Virology Serology Diagnostics Infection Training 2016

Dr Alison Watt

Total testing Pathway

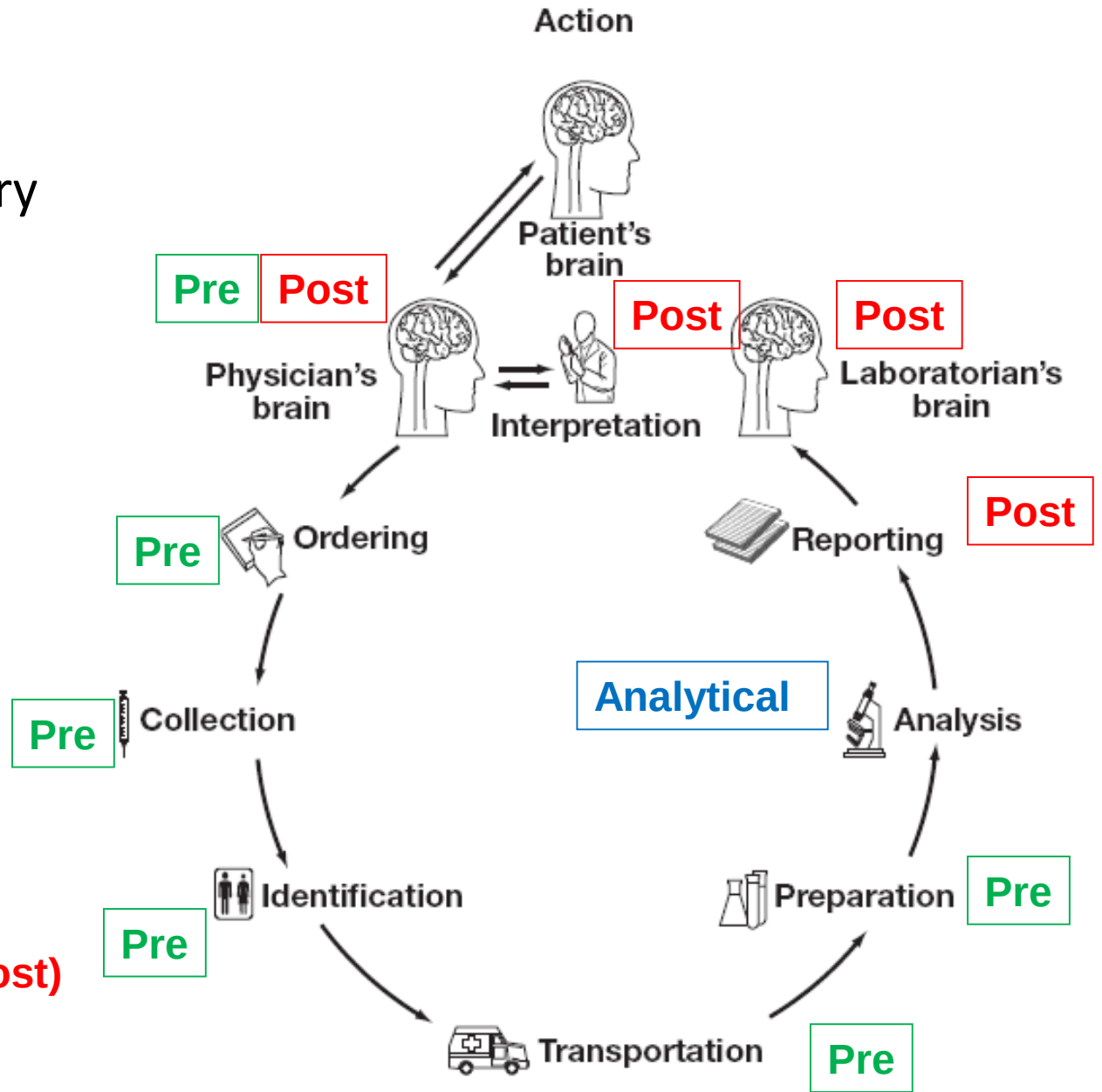
Lundberg (1981):
Brain to Brain loop
concept for laboratory testing:

Pre-Analytical

Analytical ✓

Post-Analytical

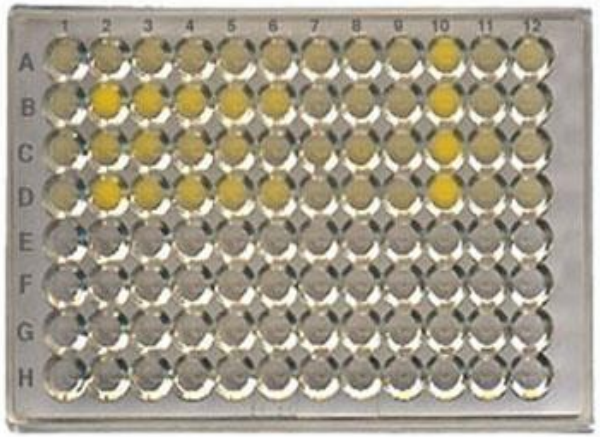
Right Test, (Pre)
Right Patient, **(Pre)**
Right Sample,
Right Time, **(Pre)**
Right Results,
Right Interpretation, **(Post)**
Right Management



What serology techniques are performed in the laboratory in your hospital?

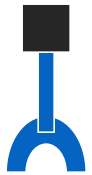
TEST	EXAMPLE OF USE
Enzyme-linked immunosorbent assays (EIA or ELISA or CLIA)	IgG/IgM antibody – rubella, measles, mumps, Hepatitis A etc Antigen – hepatitis B surface antigen, HIV p24 antigen
Immunofluorescence (IF)	IgG/IgM antibody – EBV, VZV, Measles, mumps Antigen – RSV, influenza
IgG avidity assays	To confirm recent CMV, rubella and toxoplasma infections.
Western blot and line assays (LIA)	Used to confirm HIV, HCV screen positive specimens. Borrelia, syphilis
Latex and gel particle agglutination	Antibody – rubella, toxoplasma Antigen – rotavirus, norovirus
Haemagglutination (HA) and Haemagglutination inhibition (HAI)	Detects antibody to rubella, influenzas i.e. viruses that possess a haemagglutinin antigen
Complement fixation test (CFT)	Respiratory viruses, measures total antibody, acute and convalescent serum samples are required.

Automation of serology



Enzyme immunoassays EIA or ELISA

Enzyme mediated colour change



Enzyme labelled anti-human antibody

Patient antibody

Antigen

Colourless

Coloured

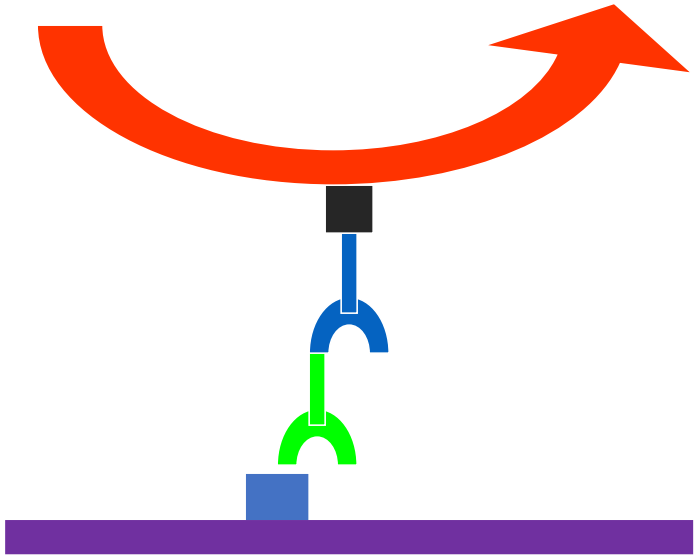


Figure 1. Direct ELISA protocol.

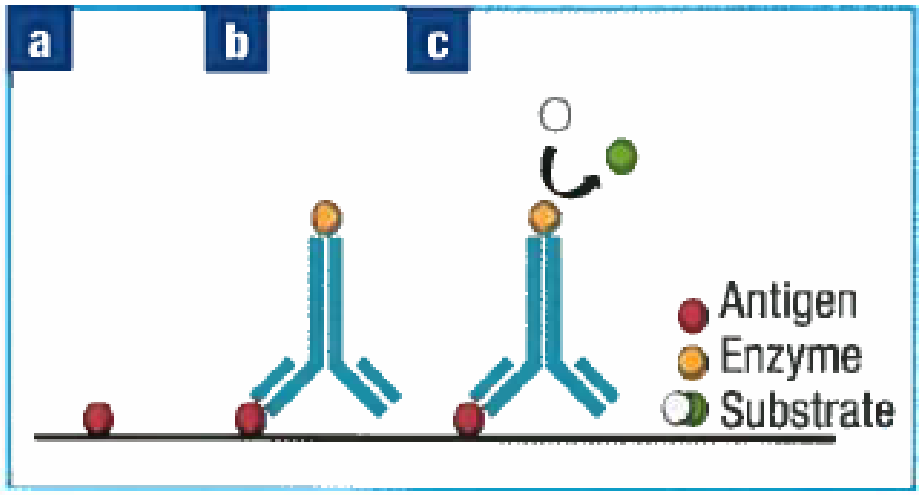


Figure 2. Indirect ELISA protocol.

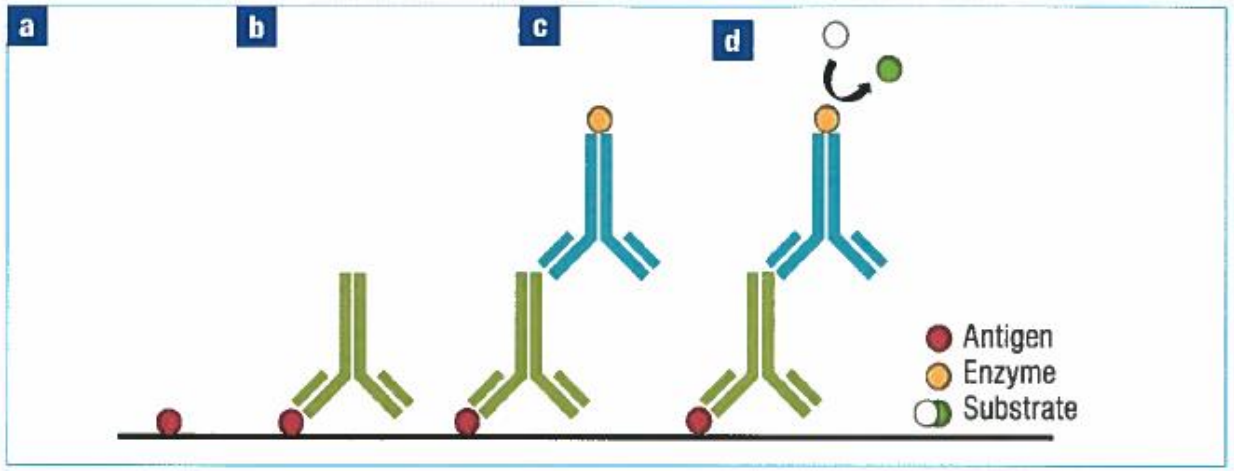


Figure 6. Competitive ELISA protocol.

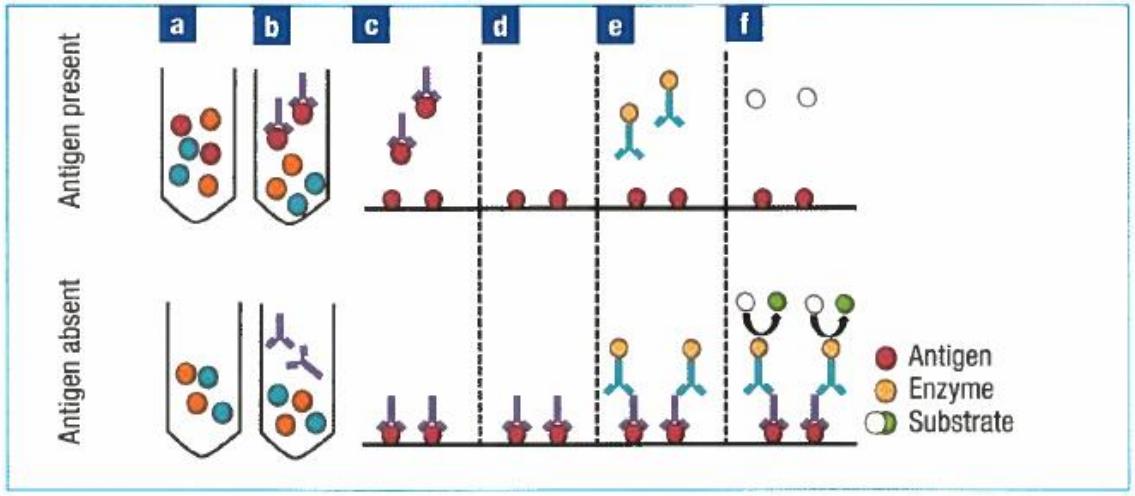
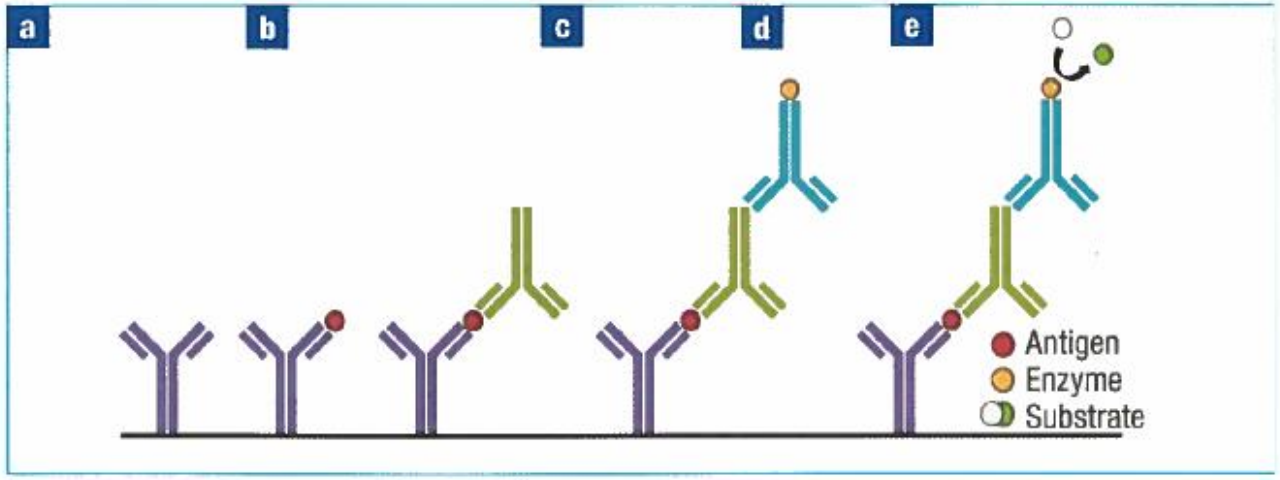


Figure 4. Sandwich ELISA protocol.



Immunoassays with different detection systems

- CLIA – Chemiluminescent immunoassay
- EIA – Enzyme immunoassay
- ELISA – Enzyme linked immunosorbent assay
- Performance of assay depends on assay format, antibodies used and assay optimization – not detection method

Table 1. Advantages and disadvantages of different types of enzyme-linked immunosorbent assays (ELISAs)

	Advantages	Disadvantages
Direct ELISA	■ Rapid ■ Secondary antibody cross-reactivity eliminated	■ Low sensitivity ■ Specific antibody for each ELISA; time-consuming and expensive
Indirect ELISA	■ High sensitivity ■ Cost-saving ■ Flexible; can use many primary antibodies	■ Risk of cross-reactivity between secondary antibodies
Sandwich ELISA	■ Minimal sample purification needed ■ High sensitivity and specificity	■ Must use 'matched pair' primary and secondary antibodies ■ Time consuming and expensive
Competitive ELISA	■ Minimal sample purification needed ■ Used to measure large range of antigens in a sample ■ Used for small antigens ■ Low variability	■ Low specificity so cannot be used in dilute samples

Shah and Maghsoudlou, 2016. ELISA: the basics Br J Hosp Med :77, 7.

- A midwife from the local antenatal unit phones you for some advice. A primigravida 23^{+1} transferred to her care from another unit recently had bloods tested for Rubella and parvovirus serology following contact with a maculopapular rash in the nursery where she works.

- The midwife is confused as the patients Rubella immunity on the current blood contradicts that found on the patients booking blood at 12 weeks, she needs clarity on this to plan patient management. She wants to know has the lab made a mistake when testing this blood?

- Review the results below – what is the most likely explanation for the discrepancy?

- Booking blood Rubella IgG 25 IU/ml
- Contact with rash serology Rubella IgG 8 IU/ml

Standardization of Anti-Rubella Virus IgG testing

- Rubella IgG tests are calibrated using the WHO 1st International Standard for Anti-Rubella Immunoglobulin (RUBI-1-94)
- Results reported International Units per milliter (IU/ml)
- Immune cut off – 10 IU/ml
- Cut off assigned using Haemagglutination inhibition
- Vaccination elicits a lower level of antibody response than wild type infection
- Test kits vary in manufacture – different antigens, detection chemistries, conjugate antibodies

Assessing Immunity to Rubella Virus: a Plea for Standardization of IgG (Immuno)assays

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Immunity to rubella virus (RV) is commonly determined by measuring specific immunoglobulin G (RV IgG). However, RV IgG results and their interpretation may vary, depending on the immunoassay, even though most commercial immunoassays (CIAs) have been calibrated against an international standard and results are reported in international units per milliliter. A panel of 322 sera collected from pregnant women that tested negative or equivocal for RV IgG in a prior test (routine screening) was selected. This panel was tested with two reference tests, immunoblotting (IB) and neutralization (Nt), and with 8 CIAs widely used in Europe. IB and Nt gave concordant results on 267/322 (82.9%) sera. Of these, 85 (26.4%) sera were negative and 182 (56.5%) sera were positive for both tests. All 85 IB/Nt-negative samples were classified as negative with all CIAs. Of the 182 IB/Nt-positive samples, 25.3 to 61.5% were classified as equivocal and 6 to 64.8% were classified as positive with the CIAs. Wide variations in titers in international units per milliliter were observed. In our series, more than half of the women considered susceptible to RV based on CIA results tested positive for RV antibodies by IB/Nt. Our data suggest that (i) sensitivity of CIAs could be increased by considering equivocal results as positive and (ii) the definition of immunity to RV as the 10-IU/ml usual cutoff as well as the use of quantitative results for clinical decisions may warrant reconsideration. A better standardization of CIAs for RV IgG determination is needed.

Sensitivity

- **Analytical Sensitivity** – ability of a test to detect very small amounts of a **substance**
- **Clinical Sensitivity** – ability of test to give positive result if patient has the disease (no false negative results)

Specificity

- **Analytical Specificity** – ability of test to detect substance without interference from cross-reacting substances
- **Clinical Specificity** – ability of test to give negative result if patient does not have disease (no false positive results)

False negative serology

- Test sensitivities typically <100%
- Interfering substances that block assay function
- Immunocompromised patients – reduced or absent humoral immune response.
- Insufficient time elapsed since onset of infection – Window period.

False positive serologic results

- Antibodies that cross react with microbial antigens used in the assay or interfering substances that interact with assay components

- Parvovirus B19 infection – implicated in false positive IgM for EBV, HSV, CMV, Measles.

IVIG/Blood transfusion

Transplacental antibody

Do not give false positives in serology assay but can considered "false positive" for patient as antibody is passively acquired i.e. not produced by patient

Interference

- Occurs when a serum component interacts with a test component to generate a false positive or false negative result.
- Heterophile antibody in patient serum binding to “reagent” antibodies.
- Rheumatoid factor – heterogeneous group of autoantibodies that recognize epitopes on the Fc region of IgG molecules
- Found in 70% of patients with rheumatoid arthritis but also 10% adults without.
- Rheumatoid factor type antibodies can also be generated during multiple infections including infectious mononucleosis, CMV, Flu A, TB, infective endocarditis.

27 year old male – Day 1

- c/o - soles of feet completely numb <24 hours
- hpc unwell 9 days
- constant headache, felt weak, temp, sweats
- no vomiting, no diarrhoea, no rash
- pmh sore throat 3 wks ago - rx penicillin
  occupation employed by water board - no sewage contact.
- travel back from 2 week honeymoon - returned 3 weeks ago
- current medication - otc paracetamol, ibuprofen for headache
- o/e
- RT - clear. RR-14/min.
- CVS clear HS I+II+0. HR-90bpm, regular. BP-124/86.
- CNS clear - CNs II-XII intact. No rash, no photophobia, no focal neurology.
- GI clear – soft abdomen, bs present.
- Looks v well. Mobile unaided

Diagnosis - flu-like illness – reassured

Day 2

- Numbness spread to arms and face - 'feels like teeth aren't his own'
- unsteady on feet
- headache (throbbing with dizziness)
- mild temp
- weak, unable to stand
- Admitted
- CSF – Protein slightly raised (0.53) otherwise normal - ? viral meningitis.

	CSF GLUCOSE	3.5	2.2-3.9 (mmol/l)
	CSF PROTEIN	* 0.530	0.15-0.45 (g/l)
Glycoprotein Ab	Anti-GM1	Negative	(Units)
	Anti-GQ1b	Negative	(Units

Day 4 Reviewed by neurology – Viral headache – Naproxan discharged.

Day 6

- Presented with headache & ascending paralysis and off feet – GBS diagnosed
- Ventilated via tracheostomy - improved following IVIG x2
- Episodes of autonomic instability.
- Severe neuropathic pain - gabapentin & amitryptillin
- Needed intensive physiotherapy.
- Developed IBD on recovery

Virology Findings

Respiratory Viral Screen including Atypical bacteria Negative

V14108339	S		BTPT syphilis total antibody	POS (OD - 2.10)
V14108339	S		TPPA	1:160
V14108339	S		VDRL	NEG
V14108338	S		CMV IgG	Pos (293)
V14108338	S		CMV IgM	Pos (31)
V14108338	S		CMV AVID	Low
V14108339	S		HBV	NEG
V14108339	S		HCV	NEG
V14108339	S		HIVN	NEG
V14108339	S		EBV qPCR	NEG
V14108338	S		CMV qPCR	28700

Intravenous immunoglobulin preparations

- Prepared from pools of at least 1000 donations of human plasma
- Contains immunoglobulin G (IgG) and antibodies to HAV, measles, mumps, rubella, varicella and other viruses currently prevalent in the general population.
- Physiologic half-life of IVIgG approx. 22 days (observed to extend to over 30 days in immunodeficient patients

- **B. Avidity** - Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies.

  - Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

  - Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities.

Avidity assays

- Addition of an agent which disrupts the Ag-Ab link (e.g. urea) during an ELISA test has little effect on the high antibody link but great effect on that of weak avidity antibodies
- Comparison of results obtained with and without a dissociating agent corresponds to one measure of avidity.
- High avidity = strong indication of a primary infection more than 3 months
- Low avidity = strong indication of a primary infection of less than 3 months

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Comparative evaluation of eight commercial human cytomegalovirus IgG avidity assays

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ABSTRACT

Background: The interpretation of a positive IgM antibody result to human cytomegalovirus (HCMV) in a pregnant woman is of major importance for the correct management of the pregnancy. Determination of HCMV-specific IgG avidity is considered an useful approach for distinguishing IgM antibody due to primary HCMV infection from IgM antibody elicited during non-primary infection.
Objective: Comparative evaluation of eight commercial HCMV IgG avidity assays currently available in Europe.
Study design: A panel of 198 sequential samples collected from 65 pregnant women at 0–90, 91–180, and >180 days after the onset of primary HCMV infection was retrospectively tested by Abbott, BioMérieux, Bio-Rad, DiaSorin, Diesse, Euroimmun, Radim, and Technogenetics HCMV IgG avidity assays according to the manufacturer's instructions.
Results: None of the 198 samples tested yielded identical scores by the kits under evaluation. The Euroimmun and Radim assays showed the best correlation with expected results in terms of low (0–90 days), intermediate (90–180 days) and high (>180 days) avidity results, respectively. The best accuracy in diagnosing a recent (<90 days after the onset) or non-recent (>180 days after the onset) primary HCMV infection was shown by Radim followed by Euroimmun and Diesse. The best correlation with a well established in-house developed HCMV IgG avidity assay was shown by Radim.
Conclusions: HCMV IgG avidity kits need to be improved and standardized. In the meantime, highly specific IgM assays are preferable for screening purposes in pregnant women.

Table 1
Characteristics of commercial HCMV IgG avidity assays included in the study.

Manufacturer Name of the assay	Technology	Dissociating agent	Range and interpretation of results (according to manufacturer's instructions)
Abbott Architect CMV IgG avidity	Chemiluminescent Microparticle immunoassay Automated	None	<50% low avidity 50–59.9% grey zone >59.9% high avidity
bioMérieux VIDAS CMV IgG avidity	Enzyme-linked fluorescent assay Semi-automated	Urea	<0.2 strong indication of a primary infection dating back <3 months 0.2–0.8 does not distinguish a recent infection from a former infection >0.8 strong indication of a primary infection dating back >3 months
Bio-Rad Platelia CMV IgG avidity	ELISA	Urea	<0.4 low avidity, more in favour of recent primary infection of <3 months 0.40–0.55 grey zone >0.55 high avidity, more in favour of past infection of >3 months
DiaSorin LIAISON CMV IgG avidity	Chemiluminescent immunoassay Automated	Urea	<0.2 low avidity, possible primary infection acquired <3 months 0.2–0.3 moderate avidity, does not rule out a recent infection >0.3 high avidity, may exclude a primary infection in the past 3 months
Diesse Cytomegalovirus IgG avidity	ELISA	Urea	<30% low avidity 30–40% borderline avidity >40% high avidity
Euroimmun CMV IgG avidity	ELISA	Urea	<40% low avidity 40–60% equivocal range >60% high avidity
Radim Cytomegalovirus IgG avidity	ELISA	Urea	<35% low avidity, strong indication of infection in the previous 3 months 35–45% mean avidity >45% high avidity
Technogenetics BEIA CMV IgG avidity	ELISA	Potassium thiocyanate	<25 low avidity, primary infection in the last 3 months 25–45 medium avidity, primary infection in the last 6 months >45 high avidity, exclude primary infection in the last 3 months

- Only 60% sera gave the same results by at least 5 kits
- Concordance was variably distributed among the kits examined.
- Level of agreement with expected results was modest.
- Cut off in one assay for excluding a primary CMV assay in the previous 3 months was too high.

Table 2
Agreement of commercial kits and the Pavia assay with expected IgG avidity results at <90, 91–180, and >180 days after onset of infection (column A), and of commercial kits with the Pavia in-house assay (column B). Calculations were performed on 198 sequential sera collected from 65 pregnant women 7–275 days following onset of primary HCMV infection.

Assay	Kappa	
	A	B
Abbott	0.28[a]	0.28
BioMérieux	0.18	0.28
Bio-Rad	0.37	0.30
DiaSorin	0.37	0.28
Diesse	0.40	0.48
Euroimmun	0.48	0.35
Radim	0.43	0.51
Technogenetics	0.15	0.07
Pavia	0.45	NA

NA, not applicable.
[a] Kappa statistics interpretation: 0–0.2 slight, 0.21–0.40 fair, 0.41–0.60 moderate agreement.

28 year old female

- 20/1/16 Pregnant 1stT - ? toxoplasmosis

SpNo	Sp Date	Codes	Result
V16054580	20-Jan-16	T gondii IgG	>650.00
V16054580	20-Jan-16	T gondii IgM	3.51

28 year old female

- 20/1/16 Pregnant 1stT - ? toxoplasmosis

SpNo	Sp Date	Codes	Result
V16054580	20-Jan-16	T gondii IgG	>650.00
V16054580	20-Jan-16	T gondii IgM	3.51
V16054580	20-Jan-16	T gondii Dye	250
V16054580	20-Jan-16	T gondii avidity	Low

28 year old female

- 20/1/16 Pregnant 1stT - ? toxoplasmosis

SpNo	Sp Date	Codes	Result
V16054580	20-Jan-16	T gondii IgG	>650.00
V16054580	20-Jan-16	T gondii IgM	3.51
V16054580	20-Jan-16	T gondii Dye	250
V16054580	20-Jan-16	T gondii avidity	Low

- 28/8/15 Patient had a script from a private clinic for Spiromycin prior to IVF
- 10/6/15 Pt is concerned because she has had enlarged cervical LNs for 6/52 – toxoplasma confirmed
- Preconception infection!

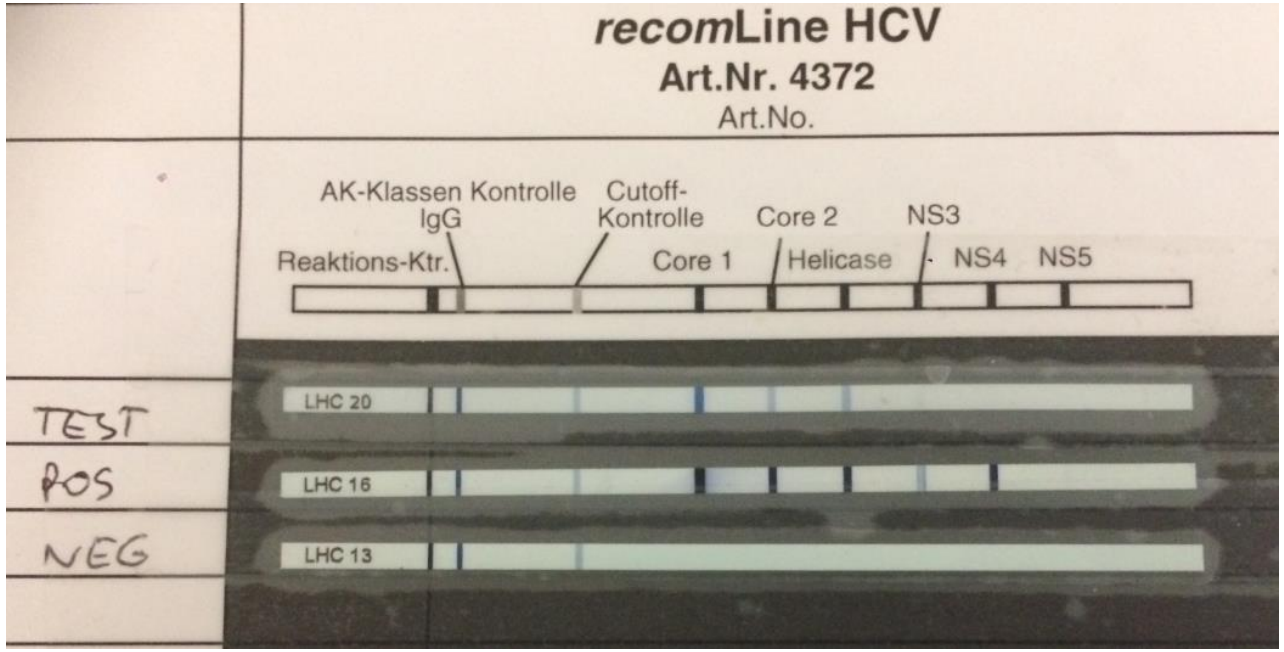
Learning Points Avidity

- Toxoplasma IgG avidity best regarded as a test of exclusion - high avidity excludes recent infection, low avidity persists
- Treatment further delays development of antibody profile
- Caution in interpretation of avidity results especially for interpretation of key pathogenetic mechanisms! E.g. transplacental CMV transmission

HCV Results

Question 5

Date	HCV Ab	HCV Ab 2	HCV PCR
08-Mar-16	101	NEG	Not detected



43 year old female

- c/o incidental raised white cell count (x2) due to a neutrophilia and mild monocytosis 4 months ago.
-
- hpc unwell ~4 months. Dental extractions (3) + Metronidazole. Fatigue. Arthralgia. Weight loss.
  No fever or sweats. No other symptoms. Bowels normal. Menstrual cycle a bit erratic recently
-
- pmh Jaundice due to HCV in 2000 (HCV antibody positive / PCR negative) – no longer attending OPs
  Giardiasis 8y ago in India.

- Occupation civil servant
- Medication none

o/e throat mildly inflamed. No palpable lymphadenopathy. Chest clear. Abdominal examination was normal. Full blood count today has also normalised.

- Conclusion Previous changes were reactive in the setting of infection. I have checked a number of additional routine blood tests and virology.

HCV Results

Date	HCV Ab 1	HCV Ab 2	C100	C22	C33	NS5	PCR
18-Mar-99	4		-	++++	++	-	
29-Mar-99	4.5		-	+++	±	-	
21-May-99	3.9		++	++	++++	-	
16-Mar-00	3.3		++	++	++	-	
16-Mar-00	4		++	++	++	-	
17-Oct-00	4.8						
08-Nov-00	3.2						ND
08-May-01	3.7						ND
06-Feb-04	4						ND
10-Aug-04	3.3						ND
24-Jun-05	4.5						ND
18-Jan-10	3						ND
26-Jan-11	3	POS					ND
08-Mar-16	101	NEG					ND

Is it important to confirm the specificity of HCV antibody in a screening assay!!

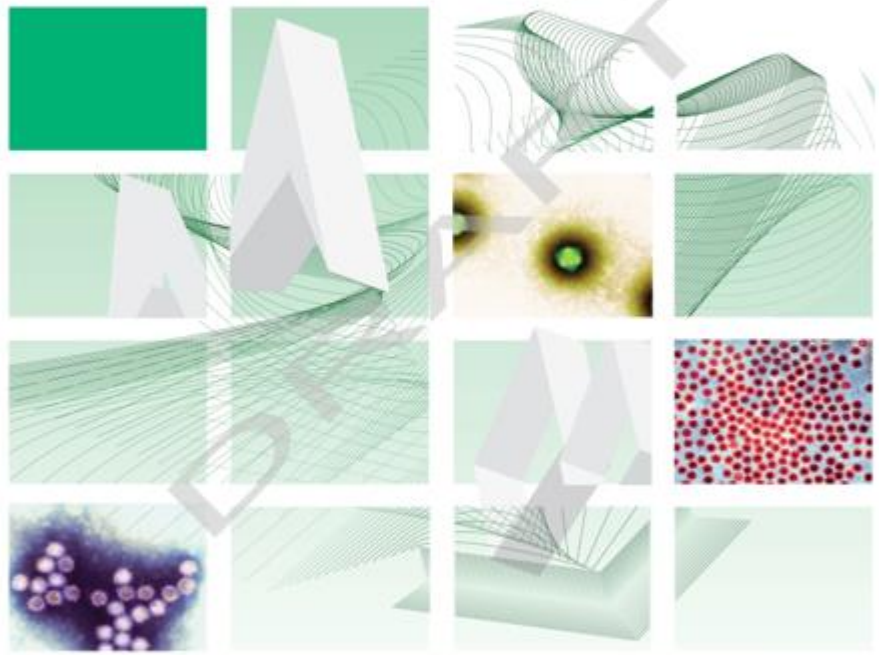
- Report to manufacturer
- Organs donors- interim measure refer to NVRL Dublin
- Audit assay performance since introduction of test
- Review verification data
- Identified 28 patients with positive HCV Ab 1 results (COI 0.82-51.68) which did not confirm using Vidas assay
- 4/28 patients records suggested true HCV positives
- On review of serology no evidence to suggest HCV Ab 2 assay was not performing with the expected sensitivity and specificity
- Continue to monitor performance – high positives on HCV Ab1 which do not confirm on HCV Ab2 should be scrutinised!

Public Health England

NHS

UK Standards for Microbiology Investigations

Screening for hepatitis C infection



NICE accredited

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation

Issued by the Standards Unit, Microbiology Services, PHE

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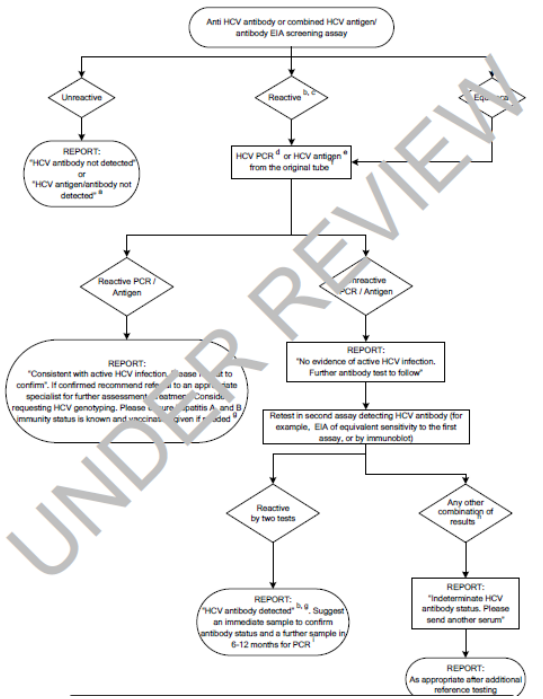
https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Investigation of Hepatitis C Infection by Antibody Testing or Combined Antigen/Antibody Assay



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