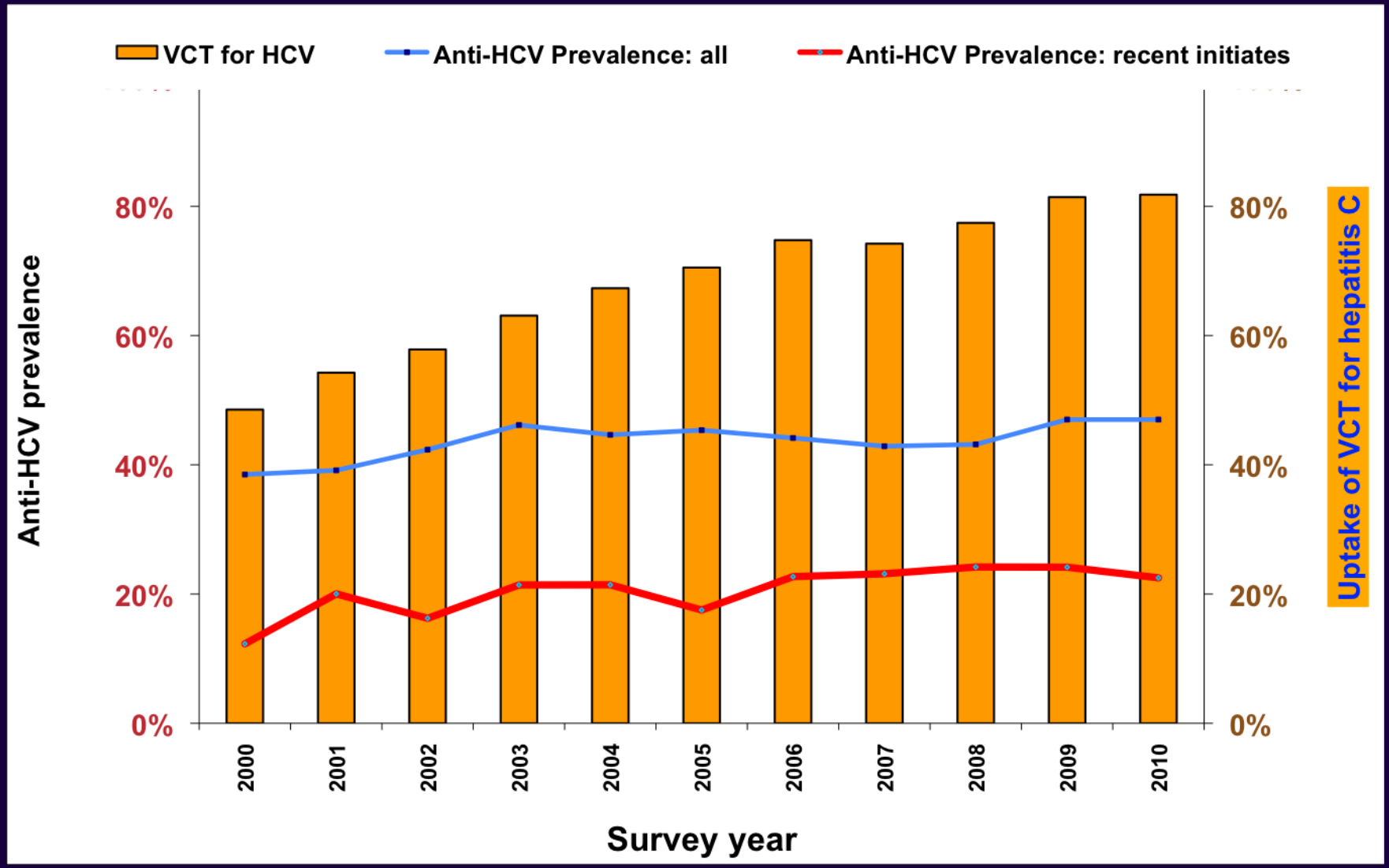
Proportion of IDUs with Oral Fluid antibodies to HCV (voluntary UA Survey)



- 50% and greater
- 40% to 49%
- 30% to 39%
- less than 30%

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

Tracking hepatitis Infection: Voluntary UA OF Survey of IDUs



PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

BBV Incidence Estimates:
Traditional capture - recapture approach

- Current IDU <30 yrs or <6yrs injecting
- London & Brighton - ORAL FLUID specimen
- Recruited in community 2001 (n=428)
- Re-captured 2002 (n=300)

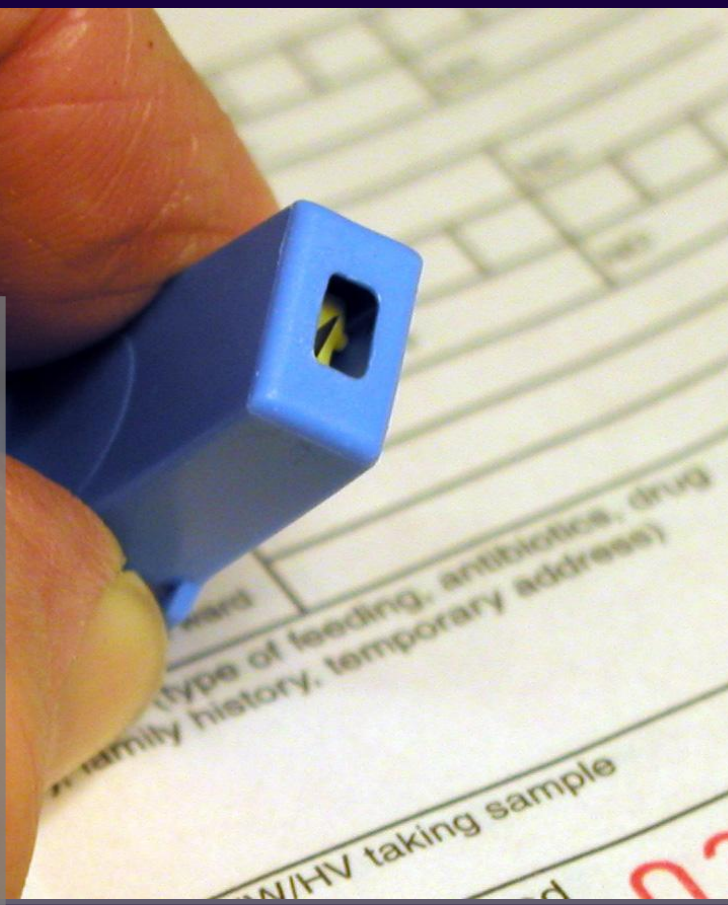
	Baseline		Follow-up	
	pos/total	prevalence	SCVNs/total	incidence
HCV	187/428	43.7%	53/151	41.8%
HIV	18/428	4.2%	9/273	3.4%

A Judd *et al* (2005) BMJ **330**: 24-25.

Oral Fluid Summary

- OF is a convenient specimen
- Can be expensive to collect (>DBS)
- Stable & easy to ship
- Anti-HCV antibody sensitivity equivocal
  - False negatives may be mostly HCV RNA negative (resolved HCV)
- HCV RNA sensitivity poor
- Applications for:
  - Prevalence
  - Incidence estimation??
  - Diagnostic screening?

Dried Blood Spots: Greater potential ?

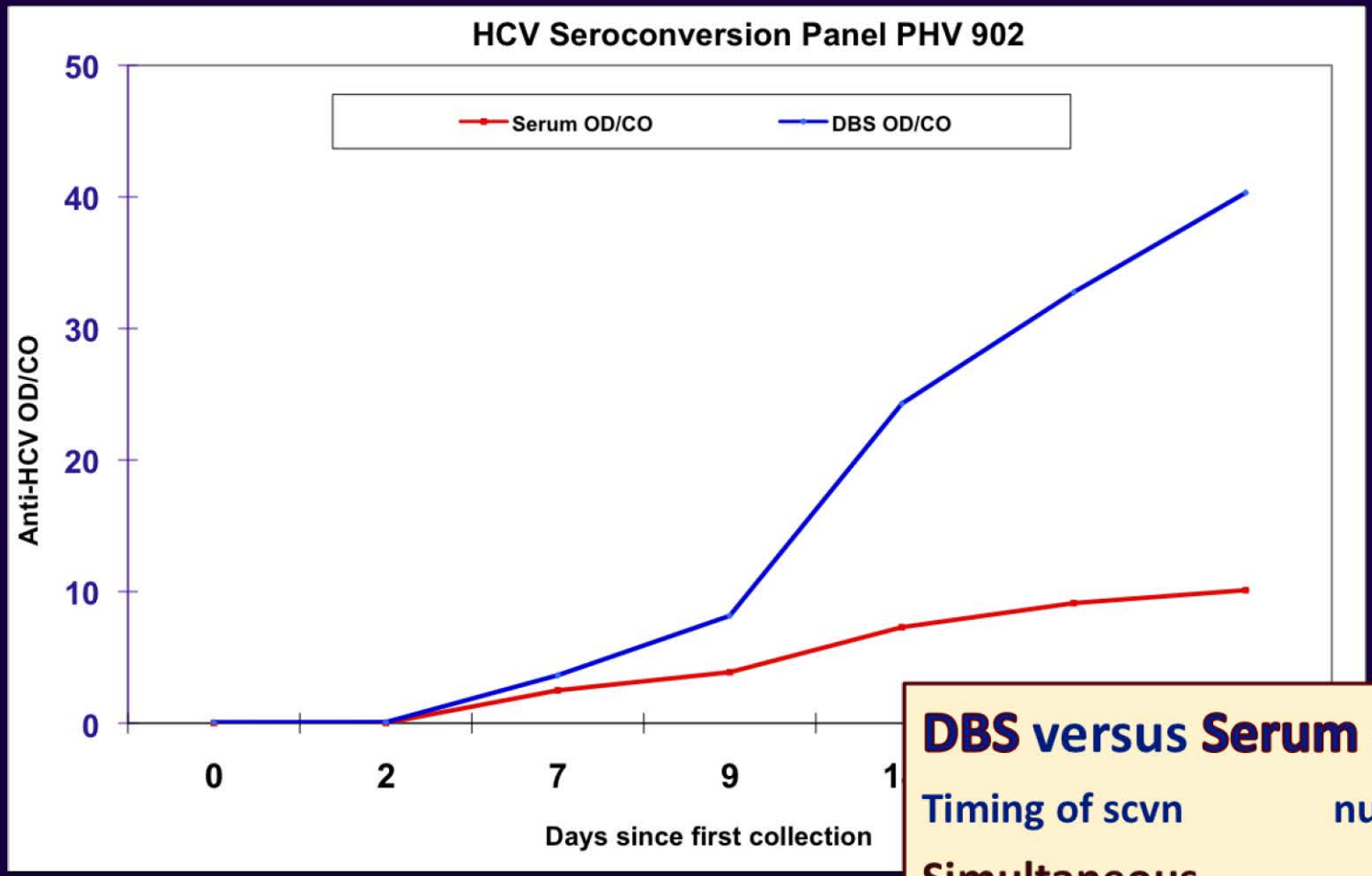


DBS Anti-HCV: Modified Ortho 3.0 EIA

	Orasure		Dried Blood Spot	
NEG mean + SD	Sens (n=252)	Spec (n= 392)	Sens (n=252)	Spec (n=381)
+2	91.7	99.2	100	96.9
+3	88.1	99.5	99.6	99.7
+5	84.1	100	99.6	100

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

Detecting anti-HCV in DBS at seroconversion

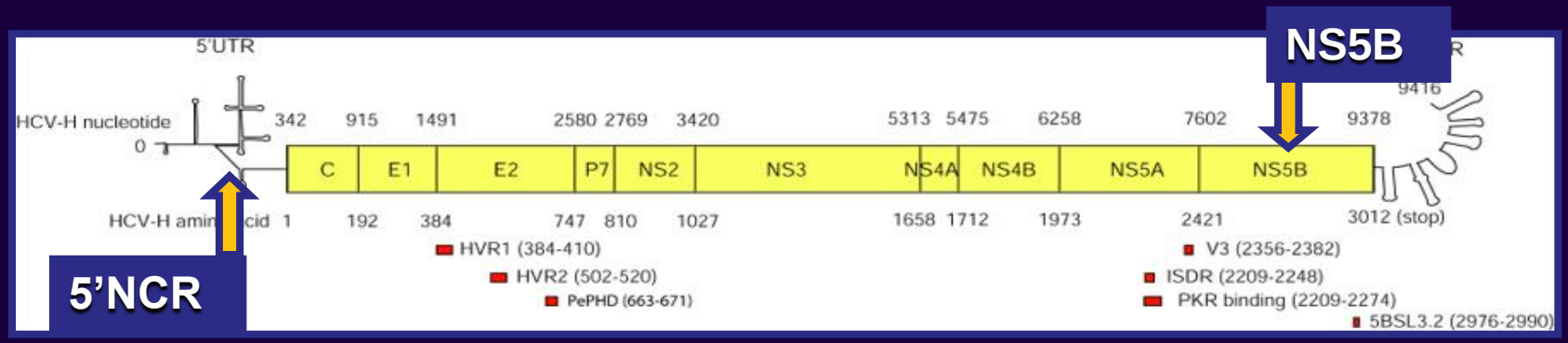


DBS versus Serum on 13 seroconv. panels

Timing of scvn	number	interval (days)
Simultaneous	9	0
DBS Later	2	9; 8
DBS Earlier	2	7; 3

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

HCV RNA detection:
targets routinely used in VRD



Genotyping by RFLP

Genotyping by sequencing

Nested PCR!!

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

‘Clinical’ sensitivity: DBS* from characterised infections

- *Serum HCV RNA concentrations: $1.2x10^3$ - $1.7x10^7$*
- *Frequency of DBS RNA positive findings:*

	HCV genotype					
	1	2	3	4	5	total
n=	*28*	*5*	*30*	*8*	*1*	*72*
PCR target						
NS5B	28	5	30	8	1	72
5' NCR	11 (39%)	3 (60%)	21 (70%)	5 (63%)	1 (100%)	41 (57%)

PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

* Simulated DBS

Pilot roll-out in HM Prison Shrewsbury & various IDU services in Bristol

	HIV			Anti-HBc			HBsAg		HCV					
									Anti-HCV			HCV RNA (NS5B) (on anti-HCV +ve)		
	tested	Detect-ed	% of all	tested	Detect-ed	% of all	Detect-ed	% of all	tested	Detect-ed	% of all	tested	Detect-ed[2]	% of anti-HCV pos
Shrewsbury	188	0	**0.0**	186	13	**7.0**	1	**0.5**	187	21	**11.2**	21	16	**76.2**
Bristol	389	3	**0.8**	400	78	**19.5**	5	**1.3**	413	177	**42.9**	176	116	**65.9**

[2] there was an additional HCV RNA positive / anti-HCV negative test in a patient who subsequently seroconverted.

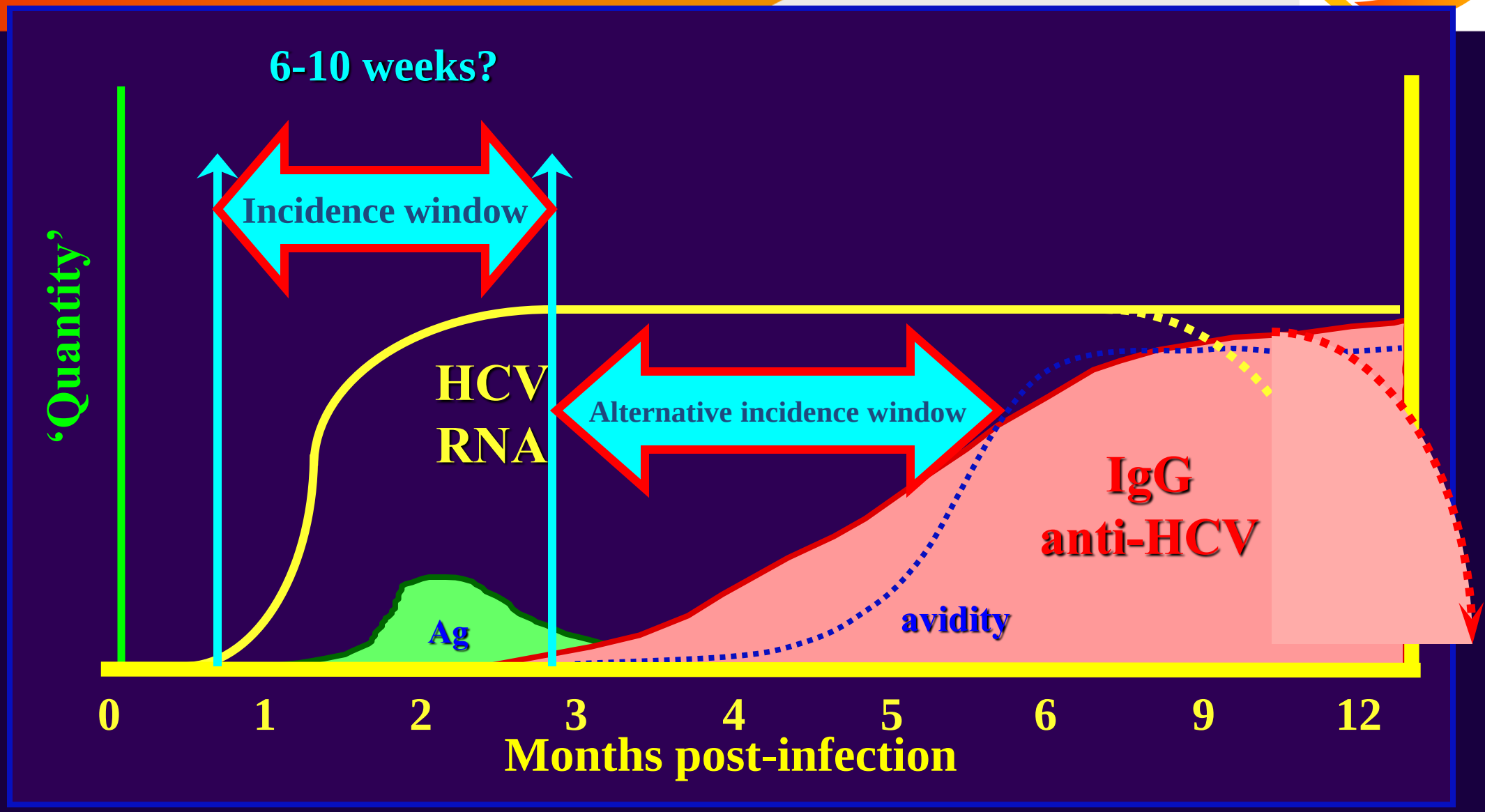
PARRY: Oral fluid & DBS HCV Testing, CDC Dec 2011

Further investigation of DBS PCR sensitivity:
Piloted on Community Survey

- Survey in large English regional city
- Current IDUs recruited from community (Respondent Driven Sampling)
- Questionnaire administered
- 288 fingerstick DBS specimens available
- Tested for anti-HCV within 2-3/12
- Anti-HCV positives: 174 (60%)
- Anti-HCV positives tested for HCV RNA 12/12 later
- of which, HCV RNA positive: 102 (59%)

Events following HCV infection:

Using them to determine HCV incidence



Investigation of HCV RNA to estimate incidence: Piloted on Community Survey

$$I = \frac{(365/T)n}{N + (365/T)(n/2)}$$

- 288 Fingerstick DBS specimens collected
- Anti-HCV positives: 174 (60%)
- Anti-HCV negatives (n=114) tested for HCV RNA
- of which, HCV RNA positive: 14 (12%)
- Employing a window (T) of 61 days (Busch *et al*) and a susceptible population (N) of 114, the estimated HCV incidence (I) was:

  54%

- Employing a window (T) of 120 days and a susceptible population (N) of 114, the estimated HCV incidence (I) would be:

  31%

Bristol RDS Survey Genotyping

114 of the 117 HCV RNA positives typed

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The genotypes found were:

1a x60,
3a x46,
2b x4,
2a x2,
4d x2.

Of the 'incident infections' (anti-HCV-negative/RNA-positive):

13 were 1a

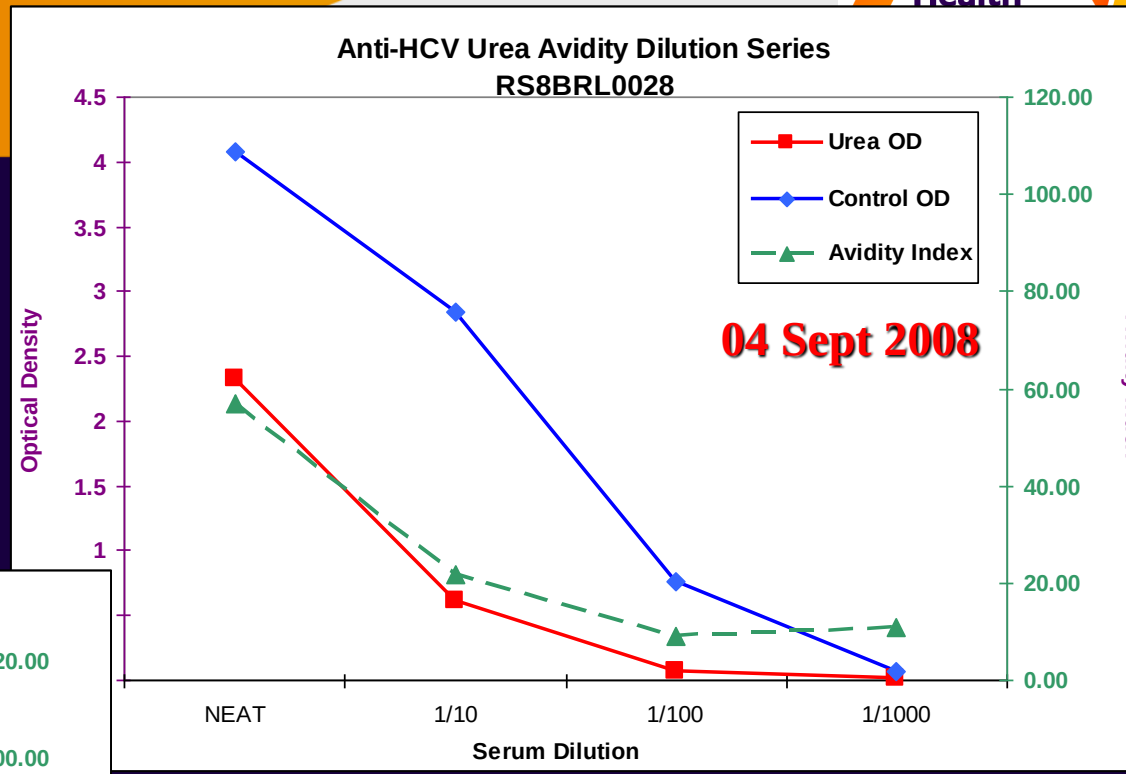
1 was 3a

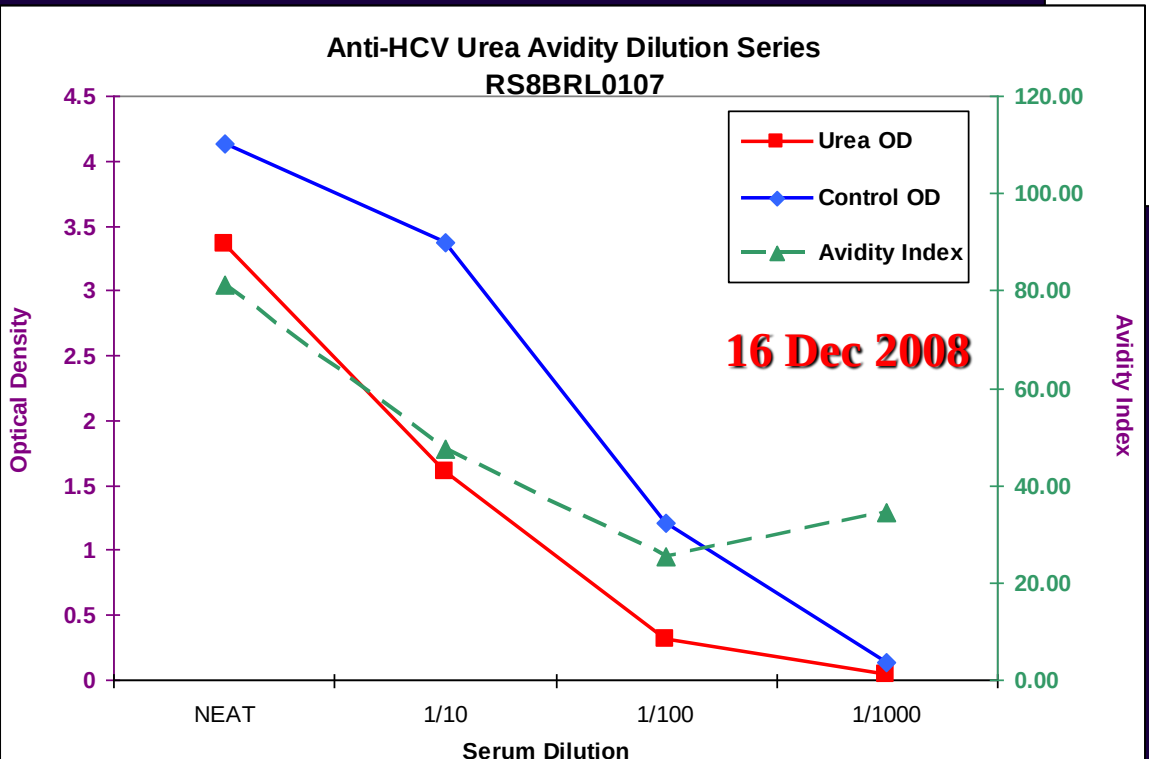
Bristol RDS Survey: HCV molecular epidemiology based on DBS samples

Is there any utility in AVIDITY testing?

DBS collected 103 days apart from a viræmic IDU

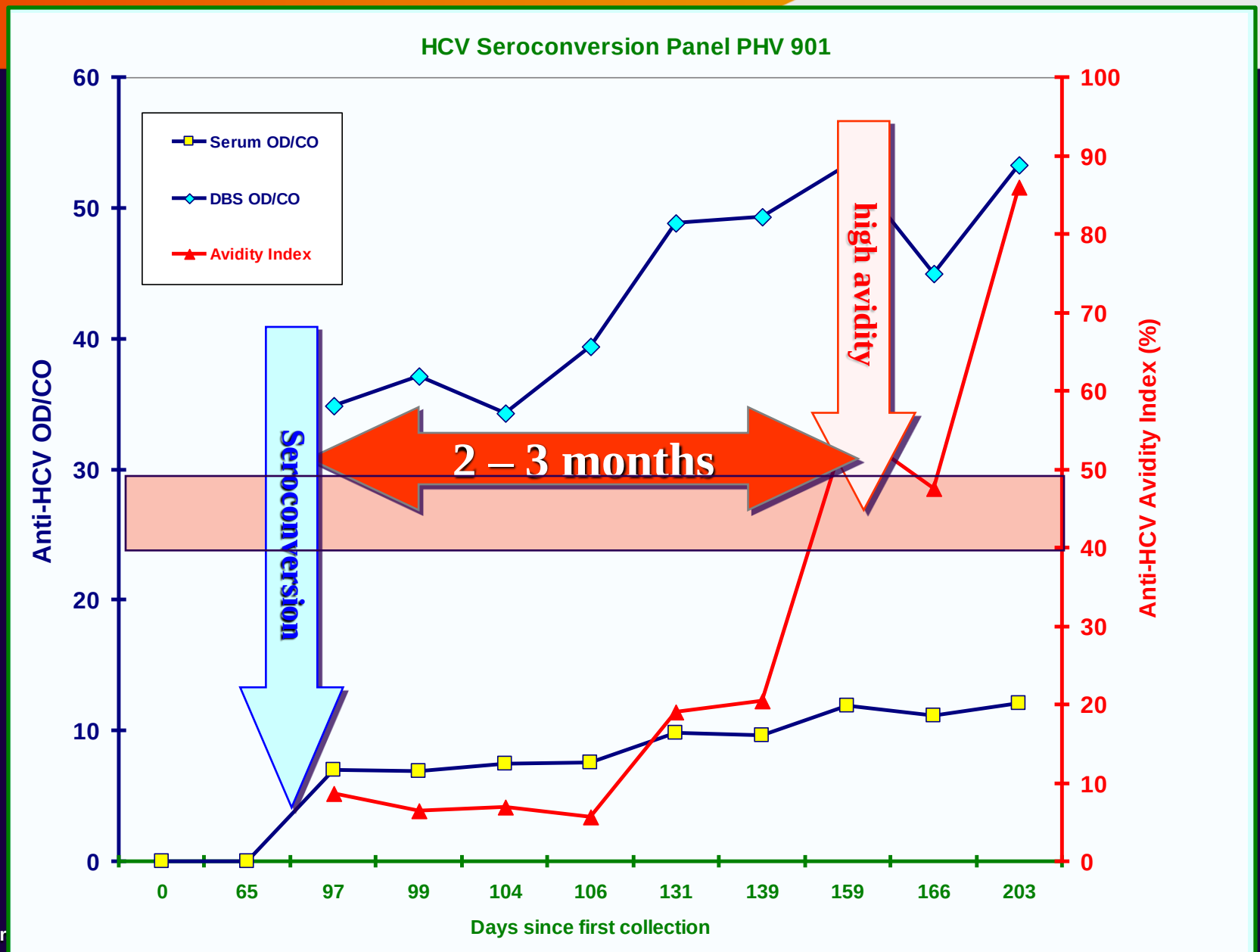
- Some increase in 'control' reactivity
- Greater increase in Urea reactivity





- Increased Avidity measurements
  - Neat: 55% → 80%
  - 1/10: 20% → 50%

Anti-HCV Seroconversion (PHV 901): Evolving reactivity & avidity (simulated DBS)



Avidity Index Measurements on All anti-HCV Positive DBS Specimens from Pilot

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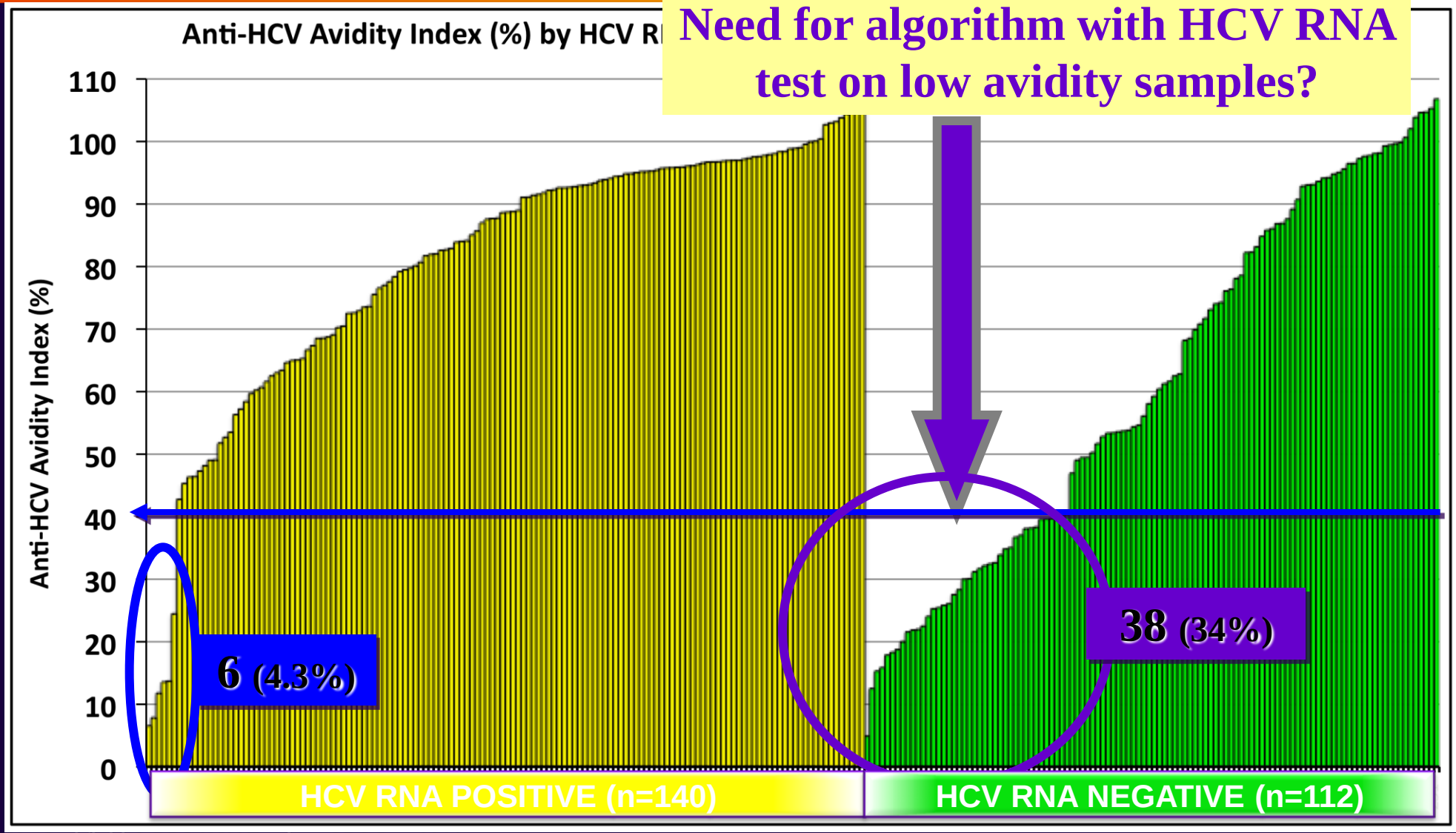
Need for algorithm with HCV RNA test on low avidity samples?



DBS Summary

- DBS is a convenient specimen
- Inexpensive to collect – cheaper than oral fluid
- Stable & easy to ship
- Excellent sensitivity for antibodies & nucleic acids (other BBVs)
- Applications for:
  - Prevalence
  - Past infection
  - Molecular epidemiology
  - Incidence estimation (possibly)
  - Diagnostic screening

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