IMMUNOLOGY, ALLERGY AND IMMUNOCHEMISTRY ANNUAL REPORT 2007
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UK NEQAS Immunology Interpretative Scheme  

Meetings and Conferences
UK NEQAS Annual Report 2007

Immunology, Allergy and Immunochemistry

We hope that you will find this report useful and informative.

The format and content will develop in forthcoming editions according to the needs of our participants

Feedback is important to enable us to tailor the format and content to meet the needs of participants and can be made via ukneqas@immqas.org.uk

We aim to highlight:

- The performance issues identified in the last year
- Hot topics (including relevant Guidelines and Best Practice statements) in the Immunology, Immunochemistry and Allergy arena which have implications for testing or interpretation
- The role of the UK NEQAS for Immunology, Allergy and Immunochemistry for participants, international and UK
- Participation in International and National Scientific Forums and Professional Meetings

We aim to make the information concise and easily digestible, with key learning points emphasised.

More detail on individual topics may be obtained on request through the Scheme Manager or Organiser via the contacts page

The schemes are subject to continuous development to make them more relevant to Quality Assurance in the 21st Century. To this end we have developed a new web-based interpretative scheme and educational tool, enhanced web-based data entry and an annual report.

We aim to make our schemes focus on clinically relevant decision points to reflect the way the assays are used, informed by practice standards such as National and International Guidelines, Protocols and Best Practice Statements. In this way we hope to assist and encourage participants to continually improve their assays, skills and procedures to improve Quality Assurance in diagnostic laboratories for the benefit of patients.
Sample acquisition and the Human Tissue Act (HTA)

EQA Needs YOU!
Sample acquisition is always a problem and UK NEQAS is grateful for all the support received from participants in the form of feedback, suggestions and sample material for distribution. In some schemes we could not continue without this support.

The use of surplus material from living patients for use in Quality Assurance is exempt from The Human Tissue Act.

Asking a patient to consider donating does not usually require ethical permission and is not research or audit.

We are always keen to receive materials for distribution - either surplus (serum or plasma from plasmaphoresis) or in the form of voluntary patient donations via participant laboratories.

We can provide you with a UK NEQAS information pack including leaflets and consent forms to facilitate the process of sample acquisition, and detailing the volumes required for distribution in each scheme. We will deal with anonymised testing for infectious disease prior to distribution.

All materials are irreversibly anonymised 48hrs after receipt - volunteer donors may withdraw consent from use within this time frame.

Please contact UK NEQAS dpatel@immqas.org.uk if you may have suitable material or wish to know more about sample collection.

The patient sera we have the most difficulty in obtaining are:

**Autoimmunity**
- glomerular basement membrane antibody
- skin basement membrane antibody
- endomysial/gliadin antibodies
- antimitochondrial antibody
- gastric parietal cell antibody

**Allergy & Immunodeficiency**
- Aspergillus and Candida infections
- avian antibodies
- Specific IgE: insect venom
  - peanut/hazel nut
  - cow’s milk
  - egg white
  - latex
- hereditary angioedema

**Tumour Markers**
- monoclonal gammopathy in serum & urine

Assistance in sourcing these would be very much appreciated
Scheme Reports

January 2006 - December 2007

Highlights

- Web-based data entry now available for all schemes
  - participants are encouraged to move to web-based data entry as soon as possible
- New Bone Marker Steering Group  p  8
- CSF Bilirubin Guidelines  p  11
- New AAT Phenotyping pilot  p  12
- Best practice in Cryoglobulin Measurement  p  16
- Further PSA calibration experiments  p  16
- Citrullinated Protein pilot scheme  p  22
- POCT sIgE Evaluation  p  25
- New European-wide sero-type specific Pneumococcal antibody scheme  p  26
- Interpretative EQA Scheme – CPD recognition  p  29
- Participants’ meetings  p  32
Programmes for Immunochemistry

UK NEQAS for AMNIOTIC FLUID (AFP)

- **Scheme Handbook 2008-2009**
- Only 31 laboratories were registered; 9 from within the UK
- No performance issues were identified in 2007 although it has not been possible to distribute amniotic fluid with elevated AFP concentrations due to lack of available material
- In view of the continued difficulties in obtaining amniotic fluid with elevated concentrations of AFP, it is possible that this Scheme may be terminated at short notice
- NHS Antenatal and Newborn Screening Programme – Working Standards for Down’s Syndrome Screening 2007 have issued the following statement on Acetyl-cholinesterase gel testing:
  “Following a raised AFP, a routine acetyl cholinesterase (gel) test on amniotic fluid is not necessary to detect neural tube defects. Instead, the diagnostic test of choice is an ultrasound scan”
- Questions remain as to why laboratories continue to offer a diagnostic service in these circumstances
- **AFP Statistics**
- **Sample acquisition**
- All samples distributed were tested and found to be satisfactorily stable and homogeneous
- **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service

UK NEQAS for ß2 MICROGLOBULIN

- **Scheme Handbook 2008-2009**
- **International Staging System (Greipp et al, 2003)**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
<th>Median survival in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Serum ß2 microglobulin &lt; 3.5 mg/L (296 nmol/l) &lt;br&gt; Serum albumin &gt; 3.5 g/dl (532 µmol/l)</td>
<td>62 months</td>
</tr>
<tr>
<td>II</td>
<td>Neither I or III*</td>
<td>45 months</td>
</tr>
<tr>
<td>III</td>
<td>Serum ß2 microglobulin &gt; 5.5 mg/L (465 nmol/l)</td>
<td>29 months</td>
</tr>
</tbody>
</table>

* There are 2 sub-categories:
  Serum ß2 microglobulin < 3.5 mg/L, but serum albumin < 3.5 g/dl, or
  Serum ß2 microglobulin 3.5 - 5.5 mg/L irrespective of the serum albumin level

**Method-related differences** in the levels measured make this categorisation difficult to apply consistently or satisfactorily, although the precision of the various commercial kits/assays is generally good (CVs approximately 10%)
• Standardisation of results across methods is critical for use in the UK-NORDIC Myeloma Guidelines - the recommended new International Staging System where low concentrations of the paraprotein need to be measured accurately to enable selection of treatment at first relapse
• Relevant references include:
• Better standardisation is needed - including a reference preparation with mass units. This protein is being included in the next version of CRM 470 which is scheduled to be available in 2008
• It is strongly advised that Guideline Working Groups recruit experts on laboratory analysis and ensure that they consider input from EQA schemes to determine that guidelines relating to measurement are technically achievable by service laboratories, before assigning action limits to guidelines
• β2 Microglobulin statistics
• Sample acquisition
• All samples distributed were tested and found to be satisfactorily stable and homogeneous
• NEW Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service

UK NEQAS for BONE METABOLISM ASSAYS

• Scheme Handbook 2008-2009
• Significant method-related variance has again been noted and scheme development will focus on this, attempting to evaluate interpretations
• NEW A Specialist Bone Marker Steering Group has been established to guide scheme development and improve the clinical relevance of the scheme
• Relevant references include:
• Sample acquisition remains a major problem. Any suitable serum and urine samples will be gratefully received
• NEW Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service

PILOT EQAS for C1 ESTERASE INHIBITOR and FUNCTIONAL COMPLEMENT ASSAYS

• Scheme Handbook 2008-2009
• The functional complement scheme continues to accrue new participants and 80 laboratories are now regularly returning results
• This scheme will again remain as a pilot in the forthcoming year
• C1inh measurement appears comparable across technologies
• The scheme is preparing samples which reflect clinically relevant analyte concentrations for future distributions, utilising novel approaches

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• Variance Index Scoring is not appropriate for C1 INH functional assays and misclassification scoring is to be introduced
• Relevant references include:
• **C1 Esterase Statistics**
• **Sample acquisition**
• All samples distributed were tested and found to be satisfactorily stable and homogeneous
• **NEW Web-based Data entry** is now available for this Scheme and *all participants are encouraged to register for this service*

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**UK NEQAS for C-REACTIVE PROTEIN (CRP)**

• **Scheme Handbook 2008-2009**
• Most methods show apparent stable performance and overall precision continues to improve (mean CV 6.3%) and biases are generally <5%
• Some anomalies of apparent bias in one method (which are variable and sample-dependent) have been satisfactorily addressed in collaboration with a manufacturer
• Latex particle agglutination is not recommended for quantification
• **Summary of Participant Questionnaire**
• **CRP Statistics**
• **Sample acquisition**
• All samples distributed were tested and found to be satisfactorily stable and homogeneous
• **NEW Web-based Data entry** is now available for this Scheme and *all participants are encouraged to register for this service*

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**UK NEQAS for ULTRASENSITIVE C-REACTIVE PROTEIN (UCRP)**

• **Scheme Handbook 2008-2009**
• No major problems have been noted with performance. Overall the profile is similar to the CRP scheme by method but this performance may not be sufficient to utilise the assay clinically in cardiovascular disease in the manner suggested in the literature
• We continue to focus on experiments examining the calibration and bias at clinically relevant decision points and the potential for antigen excess
• Relevant references:


- **UCRP Statistics**
- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - **NEW** Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service

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**UK NEQAS for CSF IgG OLIGOCLONAL BANDS**

- **Scheme Handbook 2008-2009**
  - Performance remains generally good and there are no obvious issues
  - The simulated CSF matrix technology is still proving to be successful. We will continue to focus on difficult banding patterns and their interpretation

- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - **NEW** Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service
• **Scheme Handbook 2008-2009**

**CEREBROSPINAL FLUID GUIDELINES FOR BILIRUBIN**

These have been recently revised and published in the Annals of Clinical Biochemistry


- Trial scoring of CSF Bilirubin spectrophotometric returns is ongoing. Performance criteria are being further developed and revised. There are still some participants using visual inspection and this is inappropriate

- Distributions continue to target critical performance areas in measurement of total protein and lactate where apparent bias in two methods continues to be subject to scrutiny

**Spectrophotometric Detection of CT – Negative Sub-arachnoid Hemorrhage – Ian Watson**

- An exercise to check the calibration of spectrophotometers will be conducted during 2008

**Introduction of UK NEQAS CSF Proteins & Biochemistry scheme – what effect has it had? – Dina Patel**

**Relevant references:**


- An alternative approach to spectrophotometric scanning based on the measurement of CSF bilirubin on an automated instrument had been reported to be an alternative robust screening test. This will be evaluated further in 2008

- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - **NEW** Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service

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**UK NEQAS Pilot for Alpha 1 Antitrypsin Phenotyping**

- **Scheme Handbook 2008-2009**
  - A total of 14 laboratories are currently registered to this new pilot scheme and are returning results
  - A summary of the data submitted since the inception of the scheme was presented at CAPA (Clinical Aspects of Protein Assays) in September 2007, *Pilot UK NEQAS for Alpha 1 Antitrypsin and Phenotype Identification*
  - Differences in the phenotype nomenclature used by laboratories were identified (with some laboratories reporting both isoforms for homozygotes (e.g. MM) and some not (e.g. M*)), potentially leading to confusion. Identification of the phenotypes distributed to date had been satisfactory
  - Some samples had degraded in transit due to industrial action in the postal service during the year. Although recognition of degradation is a key skill in interpretation, further effort will be made in future to ensure improved sample stability
Relevant references:
- The financial support of CPA Trust Ltd is gratefully acknowledged. The pilot will continue to be developed and extended to laboratories outside the UK
- **Sample acquisition**
- All samples distributed were tested and found to be satisfactorily stable and homogeneous
- **NEW Web-based Data entry** is now available for this Scheme and *all participants are encouraged to register for this service*
Programmes for Oncology

UK NEQAS for MONOCLONAL PROTEIN IDENTIFICATION

- **Scheme Handbook 2008-2009**
- Reports now contain method/platform specific data
- Overall performance is generally satisfactory for the identification of common isotypes but although we have previously demonstrated that many laboratories had insufficient skills or policies for recognising and acting on rare isotypes there is little evidence of improved performance overall.

- **Recent Distributions:**
  - **Distribution 074** - Serum IgD Lambda – only 55% of laboratories correctly reported IgD
  - 24% of laboratories said they would have referred the sample to another testing centre
  - 21% of laboratories failed to identify IgD or indicate that they would consider the possibility and make appropriate arrangements for testing. This is clearly unsatisfactory!
  - **Distribution 073** - Serum IgA Lambda – the majority of participants had submitted results which compared favourably with the target response. A significant number of laboratories had reported multiple IgA bands or additional light chain bands which may have been caused by some sample degradation
  - **Distribution 073** - Urine Kappa FLC – 0732 generated considerable discussion. Communication with participants has highlighted a number of potentially important issues about the sensitivity of the procedures necessary for the satisfactory detection of Bence Jones Protein and the necessity for interpretation in the light of the clinical details. This was also the subject of debate during the Participants’ Meeting which was held at the Clinical Aspects of Protein Assays (CAPA) conference in September 2007. A questionnaire was subsequently distributed to determine current practice and to identify why so many permutations had been reported. 111 laboratories had reported the presence of Kappa FLC; 168 laboratories reported the absence of light chains.

- **Relevant references:**
FREE LIGHT CHAIN ASSAYS

- The Scheme has continued to assess data submitted by laboratories using The Binding Site 'Freelite™' serum assay. Coefficients of variation have tended to remain high for this assay overall, with marked difference in the levels being reported on the same sample from different laboratories and attempts are being made in collaboration with The Binding Site to understand the reasons for this. Assessment includes studies to examine interference/matrix effects and/or antigen excess situations.
- At least one other assay for the detection of "free" light chains exists in Europe, and we will consider incorporating a method-related group if there is sufficient demand.
- Relevant references:
- Revised response criteria proposed by the International Myeloma Working Group state that serum FLC criteria for monitoring are solely for patients with disease that is not measurable with current methods. Before it displaces the M peak, the serum FLC assay must be shown to be an effective method for disease monitoring. However, given that there are clear discrepancies between quantitation of FLC assays and densitometric quantitation in individual sera, further evaluation is required.
- A study conducted in the Protein Reference Unit, Sheffield showed major and variable discrepancies with both elevated and lower values in comparison to the densitometric quantitation. Free Light Chain Quantitation: Freelite and Densitometry
- Relevant references:
  - Pattenden RJ, Rogers SY, Wenham PR. Serum free light chains; the need to establish local reference intervals. Ann Clin Biochem 2007;44:512-15
  - Ma ESK, Lee ETK. A case of IgM paraproteinemia in which serum free light chain values were within reference intervals. Clin Chem 2007;53:362-3
CRYOGLOBULINS

- The results of a questionnaire which was circulated to all participants in the Scheme has underlined the need for standardisation of the detection, analysis and reporting of cryoglobulins. The results of the survey together with an appraisal of current practice and recommendations to improve the detection and quantitation have been published
- An evaluation of current practice was presented at the annual conference of the UK Primary Immunodeficiency Network in November 2007. *Evaluation of the current practice in the detection, analysis and reporting of cryoglobulins in Europe*
- Other relevant references:

**Sample acquisition**
- All samples distributed were tested and found to be satisfactorily stable and homogeneous
- **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service

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**UK NEQAS for PROSTATE SPECIFIC ANTIGEN (PSA)**

- **Scheme Handbook 2008-2009**
- Accurate calibration and equimolarity of measurement between free and bound PSA is essential for use in the National Prostate Cancer Risk Management Programme (NPCRMP)
- **Equimolarity experiment distributed by UK NEQAS** in collaboration with NPCRMP in England previously revealed significant apparent differences in equimolarity and calibration. The *experiment was repeated during 2006 and again in 2007* (Distribution 0711), commissioned by DH Centre for Evidence based Purchasing in collaboration with the Guildford Medical device Evaluation Centre
- Relevant references:
- Assays must be able to distinguish levels around the 3µg/L trigger point reliably to be useful for patient selection in NPCRMP
- Mean CV of 8.7% was observed across methods (three assays were <5%) which is a **significant improvement on 2006**. Method mean CV% are generally <7%
- **Appropriate Internal Quality Control** requires a method CV of <4.5% to enable appropriate statistical power to detect systematic error using "Westgard-type" rules and also requires additional rules to detect random error. *Are your internal procedures up to the job?*

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• Analysis of the effect of different biases and imprecision on the referral rate for biopsy under the "informed choice programme" reveal the significant effect of minor variability in the performance characteristics of PSA testing and the need for standardisation. See UK Working Group on PSA Testing – Progress Report – Dr Cathie Sturgeon
• Free PSA CVs are generally higher than for total PSA and some assays have apparent positive biases sufficient to require NQAAP (National Quality Assurance Advisory Panel) discussion
• EQA of complexed PSA is available although there is no consensus at the moment that this is a superior measurement. Only 6 laboratories (all outside the UK) regularly return results
• We will continue to target distributions at clinically relevant action limits
• There has been improvement in total PSA and free PSA assay performance characteristics since the introduction of the IRPs and further development of commercial assays is likely during 2008
• Beckman Coulter has developed a second calibration protocol for its Access Hybritech PSA and free PSA assays, allowing traceability to WHO reference preparations
• There has also been a recent recalibration of the SMSD Immulite total PSA assay to correct a long-standing positive bias
• Although considerable progress has been made towards improving method comparatively, results from different assays are still not interchangeable
• Relevant references:
• Research is being undertaken to find alternatives to the PSA test such as Human Kallikrein 2 and genetic markers which can potentially distinguish between aggressive and non-aggressive cancers
• **Prostate Cancer Awareness Week** (19th – 25th March 2007) highlighted the largest ever global trial in the area of prostate cancer and coincided with the availability of a **Prostate Cancer case on the Interpretative EQA Scheme**
• **Total PSA Statistics**
• **Free PSA Statistics**
• **Sample acquisition**
• All samples distributed were tested and found to be satisfactorily stable and homogeneous
• **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service
UK NEQAS for TUMOUR MARKERS (CA SERIES)

Ovarian Markers (CA125)

- **Scheme Handbook 2008-2009**
  - Apparent calibration and specificity issues (with respect to glycosylation differences) continue to tax the scheme organisers, manufacturers, steering groups and participants.
  - Between-assay and within-assay precision are good, validating their suitability for use in monitoring (Method CVs are usually <5%) but results from different assays cannot be compared directly.
- **Ovarian Marker Statistics**
- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous.
  - **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service.

Breast Markers (CA15-3)

- **Scheme Handbook 2008-2009**
  - Similar apparent differences in method related bias as for CA125.
  - Between-assay and within-assay precision are good (Method CVs are usually <10%), validating their suitability for use in monitoring, but results from different assays cannot be compared directly.
- **Breast Marker Statistics**
- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous.
  - **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service.

Gut Markers (CA19-9)

- **Scheme Handbook 2008-2009**
  - One assay has a marked apparent bias, with some sample dependency. The clinical significance of this difference is unknown but means that this assay produces very different values from those of another assay from the same manufacturer. Results from different assays cannot be compared directly.
  - This major change in performance with assay migration across platforms is being investigated and was the subject of considerable discussion during the UK NEQAS Forum of the Clinical Aspects of Protein Assays (CAPA) Conference in September 2007.
- **Gut Marker Statistics**
- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous.
  - **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service.
Standardisation

**NEW** A proposal has been received for collaboration in a project to produce standards for the CA markers. Putative materials will be distributed during 2008 to determine whether they are likely to be suitable.

**Neuron Specific Enolase (NSE)**

This specialised scheme has a low, but stable, number of participants. No particular performance issues have been identified.

- **Scheme Handbook 2008-2009**
- **NSE Statistics**
- **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
- **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service
Programmes for Autoimmunity

UK NEQAS for NUCLEAR ANTIBODIES AND RELATED ANTIGENS

- **Scheme Handbook 2008-2009**
  - The nuclear antibody returns continue to demonstrate considerable heterogeneity of results for ENA and DNA assays with no obvious drift to improved standardisation
  - There continues to be a wide range of reported values in dsDNA antibody assays despite claimed standardisation against the IRP Wo80 in most methods. Laboratories using one commercial assay produce results which are significantly negatively-biased in relation to other methods, a matter which has been raised with the distributors of this product, but it remains to be seen if this clinically relevant (as either a detriment or a benefit)
  - ANA screening assays are becoming more common but remain a minority interest. They sometimes contain Jo-1, which sometimes causes confusion in EQA returns where nuclear antibody positivity is reported. Jo-1 is a cytoplasmic antigen and is not classified as an ANA in our designated responses. This will continue to generate MIS scores for such laboratories unless they differentiate the Jo-1 positives in their returns
  - Addition of centromere antigens to ENA screening EIA has generated further confusion. Centromere patterns need to be recognised as such to avoid MIS for ENA - as well as to ensure a clinically relevant report. Although it was not possible to incorporate a centromere positive in 2007, this antibody will feature in future distributions
  - Performance scoring to include subjective evaluation of ANA staining pattern continues to be challenging. A higher proportion of laboratories than expected are reporting homogeneous staining patterns in circumstances where speckled would have been anticipated and indicated by related data. **Whether this is clinically relevant depends upon the reflex testing protocol with respect to staining patterns in individual laboratories.** The use of the interpretative EQA programme for exploring the interpretation of autoantibodies will be important as there appears to be considerable variability in practice
  - Use of multi-analyte screening technologies generates a need to have a robust reflex testing protocol to ensure correct identification of clinically relevant antigens and enable the issuing of appropriate clinical reports. **The scheme will endeavour to assess the level of technical validation and clinical reporting since this is critical to the proper performance of assays and provision of clinically helpful and relevant reports**
  - Sample 0751 comprised a serum pool from some 300 healthy NBS donors. Approximately one third of laboratories reported the presence of an ANA and in many instances also of Ro. The report commentary encouraged participants to submit their comments and those are gratefully acknowledged.
  - An **additional commentary** has been produced which addresses the interpretive and technical validation issues raised by this distribution
- **Nuclear Antibodies and Related Antigens Statistics – Summary of Responses**
  - **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
• The number of laboratories using web entry (now 51%) continues to grow and all participants are encouraged to register for this service

**UK NEQAS for PHOSPHOLIPID ANTIBODIES**

**Scheme Handbook 2008-2009**

• Even though it is common practice for laboratories to report their patient results as a numerical value, EQA Scheme participants were previously required to interpret as negative, weakly positive, moderately positive and strongly positive. Misclassification scoring was based on the ‘grades’ reported with assessment in relation to consensus results. This did not achieve its objectives of improving standardisation and was therefore reviewed and participants are now required to submit their results as either positive or negative in relation to their usual local thresholds only (kit units) **NEW**

• *The Scheme Standard has been withdrawn.* Poor performance has been re-defined as MIS >3. Customer feedback has indicated that participants are more comfortable with this mode of reporting to the Scheme

**NEW** The International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome introduced a clear statement on the threshold for *positive results as >40 GPL or MPL units.* The statement also acknowledged that inter-laboratory agreement remained marginal with both home-based and commercial assays, discrepancies mainly being due to differences in cut-off, calibration and other methodological issues, which rather undermines the intention of stating a standard threshold. This limitation also applies to anti-B₂GP 1 assays. *The release of new reference monoclonal standards HCAL and EY2C9 has unfortunately been significantly delayed*

• Relevant references:

• The introduction of international standards alone is not likely to resolve the existing anomalies and inter-assay variability since there appear to be significant differences in the specificities of commercial assays which is compounded by lack of an agreed and consistent approach to interpretation of the clinical significance of any particular result

• Patient antiphospholipid antibody results should always be interpreted in the context of the clinical picture by someone with appropriate experience

**Sample acquisition**

• All samples distributed were tested and found to be satisfactorily stable and homogeneous

**NEW** Web-based Data entry is now available for this Scheme and *all participants are encouraged to register for this service*
UK NEQAS for GENERAL AUTOIMMUNE SEROLOGY

- **Scheme Handbook 2008-2009**
  - Performance is generally satisfactory
  - There is variation in the RF quantification of results even though commercial assays are nominally calibrated against the IRP. Issues relating to the specificity of one commercial assay are being investigated and dialogue with the company concerned is ongoing.
  - This also applies to TPO with another manufacturer
  - The inability of some participants to distinguish mitochondrial and LKM antibodies noted in the past continues to surface occasionally but the overall situation has improved. A proportion of participants still decline to make their minds up when they see an LKM - **please report what you see and decide whether it is an LKM or a mitochondrial antibody** (as you would have to do with a patient serum). This issue was the subject of a presentation at the Participants Meeting in November 2006 - **Mitochondrial and Liver Kidney Microsomal commentary**
- **NEW** The pilot for citrullinated proteins/peptides is progressing well and data was presented at the Participants Meeting in September 2007, **UK NEQAS Pilot for Antibodies to Citrullinated Proteins**
- Interpretation of results will be explored utilising the Immunology EQA Interpretative Scheme
- A comparative study has demonstrated that the commercially available methods do not all have the same diagnostic accuracy
- **General Autoimmune Serology Statistics – Summary of Responses**
- Sample acquisition
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - The number of laboratories using **web entry** (now 45%) continues to grow and all participants are encouraged to register for this service

UK NEQAS for Acetylcholine Receptor Antibody

- **Scheme Handbook 2008-2009**
  - Participant numbers are stable
  - There is concordance in qualitative interpretation but numerical agreement has deteriorated. The dilution regimens utilised by participants appear to vary – even by users of a given assay. Clear guidance in the manufacturers’ kit inserts is needed. Have laboratories modified their practice during the last 12 months?
- Sample acquisition
  - **NEW** **Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service
UK NEQAS for ANCA AND GBM ANTIBODIES

• **Scheme Handbook 2008-2009**
• General performance of assays is usually satisfactory. When identical samples have been distributed (Distribution 073), results had compared satisfactorily
• A regular subset of users report the "wrong" IIF pattern – this is probably substrate-related and is to be expected since this variability is seen in up to 5% of returns and is a well recognised and reproducible artefact of using different neutrophil preparations (where a C-ANCA pattern is seen where a P-ANCA would be expected and where a P-ANCA pattern is seen when a C-ANCA is expected in a minority of laboratories). *Laboratories should be aware of this and issue interpretative results in the light of this knowledge*. This requires evaluation through the Immunology EQA Interpretative scheme. Users of one commercial product consistently report anomalies and dialogue with the company has taken place
• The production of Scheme standards for C-ANCA and P-ANCA are being considered in order to address the issue of poor numerical agreement between methods. An analysis of numerical data together with an assessment of assay dynamics and linearity was presented at the Participants’ Meeting in November 2006. **ANCA Assays – where is the standardisation?**

• Relevant references:

• **ANCA and GBM Antibodies Statistics – Summary of Responses**
• **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - The number of laboratories using **web entry** (now 47%) continues to grow and all participants are encouraged to register for this service
Participants generally perform well in this Scheme and no methodological anomalies arose during 2007 except that changes in the formulation of one commercial assay led to confusion since those changes were not communicated properly to customers.

Tissue transglutaminase and endomysial antibody assays appear roughly equivalent in diagnostic performance overall, with a probable small increment in detection with the best of the tTG assays (at the probable cost of increased false positives), but this is heavily dependent on pre-test probability of coeliac disease in the population screened. Minor differences in false-positivity can result in significant differences in the number of patients referred for biopsy, and there is variability between different assay systems. *Users should evaluate and configure their diagnostic strategy in line with the service user’s clinical needs* to ensure that the assays are fit and cost-effective for local purposes.

**NEW** A rapid antibody test for use in primary care has been developed and will need to be evaluated and may require a Near-patient (NPT) or Point of Care (POCT) scheme in future


An effective diagnostic method of detecting all cases of coeliac disease in patients referred for gastroscopy without performing routine duodenal biopsy has been described


Gliadin assay usage appears to be in decline in line with their inferior diagnostic performance to IgA transglutaminase and endomysial antibodies.

A recently published survey highlights areas requiring the development of consensus guidelines


**British Society for Gastroenterology – Interim Guidelines for the Management of Patients with Coeliac Disease (revised 2002)**

**Bullous Dermatosis and Coeliac Disease Statistics – Summary of Responses**

- Bullous dermatosis samples have become extremely difficult to source

**Sample acquisition**

- All samples distributed were tested and found to be satisfactorily stable and homogeneous

- The number of laboratories using web entry (now 47%) continues to grow and all participants are encouraged to register for this service.
Programmes for Allergy and Immunodeficiency

EuroEQAS for ALLERGEN SPECIFIC IgE

- **Scheme Handbook 2008-2009**
  - Programme potentially surveys laboratory performance in the identification and quantitation of common or clinically important IgE specificities.
  - Participants were requested to return data on 15 different allergens in 2007. Requests have been received to include additional allergens - horse, wheat and olive pollen. These will be incorporated in 2008, where possible.
  - Performance in this Scheme is generally satisfactory although scoring is entirely based on the grades reported rather than numerical values.
  - Mean values, both at low and higher concentrations, often vary considerably between methods. We will continue to try to examine assay performance with respect to clinical diagnosis as *all assay results are meaningless without expert interpretation in the clinical context*.
  - Precision is also variable between methods although the precision indices are consistently ≤10% for most common allergens distributed in the scheme, this may not be true across the entire repertoire of allergens available (hundreds of different allergens are available).
  - There continues to be discussion about the introduction of an extended reporting range <0.35kU/L and the wisdom of manufacturers to encourage laboratories to measure below this concentration is questioned since the clinical relevance of this has not been demonstrated and it will inevitably erode the specificity of sIgE testing and will probably cloud interpretation. Further research on the clinical utility of this approach in expert allergy centres is required.
  - Knowledge of the existence of allergen cross-reactivity and its clinical relevance remains an important issue in allergy practice and often leads to misdiagnosis and clinical confusion if interpreted wrongly.
    - **www.allergome.org**
    - **Aalberse RC.** Assessment of allergen cross-reactivity. *Clinical and Molecular Allergy* 2007;5:2.
- **‘The Challenge for External Quality Assessment in Allergy’**
  - POCT devices are now becoming available. An evaluation of one commercially available near patient testing device was undertaken during 2007. *Near Patient testing in Allergy Diagnosis*.
  - Donors willing to give blood continued to be difficult to find. Contact from volunteers would be welcomed.
  - **We will continue to utilise the Interpretative Scheme to examine the clinical appropriateness of reporting practices**.
  - **Sample acquisition**
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous.
  - **NEW Web-based Data entry** is now available for this Scheme and all participants are encouraged to register for this service.
UK NEQAS for IgE

- **Scheme Handbook 2008-2009**
- No performance issues
- Although recovery experiments using the IRP were not performed during 2007, the relative bias of methods remains unchanged and ‘recovery’ based on materials previously distributed in the Scheme is satisfactory
- All samples distributed were tested and found to be satisfactorily stable and homogeneous
- **NEW** Web-based Data entry is now available for this Scheme and all participants are encouraged to register for this service

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UK NEQAS for SPECIFIC MICROBIAL ANTIBODIES

Pneumococcal (Streptococcus pneumoniae) antibodies

- In-house and commercial kits return broadly similar results. CVs remain around 20%
- **Absorption Experiment**
- **NEW** Despite this experimental observation there is no obvious bimodal difference in results - even though the commercial kit has an absorption step (many in-house users apparently do not CPS absorb) - suggesting that intrinsic assay variability may mask any differences due to technique overall. Nevertheless there will be individual patients with big differences in results generated by absorption where CPS reactivity predominates in their antibody response. Therefore CPS absorption remains the ideal practice and is essential for the measurement of serotype-specific responses.
- **NEW** Serotype-specific values are now published on all samples. A total of 4 laboratories are currently reporting serotype specific concentrations and a scoring scheme will be developed if the user-group expands sufficiently
- **NEW** The European Primary Immunodeficiencies (PIDs) Consensus Conference in Langen (2006) outlined the need for improved quality assurance for assays to measure specific antibodies to common pathogens and immunisation antigens. In order to assess the variability of these antibody measurements in Europe, prior to re-examining diagnostic criteria, a pilot study was undertaken. The aims were to develop quality assurance for pneumococcal assays, particularly for new laboratories in Europe, and to lay the foundation for a comparison of available assays for test immunisation in the diagnosis of PIDs. *Preliminary data from the study was presented during The Participants’ Meeting at the Clinical Aspects of Protein Assays (CAPA) conference in September 2007.* The study has since been completed and has identified the need for EQA of serotype-specific antibodies throughout Europe. **This scheme extension will be initiated by UK NEQAS during 2008.**
- **Quality Assurance for Pneumococcal Assays in Europe**
- The usage of serotype-specific results will be kept under review
Establishing generally accepted protective titres for each serotype and a robust definition of a deficient response to vaccination remains difficult. The lower 5\textsuperscript{th} centile reference threshold post-immunisation antibody concentration in healthy individuals are reported to be different for each serotype at 0.67 mg/L, 0.46 mg/L, 0.31 mg/L and 1.04 mg/L for serotypes 3, 4, 9N, 18C and 19F respectively. Levels > 0.35 mg/L are taken as evidence of a “protective response” to serotypes overall by some authorities yet clearly a normal response to some serotypes might be expected to be much higher and this may influence interpretation. Clearly further investigation and a consensus for interpretation of responses in the investigation of immunodeficiency is needed.

Relevant references:


**Haemophilus influenza B antibodies**

- Precision is less satisfactory than for Pneumococcal antibodies with CVs of approximately 30%.
- **NEW** The previously observed bi-modal distribution of in-house assay versus the single commercial kit [despite apparent calibration against an international standard for anti-HiB antibodies (FDA 1983)] has now virtually disappeared suggesting recalibration of the assay.
- Standardisation, precision and accuracy are important since long-term protective titres are established which need to be comparable across different laboratories.

**Tetanus antibodies**

- Precision is similar to Pneumococcal assays with CVs of between 20%-30%.
- Similar results are returned by in-house and commercial kits.
- The working range of these assays is usually in the region of 0.1 IU/ml to 6.0 IU/ml. UK NEQAS will continue to explore performance at the lower end of this spectrum where the absolute and long term protective titres lie.
- The clinical decision point for absolute protection is generally taken as 0.012 IU/ml.
UK NEQAS for IgG SUBCLASSES

- **Scheme Handbook 2008-2009**
  - We can now measure subclasses very well, but are the assays useful in the assessment of immunodeficiency?
  - Future developments will explore the clinical interpretation of these results via the Immunology EQA Interpretative Scheme
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - **NEW Web-based Data entry** is now available for this Scheme and **all participants are encouraged to register for this service**

UK NEQAS for ANTIBODY TO FUNGAL AND AVIAN ANTIGENS

- **Scheme Handbook 2008-2009**
  - The method-related differences in EIA versus Double Diffusion persist. The significance of these differences remains uncertain
  - Debate continues as to the most appropriate method to measure these antibodies and their clinical correlation
  - A number of publications have helped in the validation of quantitative fluorimetric assays (ImmunoCAP) in bird fanciers’ hypersensitivity pneumonitis:
  - **NEW** Analysis of numerical values was introduced in UK NEQAS reports during 2007
  - Other relevant references:
    - Van Hoeyveld E, Dupont L, Bossuyt X. Quantification of IgG antibodies to Aspergillus and pigeon antigens by the ImmunoCAP Technology: An alternative to the precipitation technique? *Clin Chem* 2006;52(9):1785-93
    - Agreement between the precipitation and ImmunoCAP technique was 86% for A fumigatus, 70% for pigeon antigens in this study
  - The sensitivity of the traditional double diffusion technique is reported to be approximately 40 mg/L, the titre below which visible precipitation lines are rarely observed
  - A series of lower reference intervals have been published by a laboratory using the ImmunoCAP (PEIA) technology which would be regarded as negative
    - pigeon serum proteins <10 mg/L
    - Micropolyspora faeni <10 mg/L
    - Aspergillus fumigatus <40 mg/L
  - Dye G. Specific IgG and ImmunoCAP IgMAGE: *News from Sweden Diagnostics (UK) Ltd.* 2006; Jan Issue 1
  - Meanwhile scoring remains based on qualitative results and consensus
  - The interpretative element requires exploration in future
  - **Sample acquisition remains a major problem.** If you know any pigeon fanciers or aviarists who would like to donate, please let us know
  - All samples distributed were tested and found to be satisfactorily stable and homogeneous
  - **NEW Web-based Data entry** is now available for this Scheme and **all participants are encouraged to register for this service**
UK NEQAS
Immunology Interpretative Scheme

www.immqas.org.uk

PILOT INTERPRETATIVE SCHEME

What is it?

This is a pilot web-based educational scheme that allows individuals to practise their clinical or scientific interpretative skills on a virtual patient’s results. ALL scientific laboratory and medical staff in participant laboratories can navigate through a series of test results and medical information to investigate a case in the same manner as they would in a real laboratory or clinical setting. All the information that would normally be available will be accessible in various formats from numerical results through to scans, images, sample information and clinical history. You may draw conclusions and make comments and compare them with the 'correct' answer as well as that produced by your peer group. This will include the normal cost of investigation and the efficiency with which you completed the case. You may repeat any case as many times as you wish to demonstrate improvement or to assist learning. All registrants' performance data will be anonymous so only you will know how you have performed in relation to your peers. The scheme will be registered for CPD.

Who can register?

This pilot scheme is available, initially at no extra cost, to ALL grades of scientific staff in participant laboratories.

How to register

To register online you need to spend a few minutes filling out a registration form available from our website.

We will be interested in comments from users to improve the web-based programme once you have tried it out!

There are now flash-demo tutorials available to demonstrate use of the scheme
Cases have been divided into categories to facilitate easy access to items of interest.

The Web-based interpretive Scheme continues to grow with cases being added regularly and with excellent feedback.

The system is designed to be used as an educational tool, a training environment and an opportunity to practice clinical interpretation in real time, benchmarked against an “expert” interpretation and your peer professional group.

It can be used to facilitate reflective learning and repeated practice to demonstrate improvement in performance and cost-efficacy with time.
The scheme is already registered for CPD with the Royal College of Pathologists and registration with the Institute of Biomedical Sciences will follow soon

If you have cases or educational scenarios which you would like to be incorporated into the educational scheme please contact eqacases@immqas.org.uk to discuss and for a case submission pro-forma

Suggestions and feedback are always welcome on eqacases@immqas.org.uk
A Participants’ Meeting for ‘Analysis of Cerebrospinal Fluid’ was held on Tuesday 12th June 2007 in the Postgraduate Medical Education Centre, Northern General Hospital, Sheffield.

The programme included formal presentations and interactive discussion on the following topics:

- **Individual Interpretative EQA System**
  - demonstration
  - future strategy and development
  - registration for CPD

- **UK NEQAS for Proteins and Biochemistry**
  - review of data
  - progress with scoring haem pigments

- **UK NEQAS for Oligoclonal Bands**
  - performance issues

- **CSF Ferritin**

- **Chemical (direct) measurement of Bilirubin**

- **National Guidelines for analysis of CSF for Bilirubin in suspected SAH**
  - update

- **National Audit of CSF Testing**
  - report

- **Latest Developments**

- **Clinical Aspects**

The meeting was open to all grades of staff and was free of charge.

*The meeting was registered with the Royal College of Pathologists and the Institute of Biomedical Sciences for CPD.*
A Participants’ Meeting was incorporated into the Clinical Aspects of Protein Assays Conference which was held at the Royal Holloway College, University of London, Egham, Surrey

Sunday 16th to Thursday 20th September 2007

Tuesday 18th September

Functional complement – C1 esterase inhibitor
Assay anomalies Alice Wiltshire
Clinical utility Bob Lock
Review of EQA data Peter White

Alpha-1 antitrypsin phenotyping
Methodology unravelled Graeme Wild
Progress with the pilot EQA schemes Dina Patel

Specific Microbial antibodies
QA of pneumococcal antibody serotype specific assays Daniel Harrison

Antibodies of citrullinated proteins
Review of EQA data Dina Patel
Utility of the assays and requesting strategy in clinical practice Jeremy Wilson

Tumour Markers
Clinical and analytical aspects of the CA markers Kristina Hotakainen

CA19-9 – a laboratory perspective Ian Barlow
Review of EQA data and discussion Peter White

The meeting was registered with the Royal College of Pathologists and the Institute of Biomedical Sciences for CPD.