



Birmingham Quality

Birmingham Quality

Previously known as the *Wolfson EQA Laboratory*,
Birmingham Quality provides primarily
UK NEQAS External Quality Assessment
Services in Clinical Chemistry



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INFORMING RECOMMENDATIONS ON DATA COMBINATION

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A project supported through a grant from DH Pathology

PHASE 2 REPORT

Web publication version, July 2011

1 Project status

The project was to be delivered in two stages. Phase 1 was completed, and an interim report prepared in June 2010

The key stakeholder meeting had already been held, and Phase 2 proceeded, informed by the comments and recommendations received

Phase 2 was completed, and a final report presented in May 2011

2 Project objectives

The project objectives were fourfold:

- to identify analytes for which the results of quantitative analyses from any laboratory and analytical/method principle (including point of care testing procedures where data exists) should be comparable across England, the UK and internationally, subject only to maintaining between-laboratory & between-manufacturer comparability through conventional ongoing quality assurance (IVD Directive traceability, evaluation, comparison, internal quality control, EQA etc) measures – 'Class 1' analytes (Dr Rick Jones)
- to identify analytes where additional constraints (eg restriction of permitted method principles) are required to permit comparability – 'class 2' analytes
- to identify analytes where the method principle (including manufacturer-related differences) limit or preclude comparability of results across time and geography; these represent the major patient safety issues – 'class 3' analytes
- to provide relevant advice and comments on harmonisation of 'semiquantitative' investigations in laboratory medicine (eg ordinal scales in urine dipstick testing, where one manufacturer's "2+" may differ substantially in meaning from another's "++")



Birmingham Quality is part of the University Hospitals Birmingham NHS Foundation Trust and a WHO Collaborating Centre for Research & Reference Services in Clinical Chemistry.
All full UK NEQAS services are CPA(UK)Ltd EQA Scheme accredited

3 Project delivery plan

The project will be delivered in two phases, for scientific and practical reasons:

- Phase 1 will be an exploratory phase, exploring and delineating principles and conclusions for analytes in the EQA services delivered by Birmingham Quality, to deliver an interim report
- Phase 2 will extend the principles and conclusions to include the other UK NEQAS services in Clinical Chemistry and those in Haematology and Immunology, with the support of the relevant Organisers, to deliver a final report

3a Phase 1

We will review systematically the features of the quantitative analytes included in UK NEQAS Birmingham's repertoire, considering methodological & metrological aspects as well as EQA data, to

- allow classification of straightforward cases
- concentrate effort on reviewing in detail analytes with special issues, to determine their appropriate classification

We will review class 2 & class 3 analytes to identify:

- particular risks to patient safety or data integrity of inappropriate combination
- potential mitigating processes (eg adoption of common method principles or calibration) which could permit more extensive combination
- any requirements for further work

We will consider semiquantitative analytes in UK NEQAS Birmingham's repertoire, to identify:

- particular risks to patient safety or data integrity of inappropriate combination
- potential mitigating processes which could permit more extensive combination
- any requirements for further work

We will then assess the process and resulting classification to identify common principles, risks, mitigating processes and conclusions. This will:

- constitute (with the classification and notes) the interim report
- provide a consistent basis for Phase 2

3b Phase 2

We will convene a meeting with key UK NEQAS stakeholders in other disciplines (primarily Haematology & Immunology) to:

- outline the objectives and implementation of the project, drawing on initial Phase 1 experience
- secure support for extension to the relevant (quantitative) analytes in other UK NEQAS centres' repertoires
- identify any barriers to extension and formulate strategies to overcome them

With the various UK NEQAS Organisers, we will repeat the review processes outlined above, presenting the information in the same format.

We will then review the interim report in the light of the additional experience in Phase 2. The final report will include any further principles, risks, mitigating processes, conclusions and examples.

4 Process

4a Phase 1

The initial stage of the work comprised a 'first pass' review using the UK NEQAS for Clinical Chemistry as an example Scheme. This was expected to include analytes of all three classes, and review continued on the UK NEQAS for Clinical Chemistry and the other Schemes for which Dr Bullock is designated Organiser, and the working spreadsheet analysis by Scheme was reviewed by the project Steering Group

They provided comments on the overall approach taken, how the supporting tabulations should be organised, and elements which had been overlooked

Examination was then extended to include all Schemes in UK NEQAS Birmingham's repertoire, data extracted from the working spreadsheet into an appropriate supporting tabulation format, and the Phase 1 report produced

Separate tabulations by class were appended. Within each tabulation analytes were arranged alphabetically within each Scheme (Schemes include analytes which are related functionally and/or analytically, and usually in the same matrix), with matrixes in the order serum (implied if no matrix is specified), blood, urine and other

The tabulation included for each analyte:

- its classification and whether this was qualified by comments
- the within-subject and between-subject biological variation [CV %; from Ricos et al (2008) at www.westgard.com ; optimal precision is one quarter of the within-subject BV]
- issues regarding nomenclature, sample preparation and reporting units
- issues regarding analytical methods
- additional comments

4b Stakeholder meeting

The key UK NEQAS stakeholder meeting was held on 25 September 2009. This was attended by UK NEQAS Organisers across the disciplines of Haematology, Immunology and Microbiology, as well as by Prof Batstone. He kindly outlined the background to the project and Dr Bullock described some of the initial findings and how we would be looking to them to contribute to Phase 2 of the project. A positive and constructive discussion followed, with support in principle from the other disciplines represented. The need for this project to follow scientific principles and the desirability of publication were confirmed. Whether the proposed timescale was achievable was questioned, however, and it was agreed that the objective should be to complete Phase 1 and await feedback on the interim report

4c Phase 2

We had requested comments on any aspect of the Phase 1 report, particularly:

- the overall approach taken
- organisation of the supporting tabulations
- any elements which appear to have been overlooked
- the principles proposed
- factors of relevance to Phase 2

The feedback was generally positive, and this Phase 2 report follows the same format

All UK NEQAS Organisers were provided with the Phase 1 report and the project scope, including Dr Rick Jones' original paper on data combination, and asked to provide an analysis of the investigations covered by their Schemes and comments for inclusion in the Phase 2 report

The appended tables incorporate information from the UK NEQASs for Peptide Hormones, Insulin, Cardiac Markers, General Haematology, Haematinic Assays, Vitamin K, Blood Coagulation, Leucocyte Immunophenotyping, Medical Microbiology, Antibiotic Assays, Andrology and Immunology & Immunochemistry, presented in that order. Not all investigations are included, as the Organisers felt that they lay outside the scope. The Organisers of the UK NEQASs for Blood Transfusion Laboratory Practice, Cytogenetics, Molecular Genetics and Histocompatibility & Immunogenetics indicated that all of their investigations lay outside the scope. Partial data only was provided by the UK NEQASs for Trace Elements, Immunosuppressive Drugs, Immunocytochemistry and Cellular Pathology Technique

As expected, extension into more specialised areas (particularly peptide hormones, tumour markers and immunology) yielded a substantially higher proportion of Class 3 analytes. However, few substantive additional issues were identified, so the comments and conclusions do not differ radically from those in the Phase 1 report

In microbiology the majority of results are categorical. Historically distinctions, such as those between immune and non-immune, were based on the limit of sensitivity of the assays. However for critical diagnoses common practice was, and still is, to use a confirmatory or second assay to confirm an original result. This practice is used for instance in determining susceptibility to rubella. An international biological standard for rubella has been available for many years and all commercially available assays are standardised against it. However, a recent review of results reported for EQA specimens with low levels of rubella antibody showed that 6 assays gave significantly higher and 5 significantly lower values than the previous market leader, emphasising the continued need for confirmation

For antibody estimations against hepatitis B surface antigen the cut off at 10 IU/L is well defined and supported by the international biological standard. Commercial assays are well standardised at the immune to non-immune interface, however absolute quantitation varies by assay at higher levels of antibody

5 Classification

5a Class 1 analytes

On review, many analytes appeared straightforward and were therefore categorised as Class 1. These classifications are not, however, unqualified. Though many refer to preanalytical aspects, several merit further explanation

- there may be good agreement between methods for the great majority of specimens, with divergence of results limited to specific situations; sodium by direct ISE diverges in abnormal states, and the superior specificity of BCP albumin is seen primarily at lower concentrations
- immunoassays are prone to changes in individual manufacturers' methods, which are inconsistent over time and thus not susceptible to manipulation – see section 6 below
- though there is a fully-validated IFCC reference system for HbA1c, results are at present still derived by calculation from the 'DCCT aligned' values produced by current instrumentation. Performance and comparability should improve when manufacturers convert to full IFCC calibration and direct measurement is possible
- results from point of care testing (POCT) systems may be less reliable than laboratory analysis, usually through worse precision due in part to operator-dependence

Creatinine presents a special case, and appears in both the Class 1 and Class 3 tabulations (see section 5c below). When measured using enzymic methods (or mass spectrometry, in a few laboratories) it is Class 1, and adoption of such methods would effectively eliminate problems of result interpretation due to non-specificity of Jaffe methods

Prothrombin time also appears in both the Class 1 and Class 3 tabulations, as does fibrinogen (see section 5c below). Results for monitoring of anticoagulant control and expressed as INR (International Normalised Ratio) are considered as Class 1, despite some variation between methods due to reagent and calibration differences. When used for diagnostic purposes and expressed in seconds, however, interpretation must be relative to method-specific reference ranges, and thus is Class 3

5b Class 2 analytes

There are substantial qualifications for these analytes, and again some common threads:

- the majority are enzyme activity assays, where the result depends entirely on the analytical method used (and on the calibration, if any). It may be possible to manipulate results to mitigate between-method differences, but there are major limitations (see sections 6 & 7 below) and method selection may be preferable
- resolution of between-manufacturer differences in calibration (eg by adoption of traceability to a single International Standard for peptide hormones) may enable reclassification in future to Class 1
- iron binding capacity should be expressed either as unbound or as total; continued use of both leads to confusion
- in some cases (eg cholinesterase phenotyping) the reportable is derived from consideration of analytical results, and reporting of this outcome (with interpretation) is more important than the component results
- parallel reporting of 'DCCT HbA1c' should be discontinued as soon as true IFCC calibration is general (see 4b above)

5c Class 3 analytes

Though this class presents greatest difficulties for data combination, a substantial number of analytes must be included on scientific grounds. Mitigation may be possible (see sections 6 & 7 below), but the limitations must be recognised

Consideration indicates several common reasons for inclusion in this class:

- non-specificity of methods
 - creatinine by Jaffe methods. Enzymic methods are currently more expensive, leading to limited adoption. Though 'compensated' Jaffe procedures are far from ideal, the problems are less than with (equally priced) 'traditional' procedures
- differences in specificity
 - this is particularly prevalent in immunoassays for peptides, and can only be resolved by manufacturers
- uncharacterised or non-homogeneous analyte
 - HDL cholesterol estimation relies on isolating a subset of lipoproteins for cholesterol measurement, so the result will depend on which subset is isolated by the method
 - 'free T4' and 'free T3'
- 'derived' results
 - fibrinogen 'estimation' by coagulation instruments, derived as a by-product of the measurement process for prothrombin time. Reporting of such results is discouraged by national guidelines, but appears to persist in practice
- difference between analyte in specimens and calibrants (non-commutability)
 - caeruloplasmin changes with age, and between-method relationships also change. Calibration against an aged Certified Reference Material (CRM) will therefore be inappropriate, and not provide comparability on fresh clinical specimens
- confusion of methods
 - a large number of formulae are used to calculate 'adjusted calcium'. Some differences may reflect use of different albumin methods, but there seems little scientific justification for continuing confusion
 - the two majority methods for cholinesterase are appropriate for different applications (phenotyping and toxicology), but it is difficult to justify continuation of the others, with insufficient users for effective EQA

- differences in interpretation guidelines
 - in antimicrobial susceptibility MIC (minimum inhibitory concentration) measurements are accurate but the interpretative breakpoints vary by guideline. EUCAST guidelines are replacing national guidelines in Europe, however CLSI guidelines are the default setting for most automated systems
- 'immature' assays
 - viral load measurements for blood-borne viruses are at different stages of development. International standards for HIV1 RNA, HBV DNA, HCV RNA and CMV DNA are available and one for EBV DNA is in development. Both biological and assay-specific variation contribute to overall variation in absolute quantitation, so results are not interchangeable between sample type or assay. These assays are used to monitor treatment efficacy within individual patients, and virologists should be aware of the lack of consistency

For urine dipsticks the lack of concordance and potential for misinterpretation (as distinct from analytical variations, such as the wide range of pH values reported on EQA specimens) is a consequence of manufacturers' decisions on product design and outcome nomenclature. Urine glucose is the most striking example, where:

- Roche '2+' and Bayer '++' (which would generally be pronounced identically in a spoken report) represent 5.5 and 28 mmol/L respectively
- 5.5 mmol/L is 'Trace' for Bayer and '2+' for Roche

5d Unclassifiable analytes

Several analytes proved unclassifiable at this stage, for two main reasons:

- for some, including vitamins and apolipoproteins, there is insufficient data yet available; the former are defined compounds and likely to be Class 1, whereas the latter are expected to be Class 2 or 3
- urinary albumin:creatinine and protein:creatinine ratios will inherently vary more than the component results, and are currently under detailed scrutiny by the DH Renal Advisory Group and NHS Kidney Care

6 Conclusions and principles

Standardisation of reporting units and nomenclature is a prerequisite for reliable data combination and interpretation, through the National Laboratory Medicine Catalogue

Even for analytes in Class 1, where combination should not present problems, laboratories need to maintain vigilance through IQC & EQA to ensure continuity of their results. Discontinuities or drift may be due to factors within the laboratory, related to software, equipment, reagent or calibrant changes or deterioration, or errors in setting instrument parameters. They should also beware of changes in the in vitro diagnostics (IVDs) used

Commercially-produced IVDs do vary over time, due to variability inherent in the manufacturing process; these should be limited by production QC, though acceptability limits appear to vary among manufacturers. Formulations and calibration may also be changed, intentionally or unintentionally, leading to changes in performance – these may be substantial, so even results by the same method may not be comparable over time. Such problems are much more prevalent in immunoassays, but can appear in apparently much simpler cases (eg a bias for serum potassium which required the manufacturer to redesign the electrode, enhance cleaning procedures and revise the calibration process). These are by their nature inconsistent over time, and thus not susceptible to correction by manipulation

Result manipulation is undesirable in principle, and to be avoided wherever feasible

If results are to be manipulated, a common specification (eg embodied in the National Laboratory Medicine Catalogue) is highly desirable, with manipulation preferably done by the laboratory producing the result rather than externally

7 Mitigation principles

For class 2 analytes manipulation may be possible to minimise the obstacles to data combination. However, manipulation must be valid, requiring for example that:

- there is no significant analyte heterogeneity among clinical specimens
 - eg manipulation may be appropriate for AST or GGT, but not for ALP or LD
- the conversion procedure has been validated and there is scientific justification
- the conversion procedure has been derived from clinical specimens representative of the full range to which manipulation will be applied
 - ie not from calibration, IQC or EQA materials, or from a subset of clinical specimens

Particular attention may be required for dry slide methods (Ortho Clinical Diagnostics Vitros), which often utilise different method principles and are more affected by physical properties of specimens. In other cases (eg prolactin, AFP and CEA) conversion factors may be manufacturer-specific, providing a further obstacle to conversion

The best way forward for some Class 2 & 3 analytes may include restriction of method choice, for example:

- agreement on method principle standardisation (selection of a method principle, not an individual manufacturer's IVD, minimises the potential problems described in section 5 above)
 - selection of 'compensated' rather than 'traditional' Jaffe for creatinine
 - ALP buffer & reagent composition
 - LD assay direction
 - discontinuation of minority methods for cholinesterase
- agreement on common algorithms
 - use of the same formula for calculating 'adjusted calcium'

Separation into multiple analytes (eg AST with and without pyridoxal phosphate) is another option, but discontinuation of methods including pyridoxal phosphate would appear preferable for both AST and ALT

Service reconfiguration may be a better approach than result manipulation where there is the potential for problems due to incompatibilities in data combination. Possible implementations include:

- the adoption of a single method, or even a single laboratory, for tumour marker assays across a Regional cancer network. Aggregation to higher levels, however, carries risks, especially lack of continuity due to changes in the IVD selected (see section 5 above) and analytical or operational issues in the selected laboratory
- local reporting only (eg only the fact that an investigation has been performed is submitted to Regional or national databases, rather than the actual result)

The urine dipstick issues appear intractable, at least at national level. Resolution would require substantial changes by multinational corporations, with vested commercial interests – an analogy would be the continued lack of harmonisation of colour coding of blood collection tubes. The matter still requires consideration, however, perhaps initially by BIVDA and professional societies, after review of the detail and potential means of resolution

8 Concluding comments

Continued stability of performance cannot be assumed and must be monitored. UK NEQAS should be a key player in this process

All the assumptions about Class 1 and mitigation of Class 2 analytes are dependent on external measures of equivalence and method & calibration stability. These may best be satisfied by participation in effective EQA for assays used in patient management, with good EQA performance and harmonisation of reference ranges. Pathology Harmony recommendations appear to be the best (albeit imperfect) easily obtainable source of such ranges



Wherever possible external recommendations for action limits should be formally scrutinised for scientific and statistical validity by the professions. For example, does the cut-off threshold match the confidence interval for probability of a significant change occurring in monitoring for the Class 1 or mitigated Class 2 values? There may be a case for trying to model the effect of a multiple of the median (MoM) approach, or using existing data in a meta-analysis for overall confidence interval

In some cases a qualitative outcome or interpretation may be considered as Class 1 even if the contributory analytical results are not. Cholinesterase phenotype provides an example of a comparable outcome, even though the inhibitor numbers used to determine it are not Class 1. However, even where there is analytical equivalence and guidelines for interpretation, these are not universally adhered to; sweat testing is such a case

It must also be remembered that the situation is not static, and changes over time do occur (ignoring the effects of ageing and shifts in performance of 'established' IVDs). For example, TSH assay sensitivity has developed substantially, with current assays having a detection limit orders of magnitude lower than 30 years ago. Similarly improvements in precision enable more reliable interpretation of troponin results

International agreements, including manufacturers, to adopt International Standards and development of appropriate material may improve the comparability of results and permit combination. However, this may have the perverse effect of invalidating the previous evidence base used for interpretation

New investigations (or improved methods) may go through a life cycle, with changes during the process. A single initial producer yields 'de facto standardisation', and comparability is assumed. As multiple providers enter the market divergence may appear, even if the analysis is based on a patented procedure which is licensed to the manufacturers. Finally, recognition of the divergence may lead to its resolution, and restoration of comparability being achieved

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Albumin	1		3.1	4.2				BCP more specific than BCG; effect more marked at lower albumin concentration	
Bicarbonate	1		4.8	4.7					
Bilirubin	1		23.8	39.0		avoid exposure to light			
Calcium	1		1.9	2.8					
Chloride	1		1.2	1.5					
Copper	1		4.9	13.6		pretested specimen collection containers should avoid contamination			
Creatinine	1 / 3	Y	5.3	14.2				enzymic methods yield quantitative recovery, and would be class 1	Jaffe methods (whether 'traditional' or 'compensated') are inherently non-specific and perform differently on different clinical specimens, so can <u>never</u> be traceable or compatible. 'Compensated' Jaffe preferred to 'traditional'
D-3-hydroxybutyrate	1								
Glucose	1		5.7	6.9		plasma differs from serum			
Iron	1		26.5	23.2					
Lithium	1								
Magnesium	1		3.6	6.4					

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Phosphate	1		8.5	9.4					
Potassium	1		4.8	5.6					
Sodium	1	Y	0.7	1.0					direct ISEs give differing results (from indirect ISE/flame photometry, and from each other) at abnormal concentrations and specimens of abnormal lipid & protein composition
Urate	1		9.0	17.6		avoid exposure to light	umol/L & mmol/L both in use		
Urea	1		12.3	18.3					
Zinc	1		9.3	9.4		pretested specimen collection containers should avoid contamination			
Bilirubinometers	1	Y	23.8	39		light-sensitive			results from POCT procedures may be less precise
Conjugated bilirubin	1		36.8	43.2		light-sensitive			EQA data limited and less secure, due to lack of glucuronide for spiking specimens
Total bilirubin	1		23.8	39		light-sensitive			
Total cholesterol	1		5.4	15.2					
Triglyceride	1		20.9	37.2					
POCT HDL cholesterol	1	Y	7.1	19.7					results from POCT procedures may be less precise

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
POCT total cholesterol	1	Y	5.4	15.2					results from POCT procedures may be less precise
POCT triglyceride	1	Y	20.9	37.2					results from POCT procedures may be less precise
Alpha-1-acid glycoprotein	1		11.3	24.9	should no longer be referred to as orosomucoid		g/L		
Alpha-1-antitrypsin	1		5.9	16.3			g/L		
Alpha-2 macroglobulin	1		3.4	18.7			g/L		
C3	1		5.2	15.6			g/L		
C4	1		8.9	33.4			g/L		
Haptoglobin	1		20.4	36.4			g/L		
IgA	1		5.4	35.9			g/L		
IgG	1		4.5	16.5			g/L		
IgM	1		5.9	47.3			g/L		
Transferrin	1		3.0	4.3			g/L		
Transthyretin	1		10.9	19.1	should no longer be referred to as prealbumin		g/L		
Paracetamol	1						umol/L now discontinued in UK		

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Salicylate	1						umol/L now discontin- ed in UK		
Thyroglobulin	1		0.2	0.4				substantial between- method differences exist	EQA data dominated by one method (DPC Immulite 2000)
Total T3	1	Y	8.7	17.2				between-manufacture differences exist, but vary over time in magnitude and direction	
Total T4	1	Y	4.9	10.9				between-manufacture differences exist, but vary over time in magnitude and direction	
TSH	1	Y	19.3	19.7				between-manufacture differences exist, but vary over time in magnitude and direction	
17OH-Progesterone	1		19.6	52.4				between-manufacture differences exist, but vary over time in magnitude and direction	
Aldosterone	1		29.4	40.1				between-manufacture differences exist, but vary over time in magnitude and direction	

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Androstenedione	1		11.1	51.1				between-manufacture differences exist, but vary over time in magnitude and direction	
Cortisol	1		20.9	45.6				between-manufacture differences exist, but vary over time in magnitude and direction	
DHA sulphate	1		4.2	29.3				between-manufacture differences exist, but vary over time in magnitude and direction	
Oestradiol [E2]	1	Y	18.1	19.7				between-manufacture differences exist, but vary over time in magnitude and direction	differing method biases make clinical interpretation difficult
Oestradiol [high-level]	1							between-manufacture differences exist, but vary over time in magnitude and direction	
Progesterone	1							between-manufacture differences exist, but vary over time in magnitude and direction	
SHBG	1		12.1	42.7				between-manufacture differences exist, but vary over time in magnitude and direction	

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Testosterone [female]	1	Y	9.3	23.7				between-manufacture differences exist, but vary over time in magnitude and direction	differing method biases make clinical interpretation difficult
Testosterone [male]	1		9.3	23.7				between-manufacture differences exist, but vary over time in magnitude and direction	
Beta carotene	1		36.0	39.7				HPLC with internal standard preferred	
Isoleucine	1								
Leucine	1								
Phenylalanine	1								
Tyrosine	1								
Valine	1								
Vitamin A	1							HPLC with internal standard preferred	
Vitamin E	1		7.6	21				HPLC with internal standard preferred	
Blood HbA1c [IFCC]	1	Y	3.4	5.1			mmol/mol	currently calculated from DCCT 'measurement'; will in future be measured directly as instruments become IFCC-calibrated	

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Blood cadmium	1					pretested specimen collection containers should avoid contamination			
Blood lead	1	Y				pretested specimen collection containers should avoid contamination	umol/L and ug/dL both in use; may be dual reported		reporting in ug/L should be discontinued
Urinary Albumin	1		36	55					
Urinary Calcium	1		27.6	36.6					
Urinary Chloride	1								
Urinary Creatinine	1		24.0	24.5					
Urinary Glucose	1								
Urinary Magnesium	1		45.4	37.4					
Urinary Phosphate	1		26.4	26.5					
Urinary Potassium	1		27.1	23.2					
Urinary Sodium	1		24.0	26.8					
Urinary Urate	1		24.7	22.1					
Urinary Urea	1		22.7	25.9					

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Urinary Free Cortisol	1							between-manufacture differences exist, but vary over time in magnitude and direction	
Urine 5HIAA	1		20.3	33.2			umol/L or umol/24h; nmol also in use		
Urine adrenaline	1						umol/L or umol/24h; nmol also in use		
Urine dopamine	1						umol/L or umol/24h; nmol also in use		
Urine HVA	1						umol/L or umol/24h; nmol also in use		
Urine metadrenaline	1						umol/L or umol/24h; nmol also in use		
Urine noradrenaline	1						umol/L or umol/24h; nmol also in use		
Urine normetadrenaline	1						umol/L or umol/24h; nmol also in use		

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Urine total metadrenalin	1						umol/L or umol/24h; nmol also in use		
Urine VMA [HMMA]	1		22.2	47			umol/L or umol/24h; nmol also in use		
Urine orotic acid	1								
Urine orotic acid/creat ratio	1	Y							ratio will be more variable than individual results
Sweat chloride	1		15.0	25.0					
Sweat sodium	1	Y	15.0	25.0					should only be used as an adjunct to chloride and/or conductivity
Total White blood count	1	Y	10.9	19.6			x10 ⁹ /l	Manual methods not recommended	Class 1 designation assumes automated counting used. Manufacturers' instructions on the limitations of counts at extreme low and high levels must be followed. Total white blood count must be distinguished from total nucleated cell count, which may include
Red blood cell count	1	Y	3.2	6.1			x10 ¹² /l	Manual methods not recommended	
Haemoglobin	1	Y	2.8	6.6			g/dl or g/l		80% of labs use g/dl. Units will remain an issue until a single system is agreed.

Analyte	Class Qualified?						Name	Sample	Units	Methods	Comments
	1	Y			BV within	BV between					
Haematocrit	1	Y					This may be described as Haematocrit (Hct) or Packed Cell Volume (PCV)	l/l		Manufacturer and manual vs automated differences exist.	There is a difference in terminology (Hct or PCV). For some analyser types this is a derived rather than a measured value. Manual methods are comparable but it is important to correct for trapped plasma.
Mean Cell Volume	1			1.3	4.8			Value less stable with specimen age than MCH	fl	intermanufacturer differences exist.	May be derived or measured value.
Mean Cell Haemoglobin	1			1.6	5.2				pg		Derived value
Mean Cell haemoglobin concentration	1	Y		1.7	2.8				g/dl or g/l		Derived value. Approx. 80% of labs use g/dl. Units will be an issue until a single system agreed for Hb.
Platelet count	1	Y		9.1	21.9				x109/l	Manual methods not recommended	Class 1 designation assumes automated counting used. Manufacturers instructions on the limitations of counts at extreme low and high levels must be followed.
Vitamin K	1			38	44		Phylloquinone (vitamin K1)		ug/L	HPLC and LC-MS	See www.keqas.com for more information.

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Prothrombin Time (PT) for anticoagulant control	1	Y					INR (International Normalised Ratio)		Must be expressed as INR values for use in anticoagulant control. INR theoretically standardised, though some between reagent/calibration differences seen
POCT Prothrombin Time	1	Y					INR		INR values for anticoagulant control theoretically standardised. Results reported in seconds not comparable between methods
Fibrinogen	1 / 3	Y					g/L		Clauss methods should give comparable results. PT-derived methods vary by reagent/instrument combination, and are discouraged in national guidelines
anti-Xa assay	1						u/ml		
Homocysteine (plasma)	1						umol/L		some methods employ different anticoagulants
Factor II	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor V	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor VII	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor VIII	1 / 3	Y					u/dL (u/L; IU/L)		some molecular defects give different results with different methods
Factor IX	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor X	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor XI	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor XII	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor XIII	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Factor VIII inhibitor	1						bethesda units		
VWF antigen	1	Y					u/dL (u/L) u/ml		50:50 split of reporting u/dL and u/L; results potentially confusable
VWF activity	1 / 3	Y					u/dL (u/L)		some molecular defects give different results with different methods
VWF collagen binding assay	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Antithrombin activity	1 / 3	Y					u/dL (u/L)		some molecular defects give different results with different methods
Protein C antigen	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Protein C activity	1 / 3	Y					u/dL (u/L)		some molecular defects give different results with different methods
Protein S total antigen	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Protein S free antigen	1	Y					u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Plasminogen	1						u/dL (u/L)		50:50 split of reporting u/dL and u/L; results potentially confusable
Factor V Leiden	1								genetic test: absent/wild type; heterozygous; homozygous
Prothrombin 20210A	1								genetic test: absent/wild type; heterozygous; homozygous
HCV RNA	1	Y				serum, plasma	IU/mL		IU/mL universally adopted
anti-HBs	1	Y				serum, plasma	mIU/mL		Well standardised at the 10 mIU/mL cut-off between immune/non immune. Absolute quantitation varies by assay at higher concentrations

Analyte	Class Qualified?							Sample	Units	Methods	Comments
	BV within	BV between	Name								
Rubella IgG	1	Y						serum, plasma	IU/mL		Variation exists between methods at the 10IU/mL cut off. In practice many labs confirm negative results by testing with an alternative assay
Amikacin	1								mg/L		
Flucytosine	1								mg/L		
Gentamicin	1								mg/L		
Teicoplanin	1								mg/L		
Tobramycin	1								mg/L		
Vancomycin	1								mg/L		
Posaconazole	1								mg/L		
IgG2	1	Y						Serum		nephelometry & turbidimetry & ELISA and RID	discrepancy between IRP CRM470 calibrations for all subclasses vs total IgG
IgG4	1	Y						Serum		nephelometry & turbidimetry & ELISA and RID	discrepancy between IRP CRM470 calibrations for all subclasses vs total IgG
total prostate specific antigen	1			18.1	72.4			Serum	mg/L	ELISA, CHEMILUMINESCENCE, Microparticle Enzyme Immunoassay	immulite 2000 poor CVS within method group for EQA scheme
Total IgE	1							Serum	IU/ml	ELISA, CHEMILUMINESCENCE, TURBIDIMETRY, EIA, NEPHELOMETRY	
CSF Albumin	1							CSF	mg/L		
CSF Glucose	1	Y						CSF	mmol/L	hexokinase/G-6-PDH, enzymatic, dry slide (ortho vitros)	ortho vitros - use different method (dry slide) resulting in differing results from other methods

Analyte	Class Qualified?							Sample	Units	Methods	Comments
	BV within	BV between	Name								
C reactive protein	1	Y		42.2	76.3			Serum	mg/L	turbidimetry, nephelometry, agglutination, ELISA, RID. Some methods also latex enhanced	85/506 in IU/mL
IgG1	1							Serum		see IgG1 and 4	
UCRP	1	Y						Serum	mg/L	turbidimetry, nephelometry, agglutination, ELISA, RID. Some methods also latex enhanced	85/506 in IU/mL
IgG3	1	Y						Serum	g/L	nephelometry & turbidimetry & ELISA and RID	discrepancy between IRP CRM470 calibrations for all subclasses vs total IgG
free prostate specific antigen	1							Serum	g/L	chemiluminescence, turbidimetry etc	
CSF Lactate	1							CSF	mmol/L	chemiluminescence, turbidimetry etc	
CSF IgG	1							CSF	mg/L	chemiluminescence, turbidimetry etc	

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
ALP [Alk Phos]	2	Y	6.4	24.8				twofold difference in results depending on buffer used	
ALT [ALAT]	2	Y	24.3	41.6	ALT, ALAT & expansions used interchangeably; also GPT				results from methods with and without pyridoxal phosphate can NOT be related
Amylase	2	Y	8.7	28.3				twofold difference in results depending on substrate used	
AST [ASAT]	2	Y	11.9	17.9	AST, ASAT & expansions used interchangeably; also GOT				results from methods with and without pyridoxal phosphate can NOT be related
CK	2		22.8	40.0		avoid exposure to light			
GGT	2		13.8	41.0				consistent relationship between nitroanilide and carboxynitroanilide substrate	
Iron binding capacity	2	Y			may be expressed as TIBC or UIBC				TIBC and UIBC are different analytes
LD	2	Y	8.6	14.7	LD & LDH in use			twofold difference in results depending on reaction direction and buffer	
Lipase	2	Y	23.1	33.1				large differences in results	results by Ortho dry slide technology differ most

Osmolality	2	Y		1.3	1.2							'calculated osmolality' should not be reported
Total protein	2	Y		2.7	4.0							analyte inherently heterogeneous
LDL cholesterol	2	Y		8.3	25.7					usually calculated by algorithm (Friedewald equation), but direct measurement methods becoming available		requires review when sufficient data from direct measurement methods available
eGFR	2	Y								correct (MDRD) algorithm for method must be used; calculation may take account of manufacturer performance		eGFR should be calculated and reported by laboratory, not calculated from creatinine by clinical systems
Butyrylcholinesterase phenotype	2	Y								analytical methods not important, provided appropriate algorithms used for interpretation		reporting of interpretative comments, and activity, essential with phenotype; inhibitor numbers are not essential in report
Chloride number	2	Y								assays should be at ~25C for maximal discrimination		reporting of phenotype, with interpretative comments, essential; inhibitor numbers are not essential in report
Dibucaine number	2	Y								assays should be at ~25C for maximal discrimination		reporting of phenotype, with interpretative comments, essential; inhibitor numbers are not essential in report
Fluoride number	2	Y								assays should be at ~25C for maximal discrimination; particularly temperature-sensitive		reporting of phenotype, with interpretative comments, essential; inhibitor numbers are not essential in report
RO2-0683 number	2	Y								assays should be at ~25C for maximal discrimination		reporting of phenotype, with interpretative comments, essential; inhibitor numbers are not essential in report
Scoline number	2	Y								assays should be at ~25C for maximal discrimination		reporting of phenotype, with interpretative comments, essential; inhibitor numbers are not essential in report

Fructosamine	2	Y	3.4	5.9							uncharacterised analyte
Blood HbA1c [DCCT]	2	Y	3.4	5.1					%		dual reporting with HbA1c [IFCC] at present; will be discontinued
Urinary (Total) Protein	2	Y	39.6	17.8							analyte inherently heterogeneous
	2	Y									
Urinary Amylase			94	46						twofold difference in results depending on substrate used	
	2	Y									'calculated osmolality' should not be reported
Urinary Osmolality											
Sweat conductivity	2										
FSH	2		8.7 (males)	18 (males)						Most methods agree well, but some do not.	IRP 78/549
LH	2		14.5	27.8						Most methods agree well, but some do not.	IS 80/552
Prolactin	2	Y	6.9 (males)	61 (males)						Most methods agree well, but some do not.	IS 84/500 Qualification concerns possible confusion with units (conversion factors are method-specific), although this may not be an issue in the UK as all labs use mU/L.
AFP [As a tumour marker]	2	Y	12.7	46					kU/L and ng/mL		IS 72/225 Qualification concerns possible confusion with units (conversion factors are method-specific)
hCG [As a tumour marker]	2	Y							U/L		IS 75/537 Methods that measure only intact hCG (i.e. those that do not also detect the beta-subunit) are not suitable for use.
Inhibin A [Screening Down's syndrome in the second trimester]	2	Y							ng/mL		Assays provided by only one manufacturer, so between-laboratory results agree well.

Hb A2	2	Y							%	Electrophoresis and automated densitometry not recommended.	Manufacturer and method differences; high level of technical skill required for manual methods. Automated methods require assessment of results and instrument output before establishing diagnosis. Some disease state specific variations. Method related fa
Hb F	2	Y							%	Automated methods (capillary electrophoresis, HPLC) available, together with manual methods (radial immunodiffusion, alkali denaturation)	Most values are reported from automated systems. Different methods/manufacturers offer different specificity and sensitivity at different analyte levels. Accuracy at low (normal) levels is not essential but method must differentiate between normal and ra
Hb S	2	Y							%	HPLC, electrophoresis and automated densitometry, electrophoresis and elution, microcolumn chromatography	Most results reported from HPLC. Results may be used both diagnostically and therapeutically but standards of precision and accuracy are not as demanding as for Hb A2.
Glucose-6-phosphate dehydrogenase	2	Y		32.8	31.8				IU/gHb, IU/106 rbc,	25, 30 and 7 degrees C; one and two stage assays	Results may be manipulated so that the value is reported at a different temperature from that at which the assay is performed. Standardisation of assay temperature at 37 degrees C is desirable. Results will also be affected by the general age of the red
Red cell volume	2	Y							ml		The labelling and re-infusion of labelled cells has no IQC or EQA. Interpretation of the results requires knowledge of the patient's body composition.
Feto-Maternal Haemorrhage	2	Y							ml	Acid elution films, flow cytometry	The methods measure different features. Flow cytometry may be used alone for quantitative assay, and is required to confirm the quantitative assay of raised specimens. Acid elution may be used for screening or quantitative assay.

Vitamin B12	2	Y				S		ng/L		Binding protein assays	Large intermethod differences. Some methods currently harmonising with CRM 03/178.
Folate	2	Y	24.0	73.0		S	Not stable at RT	ug/L		Binding protein assays not specific, measuring TFOL. Should only be used for TFOL below 20ug/l	Large intermethod differences particularly at low concentrations. Assay calibrator matrix and material variable. Some methods currently harmonising with CRM 03/178. Reference range source is an issue.
HBV DNA	2	Y					serum	IU/mL or copies/mL			0.5 log variation between main commercial assays IU/mL well adopted
Sperm concentration	2	Y					Collection into pre-tested non-spermicidal containers required	millions/ml		Differences between methods exist	
Sperm motility	2	Y					Collection into pre-tested non-spermicidal containers required	% (classification)			Classification as % rapid,% sluggish,% non-progressive,% immotile
Sperm morphology	2	Y						% normal		Differences between methods exist.	sometimes reported as % abnormal
Rheumatoid factor ab	2	Y	8.5	24.5			Serum	IU		ELISA, AGGLUTINATION, LATEX AGGULATINATION, HAEMAGGLUTINATION, TURBIDIMETRY, LATEX TURBIDIMETRY, LUMINEX	different methods of analysis now from testing of reference prep, high CVs around 20-30%
C1 INH	2						Serum	g/L		RID, ELISA, nephelometry	
CSF total protein	2						CSF				

bone ALP	2		6.2	35.6			Serum					
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Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Adjusted calcium	3							wide variety of formulae used	
Creatinine	1 / 3	Y	5.3	14.2				enzymic methods yield quantitative recovery, and would be class 1	Jaffe methods (whether 'traditional' or 'compensated') are inherently non-specific and perform differently on different clinical specimens, so can <u>never</u> be traceable or compatible. 'Compensated' Jaffe preferred to 'traditional'
HDL cholesterol	3		7.1	19.7				variety of 'direct' (onestep precipitation) procedures in use	different precipitants mean that different fractions will be included in the measurement results by OCD dry slide method differ some methods may show triglyceride interference
Caeruloplasmin	3	Y	5.8	11.1			g/L		aged serum appears to behave differently from fresh serum, so calibration of methods against CRM does not necessarily produce comparable results on fresh serum specimens
Free T3	3	Y	7.9					methods are empirical (defined by manufacturer's procedure) and inherently non-comparable	without a reliable reference method it is not possible to determine with method is right
Free T4	3	Y	7.6	12.2				methods are empirical (defined by manufacturer's procedure) and inherently non-comparable	without a reliable reference method it is not possible to determine with method is right

TgAb	3			8.5	82							interpretation is more relevant than numerical result
Butyrylcholineste	3	Y		5.4	10.3		pseudocholinesterase' may still be in use		kU/L may be in use		butyrylthiocholine at 37C and benzoylthiocholine at 25C equally popular but results differ by factor of 10	other methods should be discontinued
Urine dipstick bili	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick glu	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick ha	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick ket	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations; this is most noticeable for glucose
Urine dipstick leu	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick nit	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick no	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick pH	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick prc	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Urine dipstick sp	3	Y							arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations

Urine dipstick urc	3	Y						arbitrary (nominal/ordinal scale)			scales differ between manufacturers, with apparently similar results relating to different actual concentrations
Growth hormone	3							ug/L and mU/L	Most methods agree well, but some do not.		IS 98/574 UK NEQAS dominated by Siemens Immulite methods
PTH	3			26	24		Rapid transport to laboratory required.	pmol/L and ng/L	Substantial between-method differences exist.		IS 95/646 available but not currently in use.
ACTH	3	Y					Rapid transport to laboratory on ice required.	ng/L, pmol/L and mU/L	Substantial between-method differences exist.		Multiple reporting units in use
Calcitonin	3						Rapid transport to laboratory required.	ng/L, pmol/L and mU/L			Multiple reporting units in use Scheme dominated by Siemens Immulite methods
CEA [As a tumour marker]	3	Y		12.7	56			ng/ml	Substantial between-method differences exist.		IRP 73/601 The unit is method-specific, so values cannot be compared between-methods
hCG beta subunit [Screening for Down's syndrome in the first trimester]	3							U/L and ng/mL	Most methods agree well, but some do not.		IRP 75/551
PAPP-A [Screening for Down's syndrome in the first trimester]	3							U/L, mU/L	Substantial between-method differences exist.		

hCG beta subunit [Screening Down's syndrome in the second trimester]	3								U/L and ng/mL		Most methods agree well, but some do not.	IRP 75/551
Unconjugated oestriol [Screening Down's syndrome in the second trimester]	3	Y							nmol/L and ng/mL		Severe between-method differences.	Although a CRM and reference method exists, few assays are calibrated to them.
Inhibin A [Screening Down's syndrome in the second trimester]	2	Y							ng/mL			Assays provided by only one manufacturer, so between-laboratory results agree well.
Urinary hCG [Qualitative]	3								na		Commercially available devices have different sensitivities.	IS 75/537
Urinary hCG [Qantitative]	3								U/L		Assays have different specificities towards hCG species likely to be present in urine.	IS 75/537 Few assays are licensed for use for the determination of hCG in urine.
Insulin	3			21.1	58.3			Labile - sample handling recommendations vary from method to method	scheme units: pmol/L. Other units used are mU/L and uU/ml		Between manufacturer differences exist and these can be substantial in some clinical situations	IRP NIBSC 66/304 EQA scheme dominated by the Siemens Immulite 2000/2500 and Roche Elecsys assays. Assays vary with cross-reactivity to synthetic insulins and proinsulin etc which can be important in some clinical scenarios.

C-peptide	3			16.6	23.2		C-peptide of human insulin	Labile - sample handling recommendations vary from method to method.	scheme units: pmol/L. Other units used are ug/L or ng/ml		Between manufacturer differences exist	IRR C-peptide 84/510 EQA scheme dominated by the Siemens Immulite 2000/2500 assay. Assays vary in specificity e.g. proinsulin cross-reactivity
Gastrin	3			no data	no data			Labile - samples need to be stored and transported frozen	scheme units: mU/L. Other units used are ng/L and pmol/L		Between manufacturer differences exist and can be substantial in some clinical situations	EQA scheme dominated by the Siemens Immulite 2000/2500 assay. Assays vary in specificity for gastrin isoforms, material used for calibration etc.
IGF-I	3			9.4	27		Somatostatin C, Insulin-growth factor 1, Insulin-like growth factor I	Some debate over sample stability - assay specific sample handling protocols may be required	scheme units: nmol/L. Other units used are ug/L		Between manufacturer differences exist. Assays use IGF-I for Immunoassay, IRR 87/518 but stocks exhausted. New assays will have to use 1st WHO IS, recombinant, NIBSC code: 02/254 - still need data that this is commutable	EQA scheme dominated by Siemens Immulite 2000/2500 assay. Manufacturer differences can be significant where there are changes to IGF binding proteins, eg type 1 DM, CRF, cirrhosis. Often assay performance has not been well validated in such scenarios.
IGF-BP3	3			no data	no data		Insulin-like growth factor binding protein -3	Some debate over sample stability - assay specific sample handling protocols may be required	scheme units: mg/L. Other units used are ug/L or nmol/L		Between manufacturer differences exist	EQA scheme dominated by the Siemens Immulite 2000/2500 assay. Large differences observed between methods whether glycosylated or non-glycosylated IGFBP-3 is used for calibration. NIBSC code: 93/560 available - non glycosylated

Cardiac Troponin I	3	Y						Serum, Plasma. Some assays not validated for EDTA plasma	ug/L		Immunoassay	NIST Standard (original source of this material used in EQA for over 12 years). Values may be lower in some methods if EDTA Plasma is used. Numerical differences between manufacturers methods. Factorisation should not be used to produce a common number.
Cardiac Troponin T	3	Y						Serum, Plasma	ug/L or ng/L		Immunoassay	Method standardised. However licensed out to other manufacturers - many see numerical differences
CKMB (Mass)	3							Serum, Plasma	ug/L		Immunoassay	Method has been surpassed by more specific markers now available. Numerical differences between manufacturers methods.
CKMB (Acitivity)	3							Serum, Plasma	IU/L		Immunoassay	Method should be discontinued as more specific markers now available. Numerical differences between manufacturers methods.
Myoglobin	3							Serum, Plasma	ug/L		Immunoassay, Immunoturbidimetric	Method will be surpassed by more sensitive cardiac troponin assays. Numerical differences between manufacturers methods.
B-type Natriuretic Peptide (BNP)	3	Y						EDTA plasma	ng/L or pmol/L		Immunoassay	Assays have been shown to cross-react with pro-BNP - should use mass units (ng/L). Numerical differences between manufacturers methods. Factorisation should not be used to produce a common number.
N-Terminal B-type Natriuretic Peptide (NT-proBNP)	3	Y				wrongly termed proBNP		Serum, Plasma	ng/L or pmol/L		Immunoassay	Assays have been shown to cross-react with pro-BNP - should use mass units (ng/L). Values may be 10% lower using EDTA samples. Numerical differences between manufacturers methods.
Reticulocyte count	3	Y							x10 ⁹ /l; percent (%) still in use			Manual methods may be qualitative rather than quantitative. Different manufacturers' methods measure different markers of reticulocyte status.

Neutrophil count	3	Y							x10 ⁹ /l; percent (%) still in use especially for manual counts	Automated and manual methods available; majority of counts recorded are automated	Automated counts most widely reported; reported by 5 population and some 3 population automated counters. Some 3 population counters will include these in a granulocyte category. Manual counts are subjective and methods may be variable (total number of
Band form neutrophil count	3	Y							% or x10 ⁹ /l	Manual method	Highly subjective count, not widely used in the UK.
Granulocyte count	3	Y							x10 ⁹ /l;		Produced by some 3 population automated counters to include all granulocyte series cells (neutrophils, eosinophils, basophils). Largely equivalent to neutrophil count in healthy individuals but may be misleading in disease.
Lymphocyte count	3	Y							x10 ⁹ /l; percent (%) still in use especially for manual counts	Automated and manual methods available; majority of counts recorded are automated	Automated counts most widely reported; reported by 5 population and 3 population automated counters. Manual counts are subjective and methods may be variable (total number of cells counted etc). Automated counts may be unreliable in disease states.
Monocyte count	3	Y							x10 ⁹ /l; percent (%) still in use especially for manual counts	Automated and manual methods available; majority of counts recorded are automated	Automated counts most widely reported; reported by 5 population and 3 population automated counters. Manual counts are subjective and methods may be variable (total number of cells counted etc). Automated counts may be unreliable in disease states.
Eosinophil count	3	Y							x10 ⁹ /l; percent (%) still in use especially for manual counts	Automated and manual methods available; majority of counts recorded are automated	Automated counts most widely reported; reported by 5 population automated counters only. Manual counts are subjective and methods may be variable (total number of cells counted etc). Automated counts may be unreliable in disease states. 3 population ins

Basophil count	3	Y						x10 ⁹ /l; percent (%) still in use especially for manual counts	Automated and manual methods available; majority of counts recorded are automated	Automated counts most widely reported; reported by 5 population automated counters only. Manual counts are subjective and methods may be variable (total number of cells counted etc). Automated counts may be unreliable in disease states. 3 population ins
Immature myeloid and lymphoid cell types	3	Y						% or x10 ⁹ /l	Manual method	Important counts but subjective, accuracy dependent upon the number of cells counted, which will be limited by the number present. High level of operator experience needed, up to consultant level.
Manufacturer specific classifications	3	Y						% or x10 ⁹ /l	Manufacturer dependent	Only available on particular instrument types e.g. LUC count reported by Siemens analysers. <i>May have specific application but the equivalence to other cell types is not clear.</i>
Nucleated red blood cells	3	Y						x10 ⁹ /l or number per 100 WBC	Manual method, produced by some automated analysers	<i>There is no standardisation of units - absolute numbers tend to be used by automated methods. Some degree of subjectivity and the accuracy of the count will depend upon the counting method.</i>
Ferritin	3	Y		14.2	15	S		ug/L	Immunoassays	Large intermethod differences. Assay calibrators liver or spleen. One platform traceable to IS (94/572 Recombinant). No reference method. Some methods traceable to out of date IS.
RCFolate	3	Y		12.0	66	Eryth	Stability issues Haemolysi s and freeze thawing problems	ug/l	Use serum method for lysis analysis. Poor specificity of FBP greater problem for RC matrix.	Large intermethod differences. Intermethod differences in lysis preparation step. CRM 95/528

Erythropoietin	3	Y					S			Immunoassays	Most assays in the UK are traceable to urine IRP 67/343. Due for up dating iminantly. Other IS essentially for measuring recombinant EPO but one method traceable to 87/684. Reasonable assay agreement but potentially variable
Prothrombin Time (PT) for diagnosis	3	Y							sec		When PT used for diagnosis, expressed in seconds; different reagents give totally different results, requiring method-dependent reference ranges
Activated Partial Thromboplastin Time (APTT)	3	Y							sec (ratio)		APTT for either diagnosis or control of UFH - different reagents give totally different results in seconds
Thrombin Time (TT)	3	Y							sec (ratio)		different reagents give totally different results in seconds
D-Dimer	3	Y							results in ng and ng FEU (fibrinogen equivalent units) not interconvertable		different reagents give totally different results - no standardisation
POCT D-Dimer	3	Y							results in ng and ng FEU (fibrinogen equivalent units) not interconvertable		different reagents give totally different results - no standardisation
Fibrinogen	1 / 3	Y							g/L		Clauss methods should give comparable results. PT-derived methods vary by reagent/instrument combination, and are discouraged in national guidelines
Dilute Russel's Viper Venom Time (DRVVT)	3	Y							sec; may be reported as ratio		different reagents give totally different results - no standardisation

Kaolin Clotting Time (KCT)	3	Y							sec; may be reported as ratio			different reagents give totally different results - no standardisation
Silica Clotting Time	3	Y							sec; may be reported as ratio			different reagents give totally different results - no standardisation
Activated Clotting Time (ACT)	3	Y							sec			different reagents give totally different results - no standardisation
Clot solubility test?	3	Y							min; may be reported as hr			different reagents give totally different results - no standardisation
Factor VIII	1 / 3	Y							u/dL (u/L; IU/L)			some molecular defects give different results with different methods
VWF activity	1 / 3	Y							u/dL (u/L)			some molecular defects give different results with different methods
Antithrombin antigen	3	Y							u/dL; mg/dL (u/L)			approx 30% report in mg/dl, 70% report in u/dl, cannot be converted
Antithrombin activity	1 / 3	Y							u/dL (u/L)			some molecular defects give different results with different methods
Protein C activity	1 / 3	Y							u/dL (u/L)			some molecular defects give different results with different methods
Protein S activity	3	Y							u/dL (u/L)			should theoretically give comparable results, but consistent differences observed between methods
Activated protein C resistance	3	Y							ratio, sec, %, normalised ratio			different reagents give totally different results - no standardisation wide diversity of reporting units
Lymphocyte subsets (primarily CD4)	3							Peripheral blood	cells/ul		Flow cytometry single platform (bead based and volumetric) & dual platform	

CD34 stem cell enumeration	3						Peripheral blood, bone marrow and apheresis units	cells/ul		Flow cytometry single platform (bead based and volumetric) & dual platform		
Low level leucocyte counting	3						Blood Products	cells/ul		Flow cytometry single platform (bead based and volumetric) & dual platform		
HIV1 RNA	3	Y					serum, plasma	copies/mL or IU/mL				Assay variations in absolute quantification, also variation in quantification by subgroup between assays IU/mL not well adopted
CMV DNA	3	Y					Plasma, whole blood, buffy coat	copies/mL or IU/mL				Results not interchangeable between sample type or assay. International standard only released October 2010
EBV DNA	3	Y					plasma, whole blood	copies/mL				Results not interchangeable between sample type or assay. International standard in development; release expected October 2011
Antimicrobial MIC	3	Y					micro-organism	mg/L				MICs are accurate, but interpretative breakpoints vary by guideline EUCAST guidelines replacing national guidelines in Europe however CLSI guidelines the default setting for automated systems
myeloperoxidase ab	3	Y					Serum			ELISA, luminex/bioplex technology, EIA, Immunoblot		no international standard, the reference material is new, mixed arbitrary units, major differences in DR curves between assays
P-ANCA ab	3	Y					Serum			IIF		Semiquantitative, variable sensitivities between labs dependent on IQC strategy and chequerboarding - applies to most IIF assays

Desmosome ab	3	Y						Serum			IFF	Different substrates used, rat, guinea pig, monkey, no standard
Tetanus Toxoid (IgG)	3	Y						Serum	IU		ELISA	high CVs around 20-30% despite IRP and assay very variable at the threshold for absolute protection
CA125	3	Y		24.7	54.6			Serum	U/ml Ku/L		Microparticle Enzyme Immunoassay, ELISA, Chemiluminescence, turbidimetry,	No international standard no reference prep
IgG Tissue Transglutaminase ab	3	Y						Serum	Arbitrary units (No standard units)		ELISA, EIA, LUMINEX TECHNOLOGY	
IgA Tissue Transglutaminase ab	3	Y						Serum	Arbitrary units (No standard units)		ELISA, EIA, LUMINEX TECHNOLOGY	significant method-related differences and between generations of kits
specific IgG candida	3							Serum	Arbitrary units (No standard units)		ELISA, fEIA, LUMINEX TECHNOLOGY and ID	significant method-related differences
specific IgG Budgerigar	3							Serum	Arbitrary units (No standard units)		ELISA, FEIA, LUMINEX TECHNOLOGY and ID	significant method-related differences
TPO	3	Y		11.3	147			Serum	u/ml		RIA, ELISA, Chemiluminescence, particle agglutination, passive haemagglutination, turbidimetry, microparticle enzyme immunoassay, EIA	No international standard
liver kidney microsomal ab	3	Y						Serum	arbitrary units (No standard units)		IFF, ELISA, IMMUNOBLOT	different substrates used i.e mouse and rat ,immunoblotting & IFF visual inspection (no quantitative result), no standard units used
La ab	3	Y						Serum	arbitrary units (No standard units)		ELISA, EIA, LUMINEX, IMMUNOBLOTTING	immunoblotting visual inspection (no quantitative result), no standard units used

SCL-70 ab	3	Y						Serum	arbitrary units (No standard units)	ELISA, EIA, LUMINEX, IMMUNOBLOTTING, DOUBLE DIFFUSION	immunoblotting & DD visual inspection (no quantitative result), no standard units used
Anti B2 Glycoprotein IgG ab	3	Y			N/S			Serum	arbitrary units (No standard units)	ELISA, EIA	no standard units, reference material primarily for ACA
Interferon gamma (TB) P1NP	3	Y							IU	ELISA, ELISPOT, functional assays	
P1NP	3							Serum	mg/L	RIA,electrochemiluminescence immunoassay	no reference material,
NTX (urine)	3	Y		see comments column	see comments column			URINE	BCE nmol/L	ELISA ,electrochemiluminescence immunoassay	BV 23.% (very small study 18 individuals Hsin-Shan study 1997 day to day), circadian rhythm effects (increasing as much as 50% at night), no reference material
alpha 1 antitrypsin Phenotype Identification	3	Y						Serum	N/A	IEF: Agarose, polyacrylamide gels	For AAT levels see specific protein scheme. Phenotypes are not quantitative.
Glomerular basement membrane ab	3	Y						Serum	U/mL	ELISA, IIF, EIA, Immunoblotting	
C-ANCA ab	3	Y						Serum	IU/mL	EIA, ELISA, IIF	CDC reference sera due to be released shortly. Not currently available.
H. Influenzae type B (IgG)	3							Serum	IU/mL	ELISA	wide CV and previous dichotomous calibrations
CA15-3	3			6.1	62.9			Serum	Arbitrary units (No standard units)	chemiluminescence, turbidimetry etc	
NSE	3							Serum			
C1 INH functional	3							Serum	%NHS	functional assay - C1q binding and enzyme inhib	
IgA Gliadin ab	3							Serum	Arbitrary units (No standard units)	ELISA, IIF, EIA, Immunoblot	

IgG Gliadin ab	3							Serum	Arbitrary units (No standard units)	ELISA, IIF, EIA, Immunoblot		
Specific IgG Aspergillus fumigatus	3							Serum	Arbitrary units (No standard units)			
Specific IgG M.faeni	3							Serum	Arbitrary units (No standard units)			
CCP ab	3	Y						Serum	Arbitrary units (No standard units)	ELISA, FEIA, Chemiluminescence		clear method related heterogeneity
mitochondrial ab	3							Serum	Arbitrary units (No standard units)	IIF, ELISA, Immunoblotting		
Ro ab	3							Serum	Arbitrary units (No standard units)	ELISA, Immunoblot, EIA, Luminex technology		
RNP ab	3							Serum	Arbitrary units (No standard units)	ELISA, Immunoblot, EIA, Luminex technology		
Anti Cardiolipin IgG ab	3							Serum	Arbitrary units (No standard units)	ELISA, EIA		
Anti Cardiolipin IgM ab	3							Serum	Arbitrary units (No standard units)	ELISA, EIA		
kappa free light chains	3			4.8	15.3			Serum				
B2 Transferrin	3							? CSF	qualitative			

Total DPD/creatinine (urine) 24 hour	3	Y		13.5	17.6			Urine		HPLC, Competitive Enzyme immunoassay		circadian rhythm with higher levels of excretion at night (as much as 50%). Also day to day variation (as much as 20%)
Free DPD (urine)	3	Y						Urine		HPLC, Competitive Enzyme immunoassay		circadian rhythm with higher levels of excretion at night (as much as 50%). Also day to day variation (as much as 20%)
ds DNA ab	3							Serum	IU	IIF, ELISA, EIA, RIA, agglutination		
PR3 ab	3							Serum	Arbitrary units (u/mL)	ELISA, luminex/bioplex technology, EIA		
basement membrane ab	3							Serum	Qualitative	IIF		
Pneumococcal Cap. Polysacch.	3	Y						Serum	U/ml	ELISA		Aware of ref prep from Denmark and USA for serotypes. Serotypes reported in ug/mL. Only Binding site or in-house method options at present
CA19-9	3	Y		16	102			Serum	kU/L	chemiluminescence, turbidimetry etc		Ref prep cited in a kit insert but not referenced
IgA Endomysial ab	3							Serum	qualitative	IIF		
Deaminated IgA Gliadin ab	3	Y						Serum	Arbitrary units (u/mL)	ELISA, ImmunoCAP		Done a search for IRP 67/86 but no results
Deaminated IgG Gliadin ab	3	Y						Serum	Arbitrary units (u/mL)	ELISA, ImmunoCAP		Done a search for IRP 67/86 but no results
specific IgG pigeon	3							Serum	mg/L	DD, ELISA, FEIA, Electroimmunodiffusion		
complexed specific antigen	3							Serum	g/L	chemiluminescence, turbidimetry etc		
smooth muscle antibody	3							Serum	qualitative	IIF, ELISA		
gastric parietal cell ab	3							Serum	qualitative	IIF		
Sm ab	3							Serum	Arbitrary units (u/mL)	ELISA, luminex/bioplex technology, EIA, Immunoblot		
Jo-1 ab	3							Serum	Arbitrary units (u/mL)	ELISA, luminex/bioplex technology, EIA, Immunoblot		

Anti Phosphatidyl Serine IgG	3						Serum	Arbitrary units (u/mL)	Elisa	
Lambda free light chains	3						URINE		Immunofixation, CZE	BV within and BV between quoted as 4.8 and 18.0 for serum (not urine)
B2 microglobulin	3	Y		5.9	15.5		Serum	ug/L, mg/L, g/L	chemiluminescence, turbidimetry etc	dichotomous calibration likely
CTX	3						PLASMA	ug/L	chemiluminescence, turbidimetry etc	
Creatinine (urine)	3			11	23		URINE	mmol/L	Dry slide, Jaffe, Enzymatic	class one and three as per phase 1 report. Bv figures quoted for output 24hr
crithidia	3						Serum	qualitative	IIF	

Analyte	Class	Qualified?	BV within	BV between	Name	Sample	Units	Methods	Comments
Urine calculated ACR	-	Y					mg/mmol		ratio will be more variable than individual results
Urine calculated PCR	-	Y					mg/mmol		ratio will be more variable than individual results
Apo B	-	Y	6.9	22.8					insufficient data to classify
Apo A1	-	Y	6.5	13.4					insufficient data to classify
Lp(a)	-	Y	8.5	85.8					insufficient data to classify
Homocysteine	-	Y	9	40.3					insufficient data to classify
Total carotenoids	-	Y						HPLC with internal standard preferred	insufficient data to classify
Alpha carotene	-	Y	35.8	65				HPLC with internal standard preferred	insufficient data to classify
Beta cryptoxanthin	-	Y	36.7					HPLC with internal standard preferred	insufficient data to classify
Lutein + Zeaxanthin	-	Y	19.5	21				HPLC with internal standard preferred	insufficient data to classify
Lycopene	-	Y	40.1	33				HPLC with internal standard preferred	insufficient data to classify
Zeaxanthin	-	Y	34.7					HPLC with internal standard preferred	insufficient data to classify
Itraconazole	-	Y					mg/L		Insufficient data to classify yet
Voriconazole	-	Y					mg/L		Insufficient data to classify yet
Tryptase	-	Y				Serum	ug/L	FEIA	bit early to tell (only Phadia users in scheme)