Update on UK NEQAS Serology schemes

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What is EQA – what does it do? – what can it do?

- The challenge of laboratory procedures with specimens of known but undisclosed content
- EQA provides assessment of:
  - the overall standard of performance (state of the art; comparison with other participants)
  - the influence of analytical procedures (methods, reagents, instruments, calibration)
  - individual laboratory performance
  - proficiency of staff
  - the specimens distributed in the scheme

- Educational stimulus to improvement
- Provide an insight into the quality of the routine work of the laboratory
- Provide reassurance that all the components of the quality system are working
- ISO17025/15189 participation in EQA is required to document quality as a part of the accreditation process (NA/DANAK)

Source material of UK NEQAS serology specimens

The main matrix is serum

- National Blood Service

  plasma positive for a marker e.g. anti-HCV, anti-HIV, anti-T pallidum…

  plasma negative for anti-HCV, anti-HIV and HBsAg:

  . screened and characterised for common markers (CMV IgG, VZV IgG…)

  . used as diluent for other markers (eg CMV DNA)

- Other commercial sources

  Acute disease state plasma such as Rubella IgM, Acute EBV markers and PB19 IgM

  ➔ *Thrombinised into serum, bronidox 0.05%, screen for IM&RF*

  ➔ *Specimen preparation:*
  - *Neat/undiluted*
  - *Dilution/pool*

How do we establish our values and what instruments are used?

Instruments & Assays

- Pre-distribution testing: specimens are tested with a panel of different assays.
- Assays: the most popular manual and automated assays that reflects our participants practice (regularly reviewed)
- Reference/gold standard assay by a reference laboratory, where applicable
  - e.g. SRH (single radial hemolysis) to establish Rubella IgG potency by NIBSC *(National Institute for Biological Standards and Control)*

Intended results

- The values / intended results are established from the results obtained during pre-distribution testing.
- If pre-distribution testing shows discrepancies (‘not designated’) a decision is made on whether the specimen should be scored.

Report formats / Intended results: how do we establish our values?

Qualitative / serology schemes anti-HIV, anti-HAV, Rubella IgM…

Intended positive or negative

Semi-quantitative anti-HBs, Rubella IgG

Intended / < or > cut-off international unit

Rubella IgG < or ≥ 10IU/mL

Anti-HBs <10 or 10-100 or >100mIU/mL

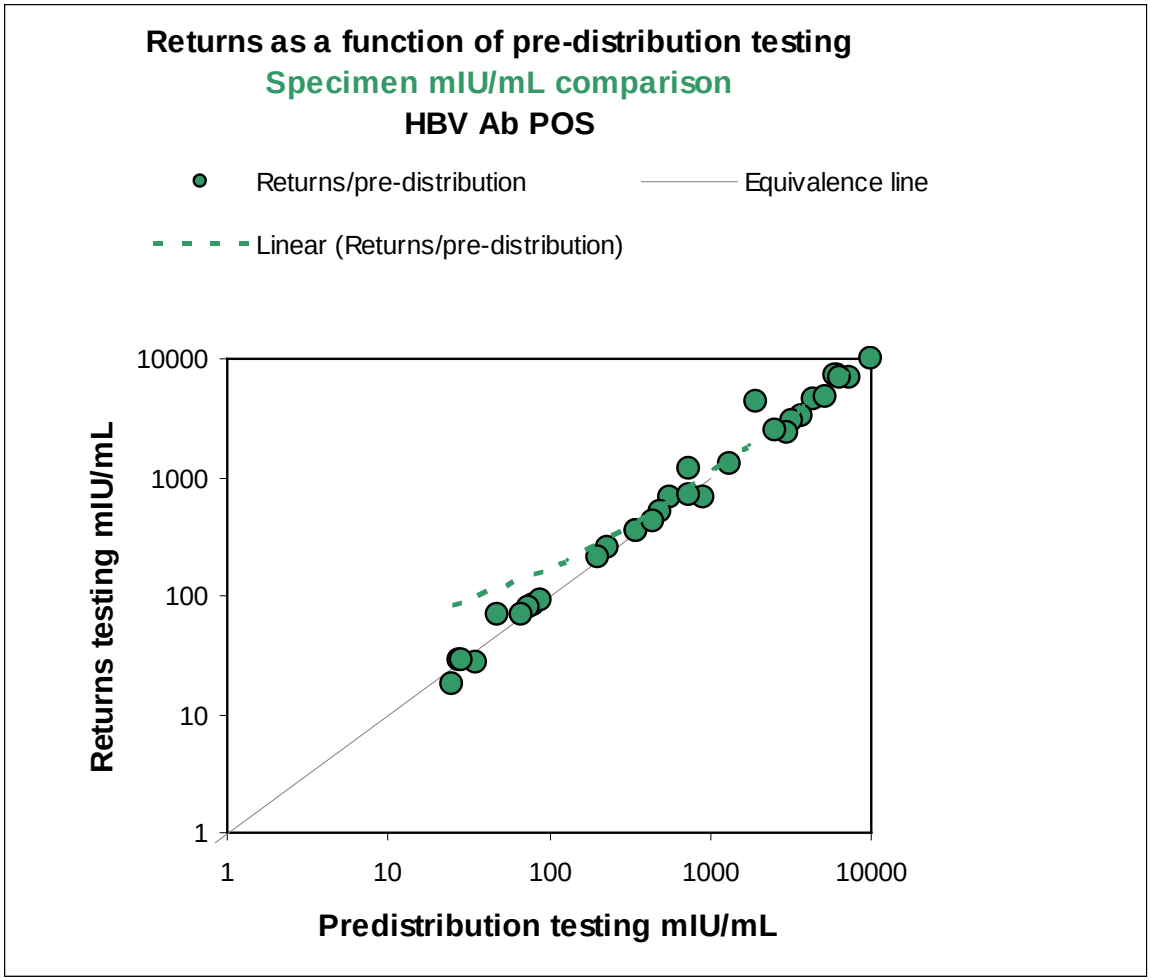
Scoring is based on qualitative results

Quantitative / molecular schemes viral load of HCV, HIV-1, HBV & CMV

Pre-distribution results are only an indication for the intended result

Intended result = A consensus of the participants results

How do we confirm specimen stability?



Process for investigation of suspected assay failure

…where an assay gives discrepant results in comparison to other assays results and for a significant number of participants.

•Report to the manufacturer listing anonymously for each result:

Qualitative result
Batch number
Cut-off
Read out (OD/index/copies)
Average-SD-
Average-SD for 2 or 3 other assays

•Manufacturer: investigations on the specimen and on any changes in the assay (feed back from participants)

•UK NEQAS & Manufacturer to establish the cause and whether this affects only EQA specimen or may affect clinical sample analysis as well

•Possible implications for clinical sample analysis: contact the MHRA e.g. batch issue

MHRA: Medical & Healthcare products Regulatory Agency

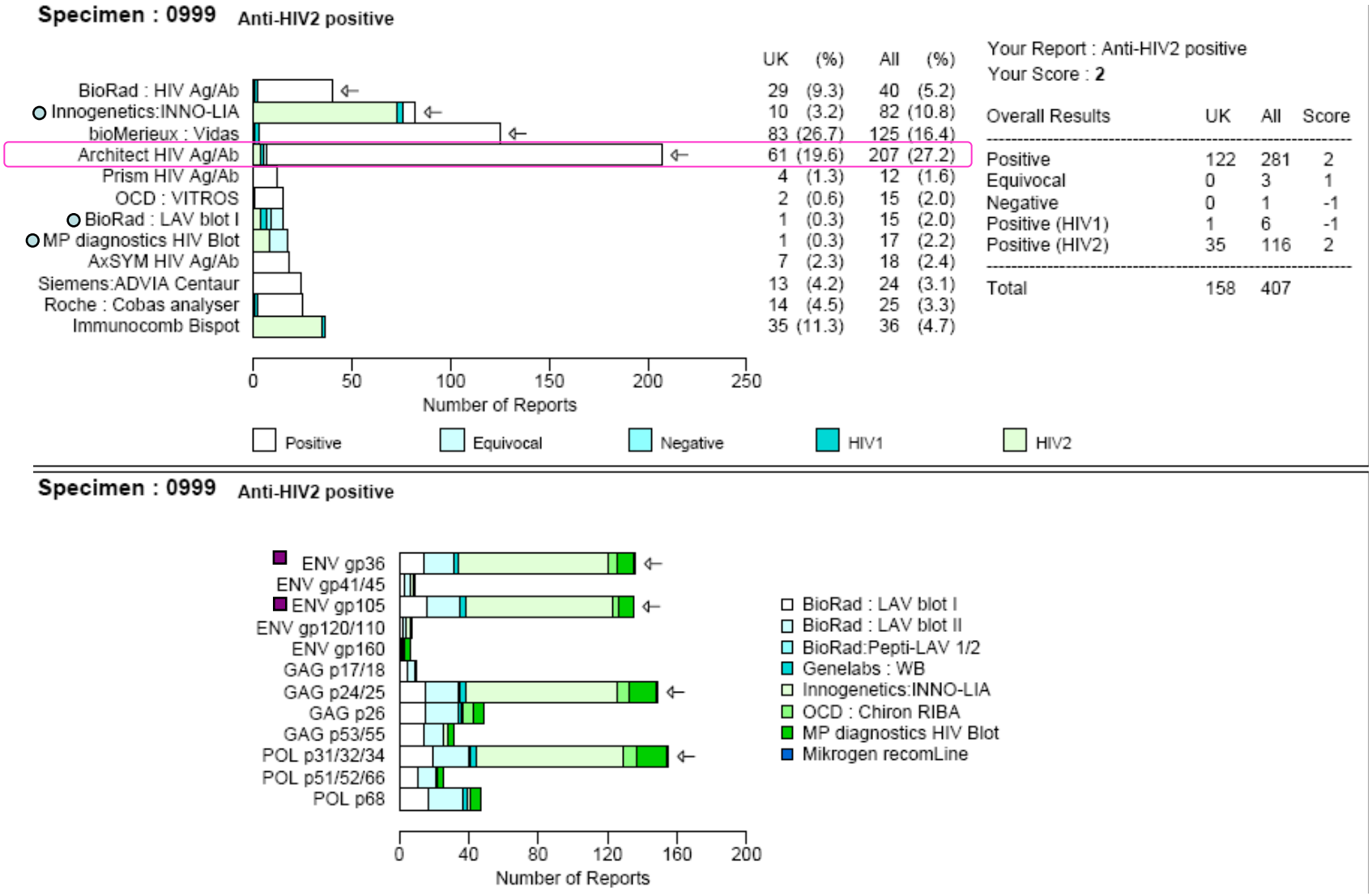
Schemes (introduction year) → YEAR / changes to schemes

Virus identification (1972)
Syphilis serology (1978)
Hepatitis B serology (1978)
Rubella IgG serology (1979)
HIV serology (1986)
Hepatitis C serology (1994)
Anti-HBs detection (1996)
Immunity screen (1996)
HIV-1 RNA quantification (2000)
Hepatitis C RNA detection (2001)
Diagnostic serology / exanthema screen (2001)
Diagnostic serology / hepatitis screen (2001)
HBV DNA quantification (2003)
Molecular detection of viruses in CSF (2003)
Blood Borne Viruses (2004)
C. trachomatis DNA (2005)
CMV DNA quantification (2007)
Measles IgG serology (2009)
Molecular detection of HPV (2009)
EBV DNA quantification (2011)
HIV Point of Care testing (2011)

Representation of Architect assays within schemes

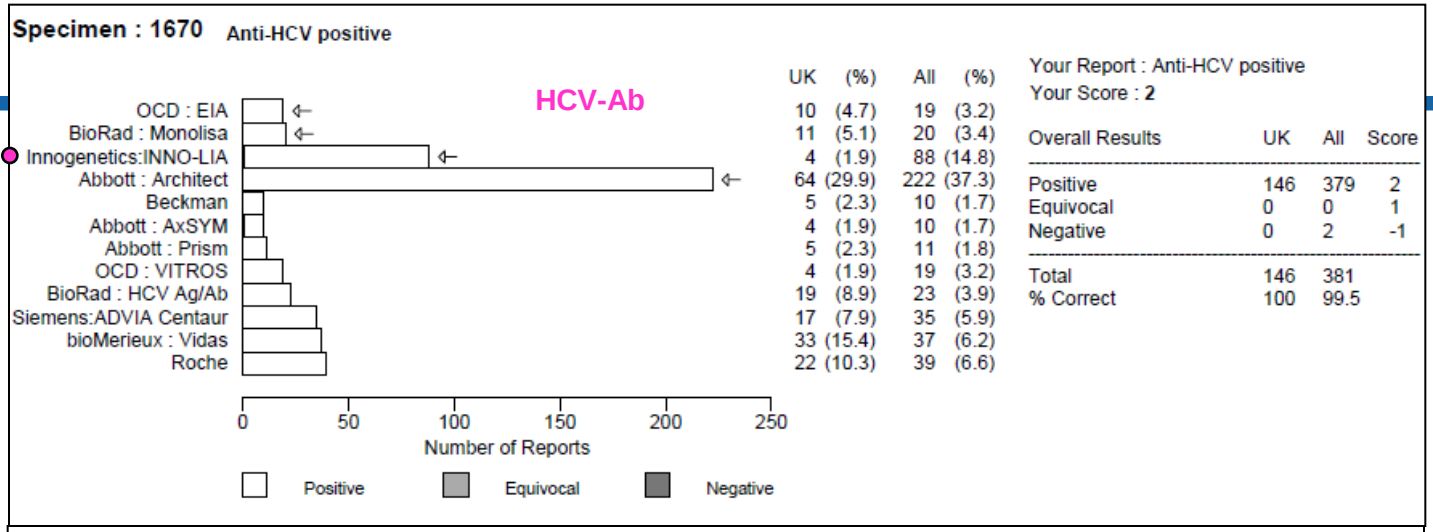
Scheme	Marker	2009	2011	2012, %	2012, n
Anti-HBs detection	HBs Ab	40%	45%	64%	198
HBV serology	HBe Ab	44%	50%	50%	140
HBV serology	HBe Ag	44%	50%	50%	137
Syphilis serology	Tp Ab	31%	43%	49%	175
HBV serology	HBc IgM	na	46%	48%	119
Immunity screen	HAV IgG	34%	43%	46%	108
HBV serology	HBc Ab	37%	46%	46%	194
Dg serology / hepatitis screen	HAV IgM	32%	42%	44%	131
Rubella IgG serology	Rubella IgG	23%	31%	36%	140
HBV serology	HBs Ag qual.	31%	36%	34%	206
HCV serology	HCV Ab/ Ag	29%	33%	34%	199
Blood Borne virus	HBsAg,HCVAb,HBVAb	30%	35%	31%	~41
Immunity screen	CMV IgG	15%	28%	29%	85
HIV serology	HIV Ab (21%)	21%	25%	27%	207
Dg serology / exanthema screen	Rubella IgM	10%	19%	25%	49
Dg serology / hepatitis screen	CMV IgM	12%	20%	22%	68
Toxoplasma serology	Toxo IgG			9%	27
Toxoplasma serology	Toxo IgM			8.5%	14
Toxoplasma serology	Toxo IgG avidity			7.7%	4
Dg serology / exanthema screen	ASO	2.1%	3.8%	4.7%	6
Dg serology / exanthema screen	ASD	na			
Dg serology / exanthema screen	PB19 IgM	na			
Dg serology / hepatitis screen	VCA IgM	na			
Immunity screen	VZV IgG	na			
Measles IgG serology	Measles IgG	na			
Mumps IgG serology	*Mumps IgG*	*na*			

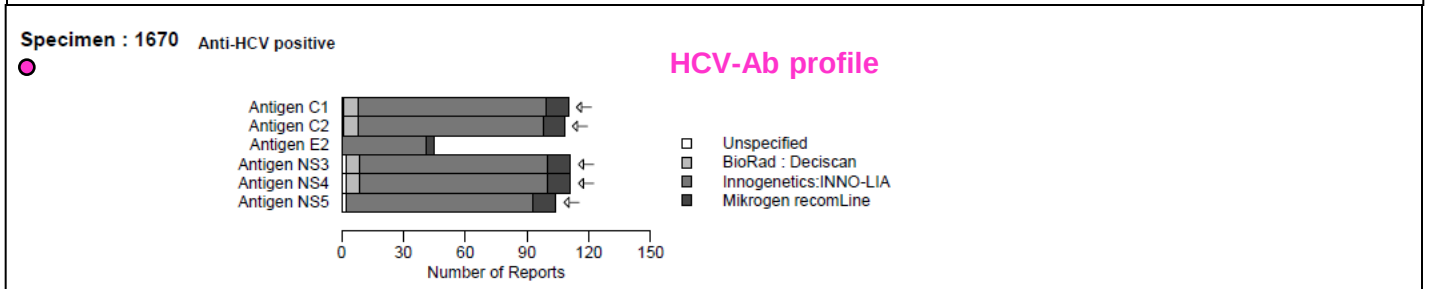
HIV serology scheme – June 2012

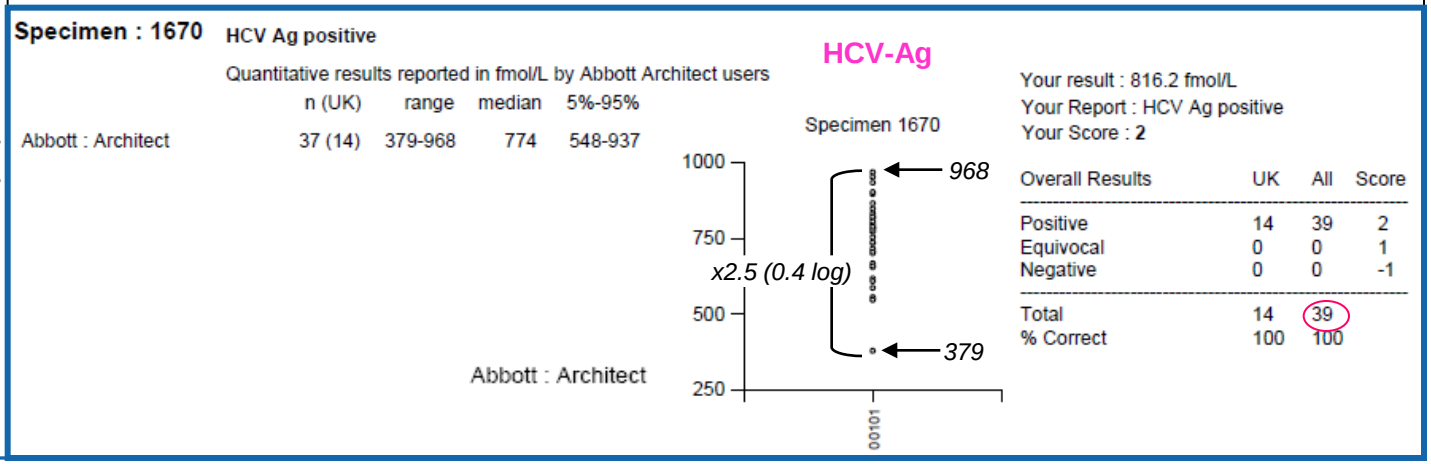


HCV serology scheme
HCV Ag
April 2013

6 serum specimens
3 distributions per year







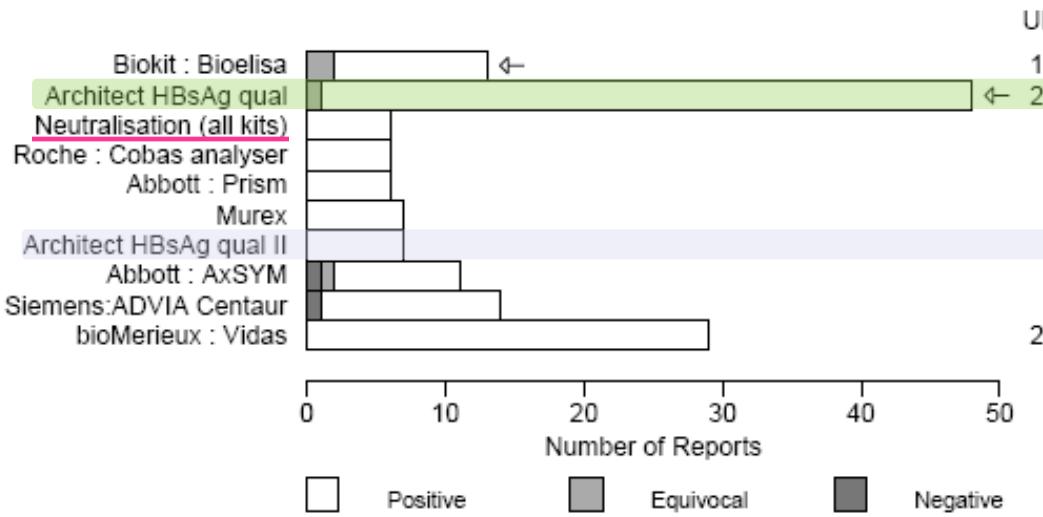
	Ag	Ab	%
All	37	294	13%
Finland	1	1	*100%*
Republic of Ireland	7	14	50%
Sweden	9	19	47%
Switzerland	2	13	15%
United Kingdom	13	150	9%
Italy	4	49	8%
Portugal	1	48	2%

Blood Borne Virus scheme, HBsAg – May 2012

- HBsAg
- 1:1000 dilution
- Pre-distribution Architect HBsAg quanti 0.1 IU/mL
- Assays to detect 0.05 IU/mL [NIBSC code: 07/286-xxx]

Specimen : 0929

Intended result
HBsAg positive (0.1 IU/mL)



	UK	(%)	All	(%)
Biokit : Bioelisa	13	(12.6)	13	(7.4)
Architect HBsAg qual	22	(21.4)	48	(27.4)
Neutralisation (all kits)	3	(2.9)	6	(3.4)
Roche : Cobas analyser	5	(4.9)	6	(3.4)
Abbott : Prism	4	(3.9)	6	(3.4)
Murex	5	(4.9)	7	(4.0)
Architect HBsAg qual II	0	(0.0)	7	(4.0)
Abbott : AxSYM	7	(6.8)	11	(6.3)
Siemens:ADVIA Centaur	8	(7.8)	14	(8.0)
bioMerieux : Vidas	21	(20.4)	29	(16.6)

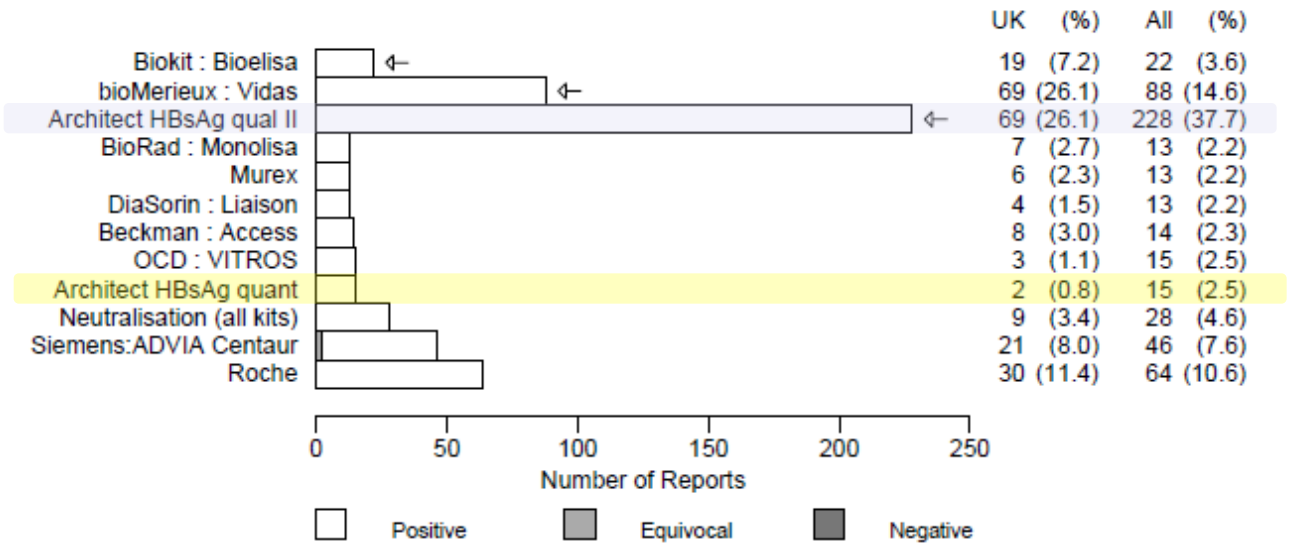
Your Report : HBsAg positive
Your Score : **2**

Overall Results	UK	All	Score
HBsAg positive	73	128	2
HBsAg equivocal	2	4	1
HBsAg negative	1	2	-1
Total	76	134	
% Correct	96.1	95.5	

→ *1 ppt reported equivocal with positive read-out (4.1 S/CO)*

The analytical sensitivity of the *ARCHITECT* HBsAg Qual II assay is 0.017 to 0.022 IU/mL

HBsAg quantitative results (01.2014)



specimen	QL	QT	average+/-SD	pre-D
A	225	**15**	1.04+/-0.06	1
B	225	**15**	**13.4+/-1.20**	**12.3**
C	225	**15**	320+/-65	214
D	225	**15**	748+/-43	>250

15 users of Architect HBsAg QT assay

Denmark	3
Italy	3
UK	2
Cyprus	1
Hong Kong	1
India	1
Netherlands	1
Portugal	1
Sweden	1
Switzerland	1

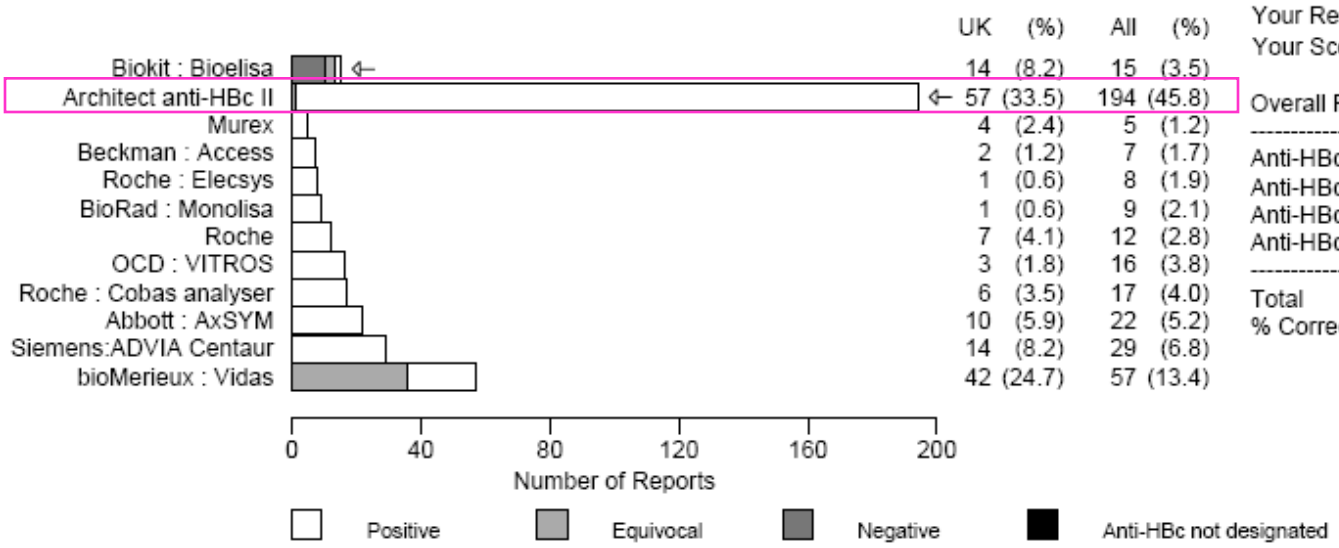
HBV serology scheme, HBc Ab – April 2012

Specimen 0895: prepared from a single donation diluted 1:200 in normal human serum leading to low level HBc Ab (other markers were not affected and normally detected)

Pre-distribution: Biokit Bioelisa equivocal, Abbott Architect positive, bioMerieux Vidas equivocal

- Intended result: 'anti-HBc not designated'

Specimen : 0895 HBsAg positive (106 IU/mL) Anti-HBc not designated



	UK	(%)	All	(%)
Biokit : Bioelisa	14	(8.2)	15	(3.5)
Architect anti-HBc II	57	(33.5)	194	(45.8)
Murex	4	(2.4)	5	(1.2)
Beckman : Access	2	(1.2)	7	(1.7)
Roche : Elecsys	1	(0.6)	8	(1.9)
BioRad : Monolisa	1	(0.6)	9	(2.1)
Roche	7	(4.1)	12	(2.8)
OCD : VITROS	3	(1.8)	16	(3.8)
Roche : Cobas analyser	6	(3.5)	17	(4.0)
Abbott : AxSYM	10	(5.9)	22	(5.2)
Siemens:ADVIA Centaur	14	(8.2)	29	(6.8)
bioMerieux : Vidas	42	(24.7)	57	(13.4)

Your Report : Anti-HBc positive
Your Score : **Not scored**

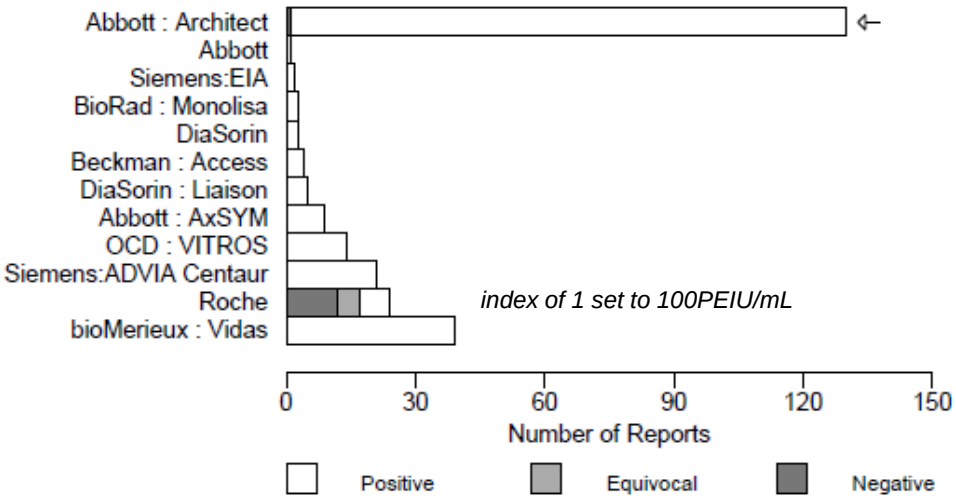
Overall Results	UK	All	Score
Anti-HBc positive	125	350	NS
Anti-HBc equivocal	22	27	NS
Anti-HBc negative	3	3	NS
Anti-HBc ND	0	0	NS
Total	150	380	
% Correct			

HBV serology scheme, HBc IgM – scored since April 2012

Challenge: find material with a good level of HBc IgM (indicating acute infection)

Specimen 1067: new material diluted 1:2.5
Pre-distribution results: Abbott Architect index 2.47 (>1 or >50PEIU/mL)
Participants median **160 PEIU/mL** (Vidas users, n=37)

Specimen : 1067 HBsAg positive (>250 IU/mL)
Anti-HBc IgM positive



	UK	(%)	All	(%)
Abbott : Architect	23	(31.9)	130	(50.6)
Abbott	0	(0.0)	1	(0.4)
Siemens:EIA	0	(0.0)	2	(0.8)
BioRad : Monolisa	1	(1.4)	3	(1.2)
DiaSorin	0	(0.0)	3	(1.2)
Beckman : Access	1	(1.4)	4	(1.6)
DiaSorin : Liaison	0	(0.0)	5	(1.9)
Abbott : AxSYM	1	(1.4)	9	(3.5)
OCD : VITROS	3	(4.2)	14	(5.4)
Siemens:ADVIA Centaur	7	(9.7)	21	(8.2)
Roche	5	(6.9)	24	(9.3)
bioMerieux : Vidas	30	(41.7)	39	(15.2)

Your Report : Anti-HBc IgM positive
Your Score : **2**

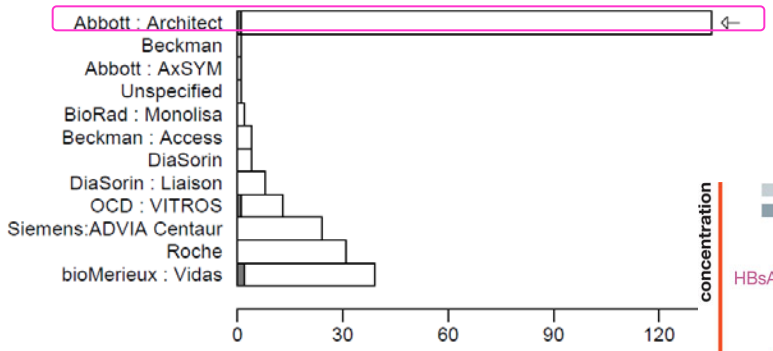
Overall Results	UK	All	Score
Anti-HBc IgM positive	68	238	2
Anti-HBc IgM equivocal	1	6	1
Anti-HBc IgM negative	3	13	-1
Total	72	257	
% Correct	94.4	92.6	

HBV serology scheme, HBc IgM – January 2014

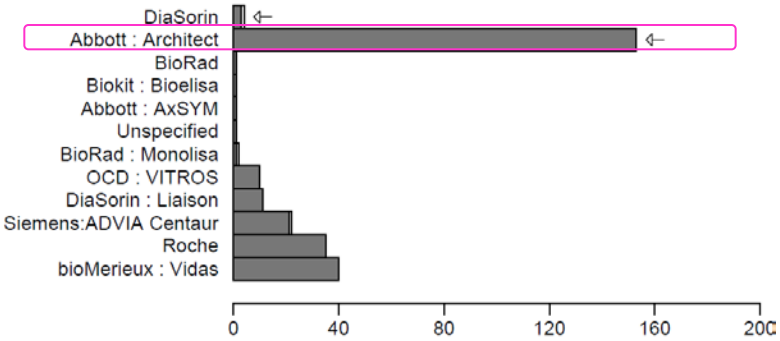
Recent distribution

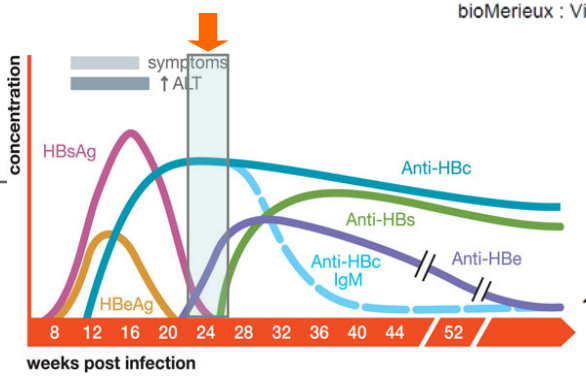
HBs Ag 1 IU/mL (anti-HBs+)

Specimen : 1810 **HBc IgM 7100 PEIU/mL**

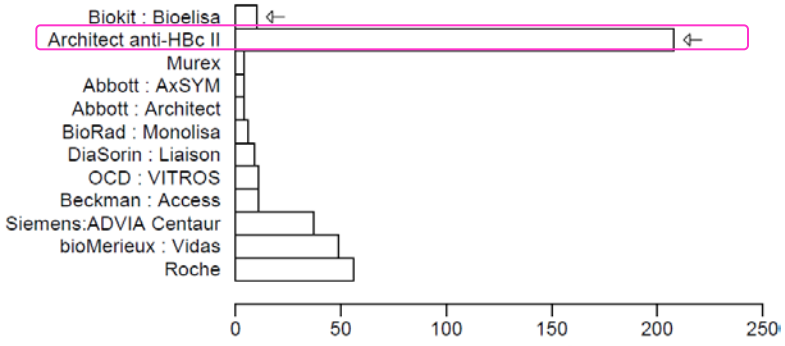


Specimen : 1810 Hbe Ag negative

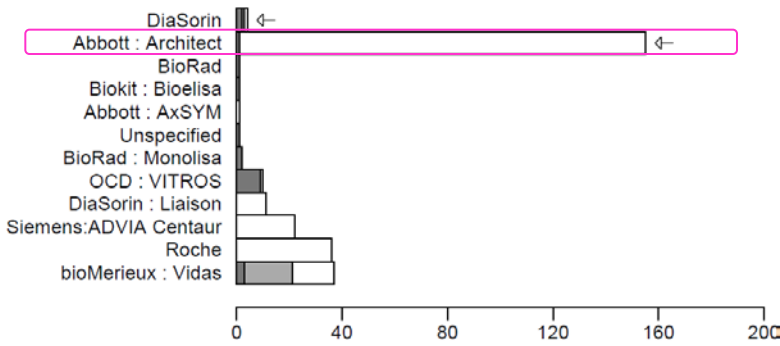




Specimen : 1810 HBc Ab, low avidity



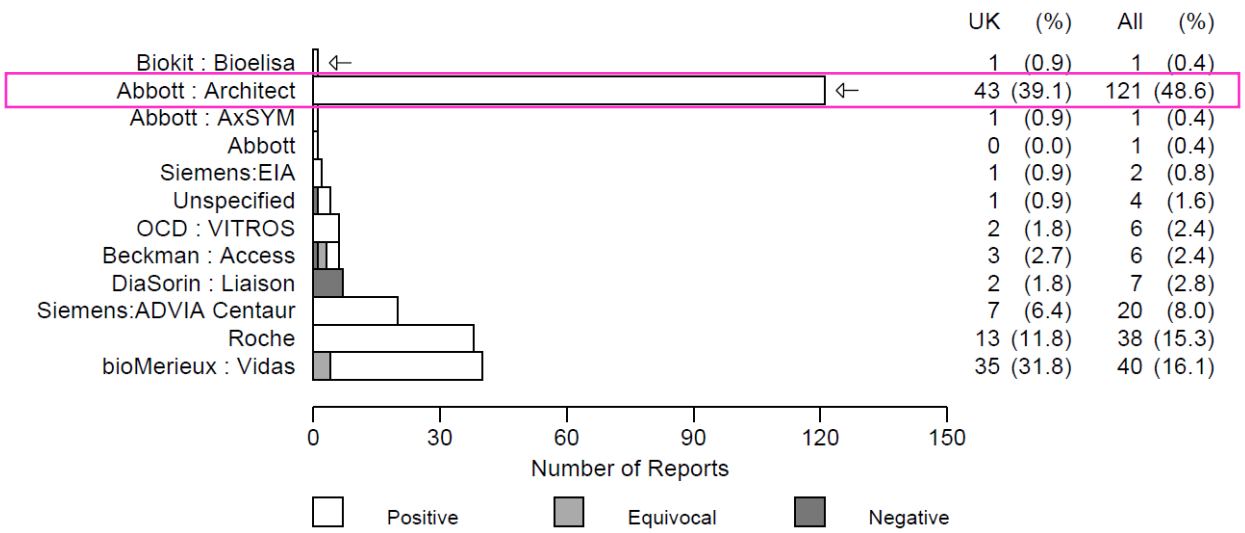
1810 Hbe Ab not designated



Immunity screen scheme, HAV Ab– October 2013

Two HAV Ab low level specimens both prepared from neat single donations

Specimen : 1730 **Intended result HAV IgG positive**



Your Report : HAV IgG positive
Your Score : **2**

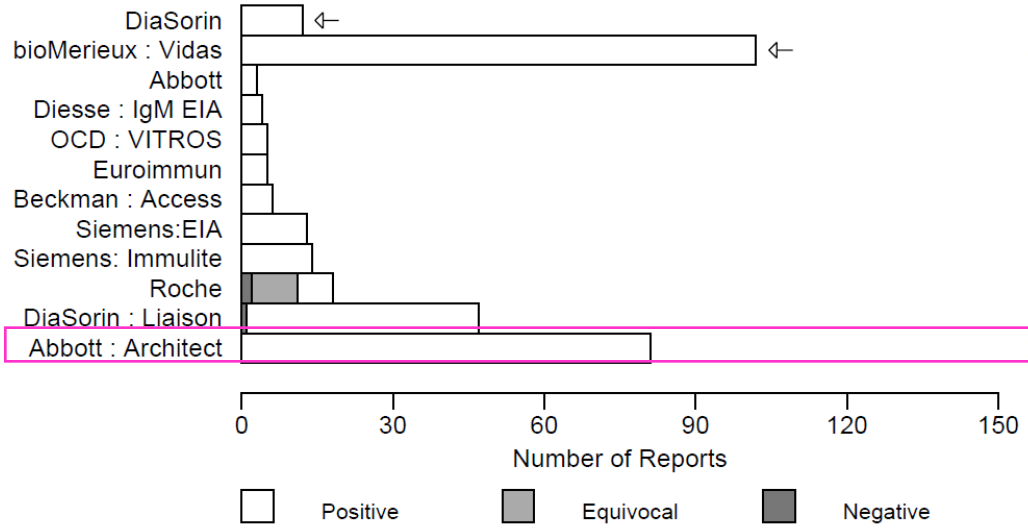
Overall Results	UK	All	Score
HAV IgG positive	101	229	2
HAV IgG equivocal	5	8	1
HAV IgG negative	3	8	-1
Total	109	245	
% Correct	92.7	93.5	

	number of participant	assay format	antibody total/IgG	cut-off grey zone	HAV Ab positive 1730 median	1729 median	1731 median	HAV Ab negative 1728 median	1732 median	1733 median
DiaSorin Liaison	7	competitive	total	1	1.67	1.56	0.13	2.64	2.41	2.47
Roche	38	non comp.	total	20	51	>60	>60	6.0	7.0	7.6
bioMerieux Vidas	39	non comp.	total	15-20	27	78	368	<15	<15	<15
Abbott Architect	115	non comp.	IgG	1	2.67	3.83	10.66	0.36	0.39	0.49

Diagnostic serology: hepatitis screen, CMV IgM– November 2013

One low level CMV IgM prepared from a single donation diluted 1:2.3 with low level CMV IgG (index 2), avidity not measurable

Specimen : 1714 **Intended result**
CMV IgM positive (low avidity CMV IgG)



	UK	(%)	All	(%)
DiaSorin	6	(5.1)	12	(3.6)
bioMerieux : Vidas	55	(46.6)	102	(30.9)
Abbott	1	(0.8)	3	(0.9)
Diesse : IgM EIA	3	(2.5)	4	(1.2)
OCD : VITROS	1	(0.8)	5	(1.5)
Euroimmun	0	(0.0)	5	(1.5)
Beckman : Access	3	(2.5)	6	(1.8)
Siemens:EIA	0	(0.0)	13	(3.9)
Siemens: Immulite	2	(1.7)	14	(4.2)
Roche	4	(3.4)	18	(5.5)
DiaSorin : Liaison	14	(11.9)	47	(14.2)
Abbott : Architect	24	(20.3)	81	(24.5)

Your Report : CMV IgM positive
Your Score : **2**

Overall Results	UK	All	Score
CMV IgM positive	106	271	2
CMV IgM equivocal	5	10	1
CMV IgM negative	0	3	-1
Total	111	284	
% Correct	95.5	95.4	

	number of participants	cut-off grey zone	CMV IgM positive 1714 median	CMV IgM negative 1713 median	CMV IgM negative 1715 median
Roche	17	0.7-1.0	0.896	0.397	0.218
bioMerieux Vidas	42	18-22	59	<5	<5
Abbott Architect	72	1	7.1	0.260	0.155

Toxoplasma serology, April 2013
scheme combines IgG, IgM and Avidity- since April 2012

Clinical details

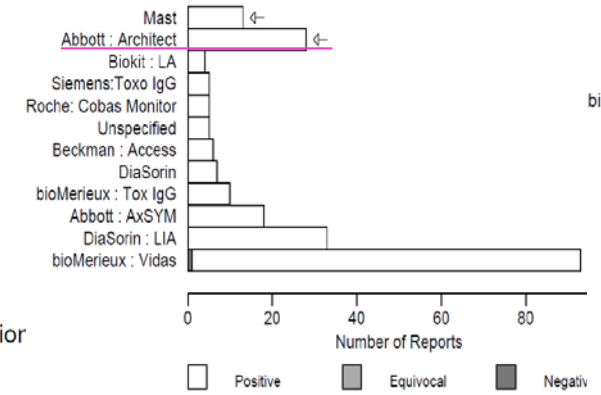
30 year old female no symptoms but concerned as her husband has toxoplasma lymphadenopathy.

Result

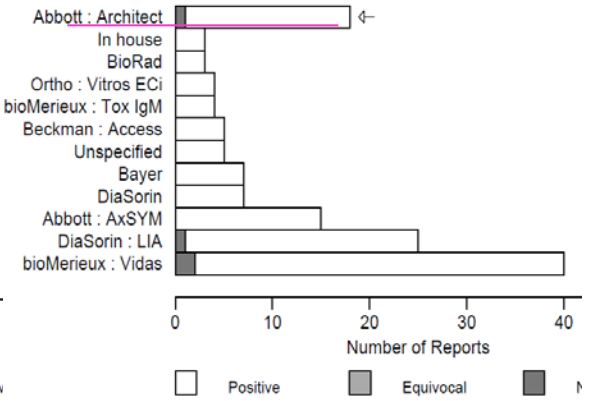
Dye test	125 IU/mL
IgG ELISA	Positive
Abbott Architect (IgG)	Positive
Abbott AxSYM (IgG)	Positive
IgG Avidity (in-house test)	Borderline
IgM ELISA	Positive
Abbott Architect (IgM)	Positive
Abbott AxSYM (IgM)	Positive

Serological evidence of current/recent toxoplasma infection

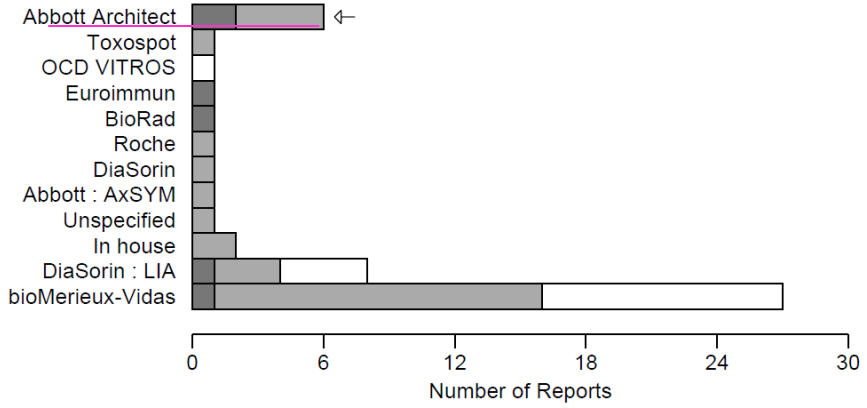
Specimen : 1437 Toxoplasma IgG/total Ab positive



Specimen : 1437 Toxoplasma IgM positive



Specimen : 1437 Toxoplasma IgG avidity borderline



	UK	(%)	All	(%)
	1	(25.0)	6	(11.3)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	0	(0.0)	1	(1.9)
	2	(50.0)	2	(3.8)
	0	(0.0)	8	(15.1)
	1	(25.0)	27	(50.9)

Your Report : Toxoplasma IgG Avidity: Borderline

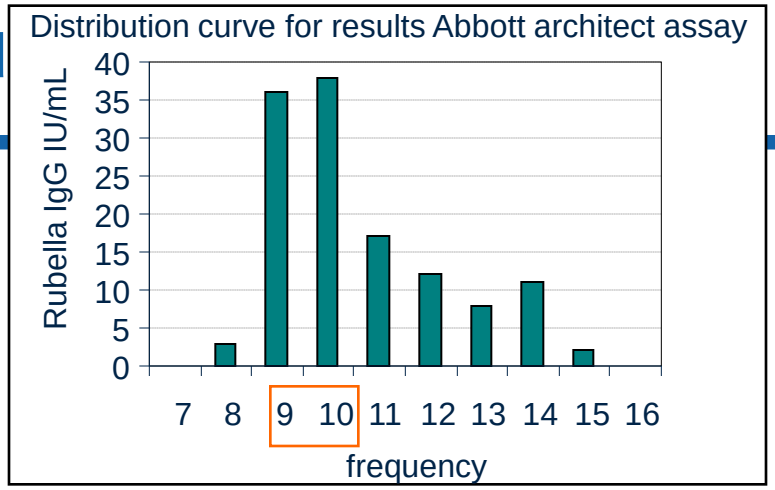
Your Score : **Not scored**

Overall Results	UK	All	Score
High	1	35	NS
Borderline	4	45	NS
Low	0	10	NS
Total	5	90	
% Correct	80.0	50.0	

Rubella IgG scheme– Janu

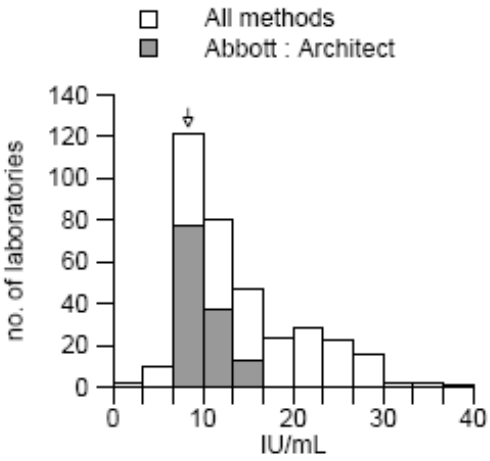
specimens are single serum donations

Pre-distribution tests:
- Rubagen + (total Ab)
- Architect 10.7 IU/mL
- Axsym 15.4 IU/mL
- Biokit 11.7 IU/mL
- SRH <15 IU/mL



Specimen : 0726 Rubella IgG positive (>10 IU/mL)

	n (UK)	range	median	5%-95%
All methods	437 (226)	0-44	13	7-28
Abbott : Architect	136 (65)	8-24	10	9-14
Abbott : AxSYM	20 (9)	5-26	18	10-25
Biokit : Bioelisa	29 (28)	6-35	17	9-29
bioMerieux : Vidas	72 (44)	7-44	26	21-32
DiaSorin	16 (9)	1-30	20	8-28
DiaSorin : Liaison	27 (9)	8-14	11	9-13
Roche	9 (5)	6-24	7	6-18
Roche : Cobas analyser	12 (8)	6-8	7	6-8
Roche : Elecsys	8 (3)	5-27	7	5-20
Siemens: Immulite	9 (1)	12-15	13	12-14
Siemens:ADVIA Centaur	22 (15)	12-22	15	13-21
Siemens:EIA	11 (3)	7-15	12	8-14



Quantitative report :
Your result : 9 IU/mL
Your method median : 10.0 IU/mL

Qualitative report :
Your Report :
Rubella IgG negative (<10 IU/mL)
Your Score : Not scored

Overall Results	UK	All	Score
Rubella IgG positive	152	289	NS
Rubella IgG negative	25	71	NS
Total	177	360	
% Correct	85.9	80.3	

7-9 IU/mL

This specimen highlights the ongoing issues with accurate quantitation of antibodies using IU/mL and the cut off value delineating 'protective' levels.

Rubella IgG scheme– results in IU/mL are not comparable between assays

Since January 2012: 24 specimens distributed
5 specimens not scored
averages of method medians= 15,13,9,6,8 IU/mL
blood donor screening= 15% detected >5-15 IU/mL

Implications:

- one cannot and must not compare IU/mL generated by different assays
  (serum may be compared if tested with the same assay & within the same run & <200IU/mL)

- 'technically' impossible to determine accurately whether low level IgG > or<10IU/mL
- ignoring intra-assay variability, inter-assay variability, cellular immunity
  →affecting ~15% of the sample tested (routine issue)

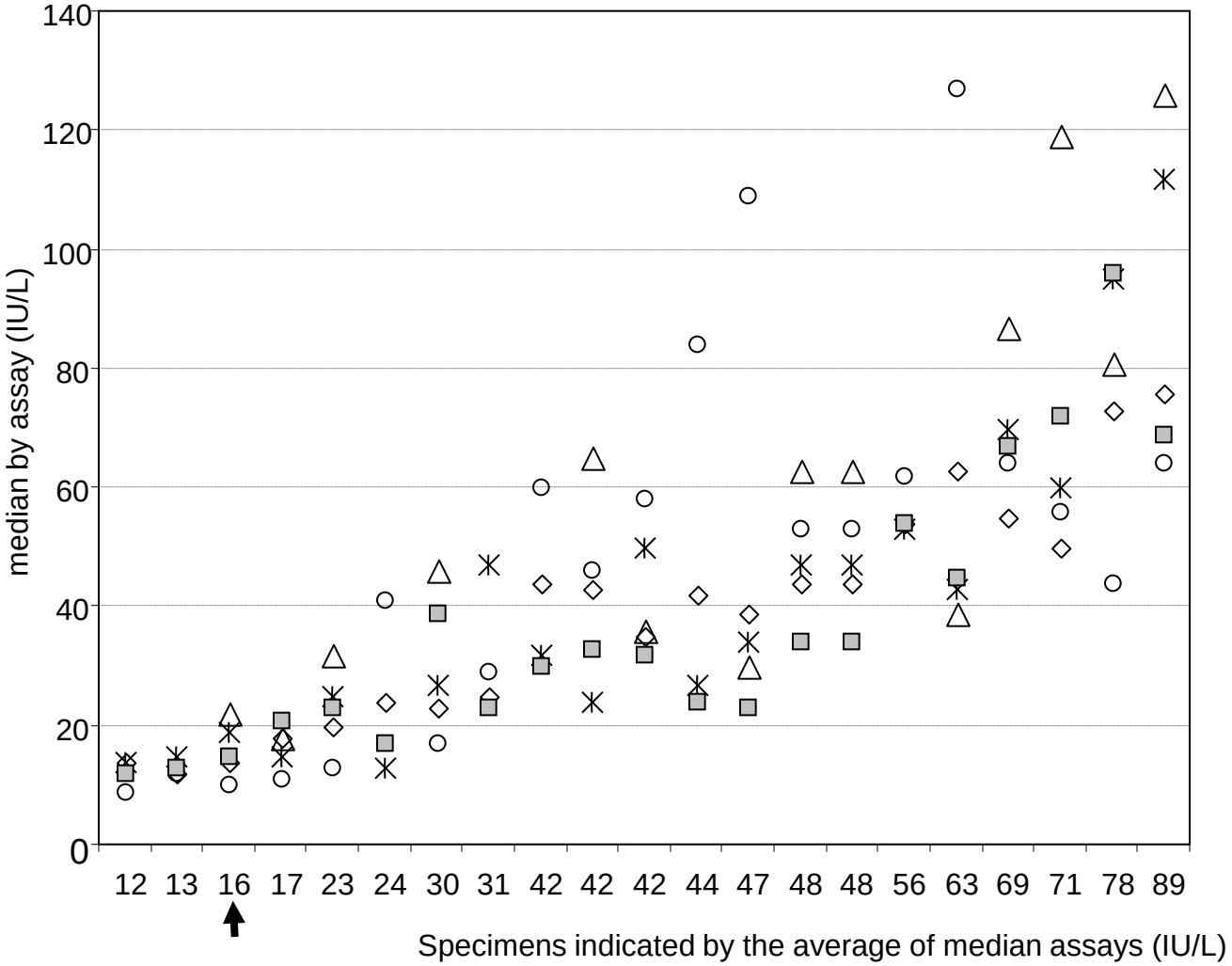
EQA data suggest that sera that are truly negative for rubella IgG antibodies (indicative of susceptibility to rubella virus) will result in quantitative values of 5 IU/mL or lower for all assays.

A cut-off of 5IU/mL?: technically achievable
100% agreement for all negative&positive EQA specimens
<5IU/mL, negative, susceptible
>5IU/mL, positive, protective immunity to be confirmed

Specimen : 0993 **Rubella IgG negative (<10 IU/mL)**

	n (UK)	range	median	5%-95%
All methods	351 (196)	0-8	0	0-3
Abbott : Architect	136 (63)	0-1	0	0-0
Abbott : AxSYM	15 (6)	0-2	0	0-1
Beckman : Access	9 (6)	0-1	0	0-1
Biokit : Bioelisa	23 (21)	0-4	2	0-3
bioMerieux : Vidas	75 (52)	0-8	0	0-0
DiaSorin	8 (4)	0-5	0	0-5
DiaSorin : Liaison	6 (2)	1-5	5	2-5
OCD : VITROS	3 (1)	0-0	0	0-0
Roche : Cobas analyser	14 (7)	0-0	0	0-0
Roche : Elecsys	8 (5)	0-0	0	0-0
Siemens:ADVIA Centaur	4 (3)	0-2	0	0-2
Siemens:EIA	6 (1)	0-5	0	0-4

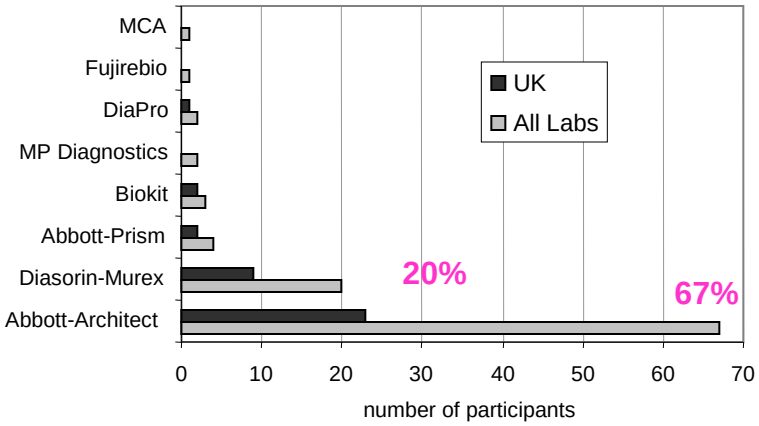
Anti-HBs detection
Agreement between 5 assays for 22 low level specimens (10-100)



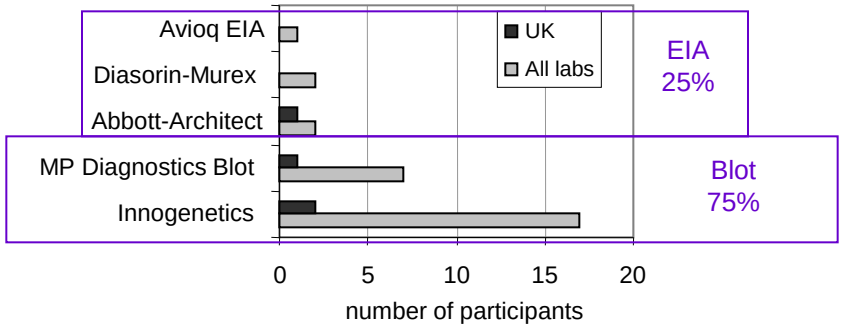
HTLV-I/II Ab marker – in development

- 2013 Questionnaire: 771 participants, 327 replies

- 102 (37 UK) routinely test for HTLV-I/II Ab



- 28% use a confirmatory assays (33% send away for confirmation)



- the majority of laboratories test less than 100 samples per month , mainly from B&T donors

58%

Category	Tests per month	
<25	21	23%
25-100	33	35%
100-1000	31	33%
>1000	8	9%
Total	93	100%

	Blood and tissue donors	Transplant recipients	Adult T-cell leukaemia/lymphoma	Myelopathy
mostly	63%	24%	9%	4%
frequently	23%	23%	19%	23%
rarely	7%	36%	60%	52%
never	7%	16%	13%	20%

- most of the laboratories test for HTLV-I/II Ab as part of a screening panel which includes HIV-Ab, HCV Ab, HBsAg and T pallidum Ab.

- *2014: pre-pilot distribution*

tak, takk,giitu, kiitos... spørsmål?

PARTICIPANTS

discussion, feedback, returning questionnaires and testing pilot specimens

MANUFACTURERS

Abbott · Ortho-Clinical Diagnostics · SIEMENS · Trinity Biotech · DIESSE · BECKMAN COULTER · Warfen Group · biokit · Roche · BIO-RAD · Mast Group · BIOMÉRIEUX · NovaTec Immundiagnostica GmbH · virion\serion

UK NEQAS

Virology&Molecular team

CHARITY · EVIE · SERGIO · ANNELINE · HABIB · VIVIENNE · ELLI · PRIYA · TILANI · MIHAELA

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