

## Calibration Issues - ● Phospholipid Antibodies and ANCA

Workshops in Portugal 2006

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## Neutrophil Cytoplasmic Antibodies - 2006

● UK Participants 108

● Non-UK Participants 308

Web entry system: 35%

Response rate:                      Approx 80%

## Neutrophil Cytoplasmic Antibodies - 2006

	Pos	Neg	Equivocal
C. ANCA	4	5	1
P. ANCA	1	9	
PR3	5	5	
MPO	1	9	

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## Neutrophil Cytoplasmic Antibodies - 2006

Manufacturer	0611	0612	0631	0632	0651	0652
All Manufacturers	21	109	180	169	47	126
Binding Site	18	64	168	144	37	106
BioDiagnostics		20	20	20	15	15
Bio-Rad/Kallestad/S	26	68	120	108	40	60
Euroimmun	15	75	118	168	36	96
Immco	40	143	304	176	56	186
Immunoconcepts	0	80	160	200	120	240
In House	25	119	185	150	105	154
Inova	23	127	207	166	44	132

C. ANCA IIF Titre  
Minimum 10 users

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## Neutrophil Cytoplasmic Antibodies - 2006

Manufacturer	0611	0612	0621	0622	0631	0632	0652
All Manufacturers	21	28	88	19	27	21	40
Binding Site	18	20	89	16	24	24	22
Bio-Rad/Kallestad/S	26	20	131	26	26	26	20
Euroimmun	16	14	85	15	14	12	14
Immco	40	40	110	20	40	40	40
Immunoconcepts	0	0	133	0	0	0	0
In House	258	25	64	20	20	20	13
Inova	23	52	69	20	29	28	80

P. ANCA IIF Titre  
Minimum 10 users

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## Neutrophil Cytoplasmic Antibodies - 2006

Manufacturer	0612	0631	0632	0651	0652
All Manufacturers	39	93	52	28	39
Axis Shield/Diastat	2	15	4	4	2
Binding Site	25	56	33	16	26
BioDiagnostics	59	106	83	57	68
BMD	161	362	242	123	172
Diamedix	44	144	58	47	34
Euroimmun	90	142	108	46	88
Fenning	15	50	25	19	18
Grifols AESKU	45	241	125	26	47
Hycor Biomedical	30	100	43	26	29
In House	33	136	48	45	35
Inova	50	81	56	34	42
ORGen Tec	21	53	30	18	24
Phadia Elia	17	97	23	21	14
Phadia Varelisa	11	40	15	11	10
Wieslab	102	117	105	28	111

PR3 Units/ml

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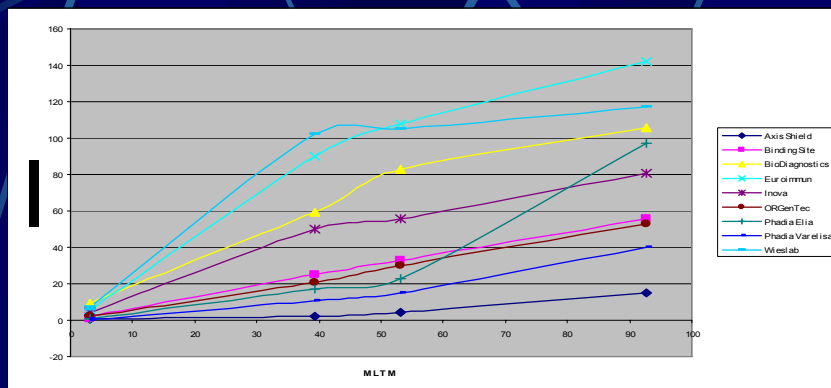
## Neutrophil Cytoplasmic Antibodies - 2006

Manufacturer	0611	0612	0621	0652
All Manufacturers	3	3	35	3
Axis Shield/Diastat	1	2	7	2
Binding Site	4	4	13	2
BioDiagnostics	3	3	25	2
BMD	0	0	67	0
Diamedix	1	1	16	1
Euroimmun	6	5	69	5
Fenning	2	1	25	3
Grifols AESKU	0	0	19	1
Hycor Biomedical	1	1	28	2
In House	5	5	29	3
Inova	4	3	23	4
ORGen Tec	2	2	13	2
Phadia Elia	1	1	32	1
Phadia Vareliisa	1	1	32	1
Wieslab	10	8	38	2

MPO Units/ml

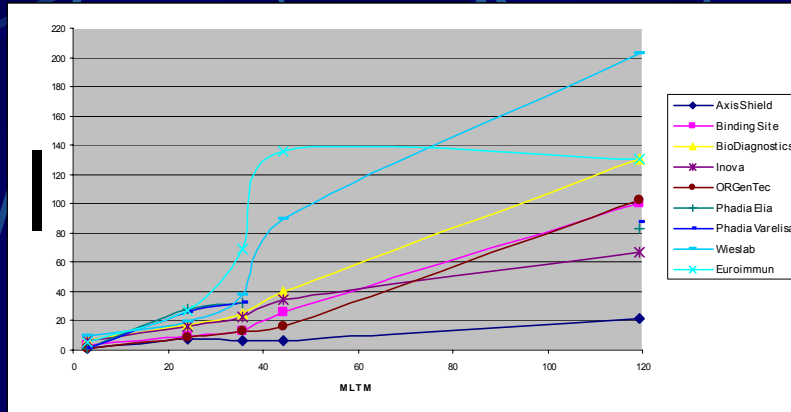
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## Neutrophil Cytoplasmic Antibodies – PR3 'Linearity/Calibration'



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## Neutrophil Cytoplasmic Antibodies – MPO 'Linearity'/Calibration



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## Phospholipid Antibodies - 2006

- UK Participants 135
- Non-UK Participants 255

Response rate: approx 83%

Incorporates IgG and IgM assays for antibodies to

Cardiolipin  
 $\beta_2$  Glycoprotein 1  
 Phosphatidylserine

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## Phospholipid Antibodies - 2006

- Some laboratories using 'in house' ELISAs

**but**

- most use one the commercially available products

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## Phospholipid Antibodies - 2006

- Commercial assays usually calibrated with 'Harris Standards'  
Louisville, Kentucky
  - Provided as a series of 'calibrators' to construct a calibration curve
- but
- there have been successive versions of the Harris Standard which are not cross referenced
  - Different versions have different values of GPLU/ml and MPLU/ml

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## Phospholipid Antibodies - 2006

- Scheme standards (IgG) produced in an attempt to allow analysis of EQA data in common unitage
- Calibrated against Harris version of the time (? Version 1) in GPLU/ml
- Current standard 97/514 produced from the same single donor source as original material and calibrated carefully from it – provides continuity of calibration and antibody specificity
  - Used by some manufacturers for calibrating their commercial assays

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## Phospholipid Antibodies - 2006

- Generally acknowledged that the Scheme standards 97/656 and 97/514 have not achieved the degree of uniformity which had been expected

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## Phospholipid Antibodies - 2006

- IUIS/WHO/AF/CDC Committee for the Standardisation of Autoantibodies
- Initiative to produce monoclonal IgG and IgM anticardiolipin standards
- Proposal to proceed with the release of the standards approved

But

- Some confusion as to whether they will only be suitable for quantitation of anti- $\beta_2$ -GP1

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## Phospholipid Antibodies - 2006

- Current position appears to be that lyophilised standards have been produced and are scheduled to be released by CDC Atlanta but may already be purchased from Inova
- Plans to quantitate and assign values as  $\mu\text{g/ml}$  rather than GPL or MPL units

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## Phospholipid Antibodies - 2006

### Intended Use

97/514 is a serum standard for cardiolipin antibody (IgG) for use in performance evaluation only in the UK NEQAS.

97/514 is prepared from human serum obtained from volunteer donors.

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## Phospholipid Antibodies - 2006

### Quality Control

Appropriate internal controls should be included in any assays performed where possible. If controls are out of range then potential problems with the assay should be investigated before releasing any results. The scheme standard should not be used as a primary internal control material

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## Phospholipid Antibodies - 2006

### Instructions for reconstitution and storage

Upon arrival the material shall be stored at below  $-18^{\circ}\text{C}$ . To make it ready for use, the freeze dried standard has to be reconstituted according to the following procedure by an appropriately experienced member of staff – this is a critical step and an error will impact on your EQA returns

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## Phospholipid Antibodies - 2006

Product	: 97/514 NEQAS scheme standard (lyophilised)
	: Reconstitute in 1mL of distilled water
Lot	: 001
Expiry Date	: 2007-03

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## Phospholipid Antibodies - 2006

### Assigned value of the standard

Positive test result for cardiolipin antibody (IgG) – **assigned value 61 GPLU/ml**

Autoantibodies of other specificities may be present

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## Phospholipid Antibodies - 2006

97/514 should be assayed in parallel with the two UK NEQAS samples sent on each distribution. Your assay values can be converted to 97/514 related values by following the worked example

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## Phospholipid Antibodies - 2006

If the value obtained for 97/514 in the assay run was 65 kit units/ml and the value for the QA sample was 78 kit units/ml then:

$$\frac{61}{65} \times 78 = 73 \text{ (97/514) GPLU/ml}$$

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## Phospholipid Antibodies - 2006

### Interpretation

The following reference intervals should be used for the semi quantitative interpretation of results:

• <20	GPLU/ml	Negative
• 20-40	GPLU/ml	Weak positive
• 41-60	GPLU/ml	Moderate positive
• >60	GPLU/ml	Strong positive

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## Anticardiolipin Antibody - 2006

0642	ACA 'G'			
	n	mean	SD	CV%
Binding Site	8	47.9	6.4	13.4
CLS Autozyme	21	38.5	8.7	22.6
Diamedix	9	76.5	29.5	38.6
Grifols	20	62.6	25.2	40.2
In House	14	43.6	20.0	45.8
Inova	18	52.9	11.4	21.6
Orgentec	89	69.2	16.8	24.3
Phadia	31	27.4	12.0	43.7

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## Anticardiolipin Antibody - 2006

0642	ACA 'G'			
	Neg	Wk Pos	Mod Pos	Strong Pos
Binding Site	1	0	8	0
CLS Autozyme	0	7	13	0
Diamedix	1	0	1	7
Grifols	2	0	5	13
In House	0	4	9	1
Inova	0	2	10	5
Orgentec	1	1	10	79
Phadia	2	6	18	4
<b>Overall Total</b>	<b>12</b>	<b>36</b>	<b>103</b>	<b>145</b>

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## Anticardiolipin Antibody - 2006

0641	ACA 'G'	
	n	mean
Binding Site	6	7.6
CLS Autozyme	21	6.1
Grifols	20	26.5
In House	11	7.8
Inova	18	13.8
Orgentec	91	19.8
Phadia	29	7.9

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## Anticardiolipin Antibody - 2006

0641	ACA 'G'			
	Neg	Wk Pos	Mod Pos	Strong Pos
Binding Site	9	0	0	0
CLS Autozyme	20	0	0	0
Diamedix	1	8	0	0
Grifols	3	15	2	0
In House	12	2	0	0
Inova	16	1	0	0
Orgentec	35	50	5	1
Phadia	27	2	0	1
<b>Overall Total</b>	<b>180</b>	<b>101</b>	<b>9</b>	<b>6</b>

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## Anticardiolipin Antibody - 2006

0641	ACA'M'	
	n	mean
CLS Autozyme	10	108.0
Grifols	17	75.4
In House	12	76.3
Inova	12	143.9
Orgentec	58	114.0
Phadia	19	83.4

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## Anticardiolipin Antibody - 2006

0641	ACA 'M'			
	Neg	Wk Pos	Mod Pos	Strong Pos
Binding Site	0	1	0	7
CLS Autozyme	0	0	0	13
Diamedix	1	0	0	8
Grifols	1	0	6	10
In House	1	0	3	9
Inova	0	0	0	15
Orgentec	1	1	2	74
Phadia	0	0	2	18
<b>Overall Total</b>	<b>6</b>	<b>6</b>	<b>24</b>	<b>213</b>

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## Phospholipid Antibodies - 2006

- International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)

Journal of Thrombosis and Haemostasis, 4: 295-306

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## Phospholipid Antibodies - 2006

- 'threshold used to distinguish moderate – high levels from low levels has no standard'
- 'definition of the level that best corresponds to the risk of clinical manifestations is difficult'
- 'the committee introduces a clear statement on threshold for positive:  
>40 GPL or MPL units

Journal of Thrombosis and Haemostasis, 4: 295-306

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## Phospholipid Antibodies - 2006

### Anticardiolipin assay

- 'Interlaboratory agreement on aCL measurement remains marginal with both home-based and commercial assays'
- 'Discrepancies are mainly because of cut-off, calibration and other methodological issues'
- 'Expression of aCL assays in ranges of positivity achieves better interlaboratory and inter-run agreement than do quantitative read outs'

Journal of Thrombosis and Haemostasis, 4: 295-306

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## Phospholipid Antibodies - 2006

- 'Methodology and standardisation limitations expressed for aCL also apply for anti- $\beta_2$  GP1'

Journal of Thrombosis and Haemostasis, 4: 295-306

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## Phospholipid Antibodies - 2006

- 'To optimise standardisation, new reference samples (monoclonal antibodies, named HCAL and EY2C9) will be distributed from the Centre for Disease Control ..... free of charge'
- 'They will have to be validated against existing calibrators, and their specificity, avidity and stability over time should be monitored'

Journal of Thrombosis and Haemostasis, 4: 295-306

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## Phospholipid Antibodies - 2006

### Cardiolipin Study

	ACAG Menarini	ACAG Orgentec	Ratio
97/656	84.4	53.5	1.6
97/514	84.6	54.7	1.6
HCAL	102.5	61.9	1.7
0531	51.1	29.9	1.7
0541	92.1	44.4	2.1

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Information about the programmes  
can be found on website  
[www.immqas.org.uk](http://www.immqas.org.uk)

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