Chapter 5 Inter laboratory variation of biochemical data and the Renal Association Standards

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The inclusion of laboratory results within the UK Renal Registry data collections sets it apart from other Renal Registries, and whilst this will provide an invaluable clinical and research database it may lead to significant difficulties in data interpretation

5:1 The Renal Association Standards

The Renal Association Standards document recommends specific target limits for some analytes (e.g. phosphate), and recommends the use of local laboratory reference ranges for others such as serum albumin, calcium, and iPTH.

For each analyte, different laboratories use different methods of analysis which give slightly differing results for the same sample. Where the Standards document quotes specific limits for an analyte, it is possible that the ability of a unit to meet these standards may be compromised not only by clinical efficiency or case mix but also by the analytical method used and the bias contained within the laboratory data.

With the use of local laboratory reference ranges, the interpretation of a result may depend upon the choice of normal reference range. For many analytes, the local laboratory reference range is derived from a population distribution; for others (e.g. iPTH), this may alternatively be derived from a reference text book, or the manufacturers kit specification (which would be derived from a US population distribution). While the laboratory data may be appropriate and valid for use within the local hospital environment, it is possible that the ability of a unit to meet the Renal Association standards may be compromised not only by clinical efficiency or case mix, but also by the derivation of the local reference range.

Many are aware of this issue with acknowledged “difficult” analytes such as PTH, but this is also a significant problem with some of the other analytes on which the Renal Registry is collecting data.

5:2 Errors in transfer of results from laboratory to renal unit data systems

The Renal Registry makes significant efforts, in collaboration with contributing renal units, to ensure the accuracy of transfer of the data sets, but with regard to the laboratory data there is an earlier transfer of information between the laboratory(ies) and the units. In this link by which clinical results are transferred for local use, accompanying error messages e.g. “haemolysed sample”, comments or flags such as “pre-dialysis”, may be lost. Manual transcription steps are still sometimes found in the chain linking the laboratory generated result and the renal unit database, with the

inherent possibility of transcription errors. The Association of Clinical Biochemists (ACB) supports the aims of the Renal Registry, but some individual laboratory consultants have expressed significant concerns about transfer these potentially corrupted data from the renal unit databases. Nevertheless there is considerable goodwill within laboratories to support the Renal Registry. The interdisciplinary nature of this process needs to be recognised, in order for renal units and laboratories to work closely together, ensuring that accurate data is supplied to the Renal Registry.

5:3 Inter-laboratory variation and quality assessment schemes

Clinical laboratories are all required to participate in national external quality assessment schemes, in which samples are distributed to all participating laboratories for analysis and then results compiled by organisations such as UK NEQAS to evaluate the degree of agreement between methods and between laboratories. These schemes act as an objective management tool for maintaining and improving professional standards, analogous to the Registry's own aims.

On behalf of the ACB the Clinical Biochemistry laboratories contributing results to Registry linked renal units were approached for permission to look at their External Quality Assessment data, access to which is only given if permission is granted. Out of the 11 units, which are Registry, linked we have obtained permission from 10 laboratories and the results discussed represent the available data from these laboratories. The individual laboratories, and therefore renal units, will not however be identifiable.

5:4 UK NEQAS data

Quality assessment schemes use stabilised specimens, and since the behaviour of these may differ from that of clinical specimens, in most cases method-related target values are used for performance assessment. This limits the use of UK NEQAS data to harmonise the results from laboratories employing significantly different methods.

5:4.1 Variation between results from different laboratories

To illustrate the distributions of results obtained nationally, example data for 1998 from the UK NEQAS Clinical Chemistry scheme for selected analytes are shown in Table 5.1. The coefficient of variation (CV) has been calculated from the geometric mean.

	N	Mean	CV (%)
Albumin (g/L)	535	36.4	4.6
Calcium (mmol/L)	546	2.05	3.1
Phosphate (mmol/L)	513	1.52	3.7
Cholesterol (mmol/L)	504	3.90	4.0
Urea (mmol/L)	553	9.67	4.1
Creatinine (umol/L)	558	346	3.0

Table 5.1 Laboratory agreement data from the UK NEQAS Clinical Chemistry

5:4.2 Creatinine

Data in Table 5.2 are shown classified by method principle and by instrument for creatinine. These data show predominantly the influence of different methods, but also highlight the subtle differences found between results for the same method principle implemented on different instruments with different reagent and calibration materials.

	N	Mean (umol/L)	CV (%)
All methods	558	346	3.0
Endpoint Jaffe	63	346	4.7
Centrifugal analyser	5	334	6.2
Other discrete analyser	42	344	5.1
Olympus systems	14	352	4.2
Beckman Creatinine Analyser	71	360	2.6
Beckman Astra	12	361	1.9
Beckman CX3/CX7 systems	58	360	2.8
DuPont Analyst	7	356	2.3
Other kinetic Jaffe	250	341	4.5
Bayer Axon	12	349	3.6
Bayer DAX	13	344	2.7
Bayer RA/Opera systems	15	342	4.3
Beckman CX4/CX5 systems	14	355	2.7
DADE Behring Dimension	7	352	1.8
Hitachi 717	25	339	3.2
Hitachi 737/747	25	334	4.0
Hitachi 911/917	39	335	3.8
IL Monarch	7	354	6.4
Olympus systems	22	333	6.6
Kone systems	6	348	6.4
Roche Integra	25	341	3.5
Roche Cobas Mira	15	362	11.4
ILab 900/1800	4	315	9.6
Other instrument	5	348	3.6
J & J Vitros systems	142	348	2.3
Shield DT60	7	348	5.5
Vitros 700/750/950	80	348	2.2
Vitros 500	4	350	1.4
Vitros 250	49	348	2.0
O'Leary method	17	344	3.9
Other method	7	346	7.4
Enzymic (creatininase)	5	337	7.6

Table 5.2 Example between-laboratory agreement data from the UK NEQAS for Clinical Chemistry for creatinine, classified by method

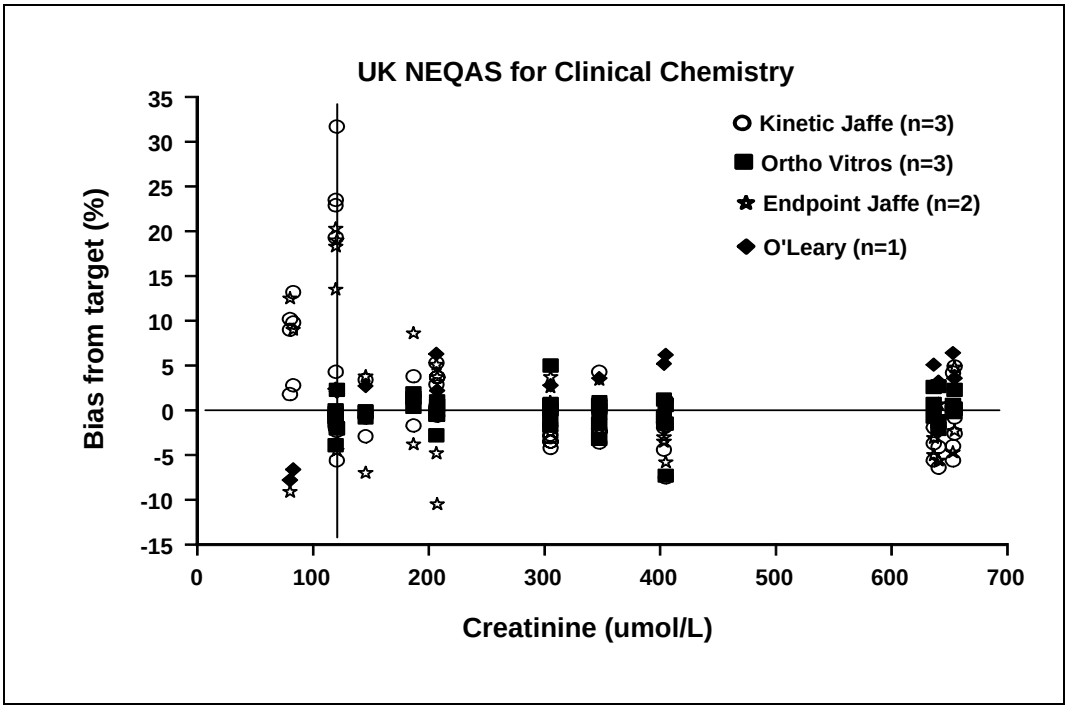

Figure 5.1 Creatinine measurement: bias from the relative target concentration by method

Above a creatinine of 200 µmol/L the range of individual laboratories' bias is of the order of 10 –15%

5:4.3 Albumin

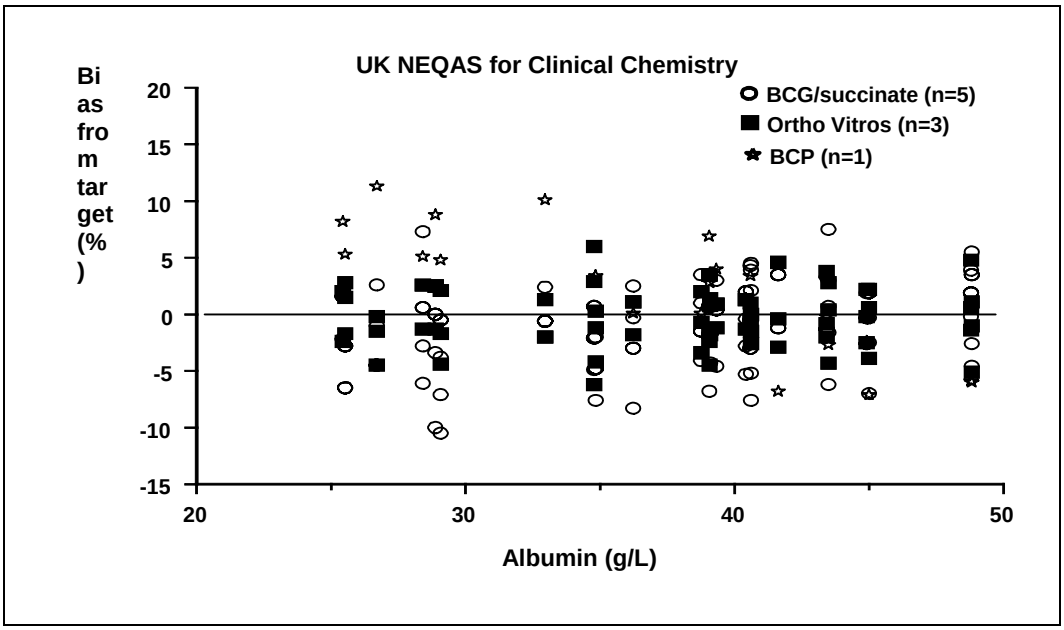

Figure 5.2 Albumin measurement: bias from the relative target concentration by method

Figure 5.2 illustrates that for albumin, the variation in bias, from the relative target concentration by the method used, varies by $\pm$ 12%.

5:4.4 Intact parathyroid hormone assay

For other analytes the influence of method is more marked. Table 5.3 shows the between-laboratory agreement (expressed as a geometric CV) for iPTH, classified by method. The specimen comprised of a mixture of sera from normal subjects and patients with chronic renal failure. Although laboratory performance with scheme specimens may not truly reflect performance with specimens from patients, these data suggest that significant method differences exist. Furthermore, these differences may not be consistent between different disease states.

	n	**Mean (pmol/L)**	**gCV (%)**
All methods	94	14.8	27.5
Method A	7	10.6	9.6
Method B	9	7.8	18.0
Method C	38	16.5	9.6
Method D	5	16.0	3.9
Method E	25	15.8	7.3
Method F	7	18.9	20.8

Table 5.3 Example between-laboratory agreement data for PTH from the UK NEQAS for Peptide Hormones, classified by method (reproduced with permission)

5:5 Harmonisation of laboratory results

5:5.1 Local laboratory methodology

Table 5.4 gives a breakdown of method, reference range and, for calcium measurements, correction formulae differences, for the laboratories contributing data to renal units included in this report.

	Albumin (g/L)		Bicarbonate (mmol/L)		Calcium (mmol/L)		Phosphate (mmol/L)		PTH	
Lab	Method	Ref Range	Method	Ref Range	Method	Correcting Formula	Method	Ref Range	Method	Ref Range
A	BCG	35-48	Actual	22-30	CPC	+0.025(40-Alb)	PMb	0.80-1.45		
B	BCG	35-53	PEPC	24-32	CPC	+0.02(40-Alb)	PMb	0.82-1.55	Cardiff	0.9-5.4 pmol/L
C	BCG	35-50	PEPC	22-29	Arsenazo	+0.02(40-Alb)	PMb	0.80-1.40	DPC	12-72 ng/L
D	BCG	35-55	PEPC	22-30	Arsenazo	+ ((40-Alb)/40)	Fish/Sub	0.80-1.40	DPC	1.3-7.6 pmol/L
E	BCG	36-50	PEPC	22-31	Arsenazo	+0.0175(40-Alb)	Fish/Sub	0.8-1.40	Chiron	10-65 ng/L
F	BCG	35-50	PEPC	20-29	CPC	+0.02(40-Alb)	PMb	0.75-1.35	Chiron	<4.0 pmol/L
G	BCP*	30-52	PEPC	19-28	CPC	+0.017(43-Alb)	PMb	0.80-1.40	DPC	12-72 ng/L
H	BCG	37-49	PEPC	20-28	CPC	+0.06(46-Alb)	PMb	0.80-1.30	Nichols	10-65 ng/L
I	BCG	35-50	PEPC	20-30	CPC	Not applicable	PMb	0.80-1.40	Nichols	10-65 ng/L

Table 5.4 Laboratory methodologies and reference ranges

5:5.2 Harmonisation method

In an initial approach, to reduce the effects of such variations on Registry assessments, the mean bias, from their NEQUAS EQA samples, over the preceding 12 months was calculated The number of samples to calculate this figure ranged from 15 to 22. This developed an adjustment factor for each laboratory to bring their method in line with the national consensus for their method principle.

Some example of the distribution of the reported results before and after this adjustment is shown below. Many of the centres on the Registry are close to the mean bias, and the maximum bias variation is 4%. This bias range will increase and the harmonisation factor become more important as more centres join the Registry.

After a harmonisation factor has been applied the local laboratory reference range is no longer applicable, and the Renal Registry will need to apply a 'standard' reference range.

5:5.2 Serum phosphate measurements

The phosphate bias correction factor for centres on the Registry ranges from 0.9780 to 1.0403. This is small, but other centres joining the Registry may require larger corrections. Harmonisation does slightly alter the percentage achieving the standards at some of the centres.

This is illustrated by the following example from haemodialysis data collected by the Registry. Figures 5.3 and Fig 5.4 show the distribution of phosphate concentration a) uncorrected for method-related bias and b) harmonised.

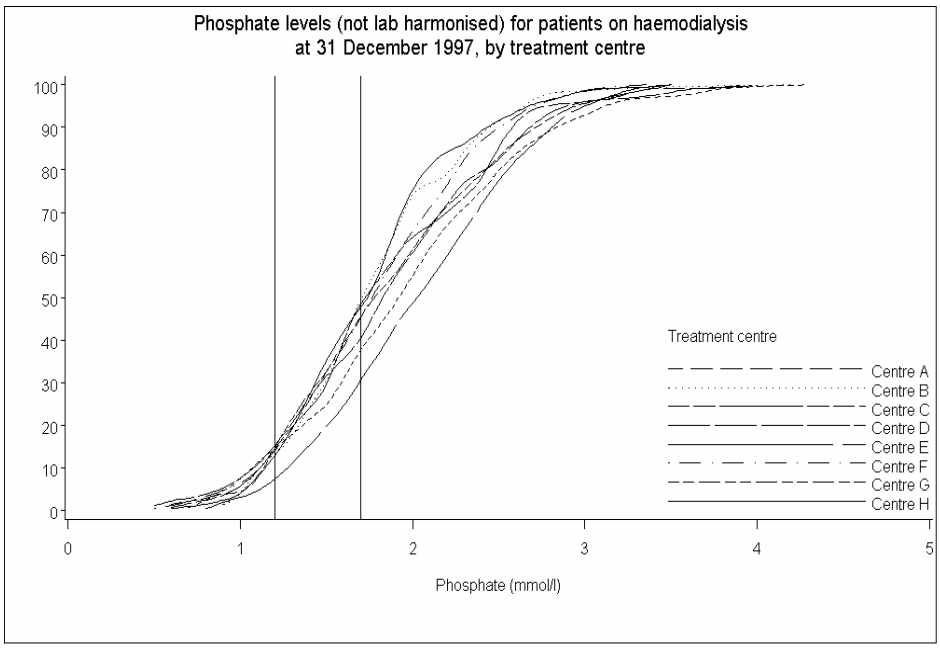

Figure 5.3 Cumulative distribution of non-harmonised serum phosphate for patients on haemodialysis.

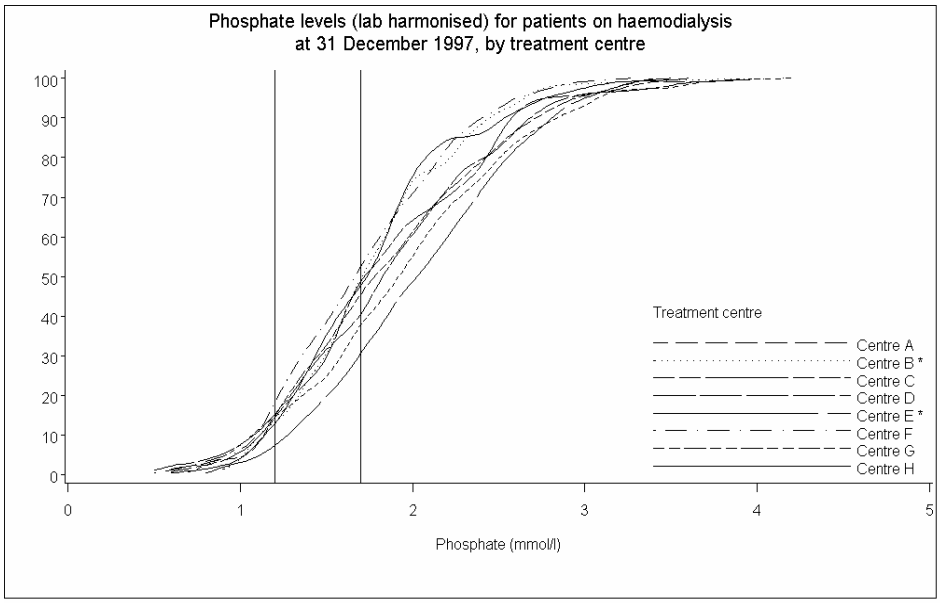

Figure 5.4 Cumulative distribution of harmonised phosphate for patients on haemodialysis.

5:5.3 Serum albumin

The harmonisation factor for centres ranged from 0.9655 – 1.0002, using non-uraemic samples. Most centres were about 1.00, but the NEQAS data shows that the harmonisation factor could range from 0.8 to 1.2 as more centres are included.

There are essentially two methods for albumin measurement in clinical use. Both use dye binding, but with different dyes, Bromocresol Green (BCG) and Bromocresol Purple (BCP). The latter method is acknowledged to be more specific for albumin (but is more expensive) whilst BCG measures additionally other proteins, but is cheaper and more widely available. External quality assessment studies have shown that this difference is exaggerated at low albumin concentrations, but overall BCP methods report lower albumin concentrations than BCG. The mean difference has been of the order of 5 g/L.

From the information supplied by the laboratories to the Registry it is clear that significantly different methods are being used to measure albumin. This is illustrated by the following examples from data collected by the Registry.

Haemodialysis

Figure 5.5 shows the non-harmonised distribution of patient results from patients on haemodialysis for serum albumin. One centre (method), G, stands out from the rest.

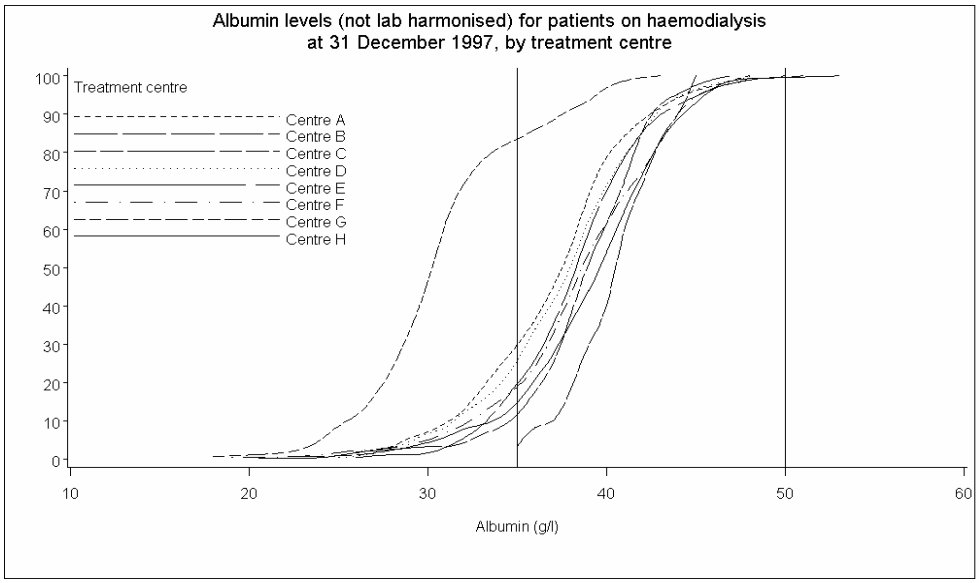

Figure 5.5 Cumulative distribution of serum albumin, non-harmonised, for patients on haemodialysis

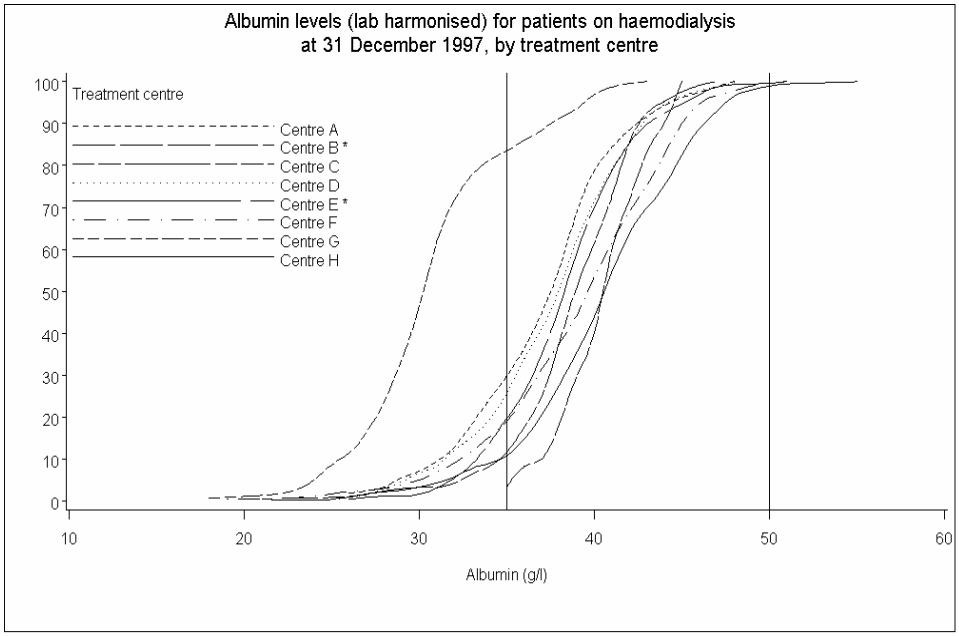

Figure 5.6 Cumulative distribution of serum albumin, harmonised, for patients on haemodialysis

Correction for method group bias reduces the scatter but the same pattern remains.

Peritoneal dialysis

The cumulative distribution curves for serum albumin of peritoneal dialysis patients are shown in figures 5.7 and 5.8

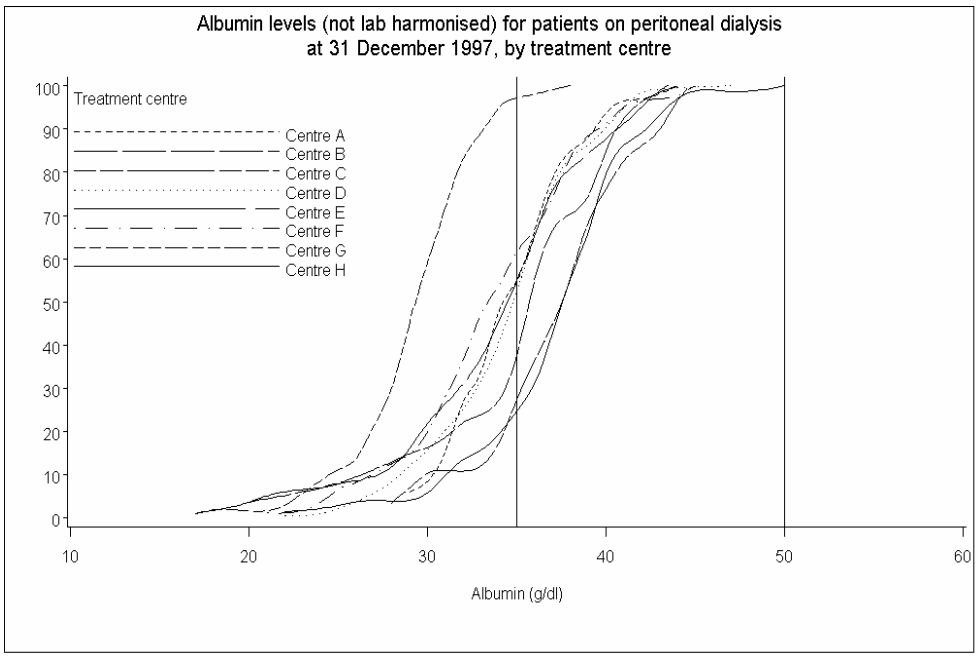

Figure 5.7 Cumulative distribution of non-harmonised serum albumin of patients on peritoneal dialysis

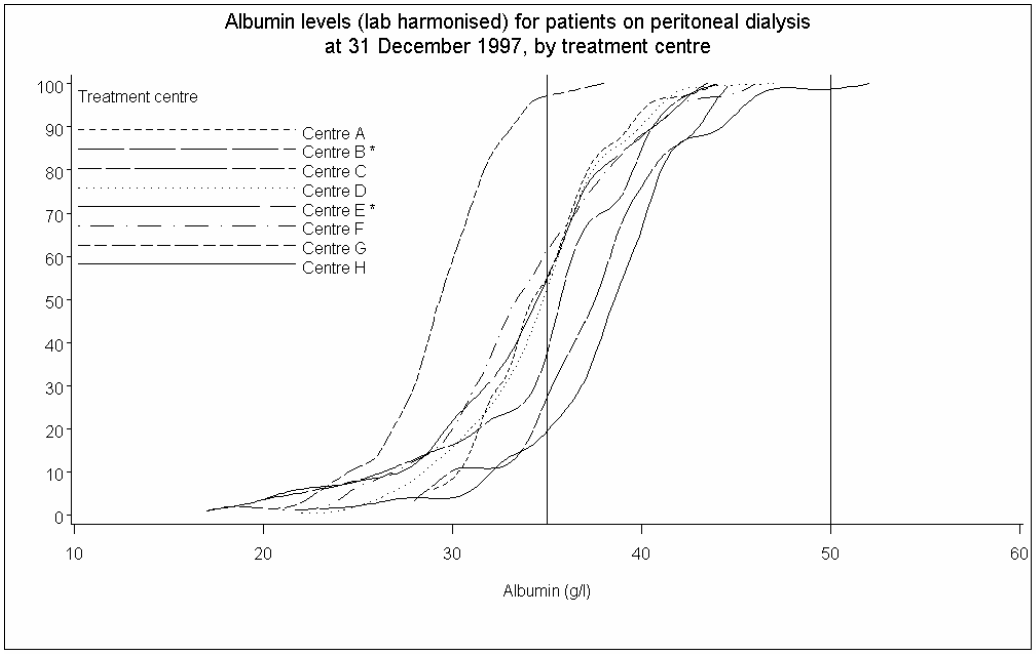

Figure 5.8 Cumulative distribution of harmonised serum albumin of patients on peritoneal dialysis

A laboratory using a BCP assay supports centre G. and reports lower results, shown in figures 5.5 to 5.77. For haemodialysis patients centre G has the lowest number of patients achieving the Renal Association standard, even using their lower reference range of 30 g/l as compared with 35g/l for most other centres. This is in contrast to peritoneal dialysis, where using the lower reference range, the compliance with the standard for centre G appears to be more comparable to other centres.

The large discrepancy between BCP and BCG could not have been predicted from the EQA data and indicates that serum samples from patients with end-stage renal failure contain substances which interfere significantly with one or other of the methods. Unfortunately there is only one unit using the BCP method and this result needs confirming by other centres. There is some literature suggesting interference with the BCP method in sera from haemodialysis patients, but not peritoneal dialysis patients.

The implications for the laboratories are that a special distribution of EQA samples based around renal patients is required to explore the methodological differences. There may need to be a recommendation made as to which method is most appropriate for monitoring renal patients. The Renal Association Standards committee may need to redefine the guidelines on serum albumin measurement.

5:5.4 Serum Calcium

Total calcium is calculated by laboratories by adjusting for the serum albumin. There are many different formulae used and these are listed in Table 5.4. To standardise the data for comparative audit, the Renal Registry requires to unadjust calcium, apply the calcium harmonisation factor, and then apply a consistent correction formula. This data is also dependent on the method the laboratory uses to measure albumin, and the bias from the NEQAS mean. The 'standard' formulae in use to correct calcium do not take this variation in albumin measurement into account. Application of this technique to the data from centre G, which reads albumin on average 5 g/l lower than other centres, still leaves a discrepancy in the data.

5:5.4 Intact parathyroid hormone assay

The Standards document specifies that iPTH should be < 3 x (upper limit of reference range).

All laboratories appear to be using assays that measure only the intact PTH. Only one laboratory (centre F) calculates its own population based reference range. This results in a much lower upper limit of the reference range and accounts for the discrepancy between centre E and F using the same manufacturer's kit. The other laboratories either use a range taken from a standard reference textbook, or the assay kit manufacturer's specified range. This discrepancy in defining the reference range markedly affects how the centre 'achieves' the Standards, as shown in figure 5.9 and table 5.5. Centre F appears non-compliant, but when compared against an upper limit of 7.6 pmol/l has one of the highest compliances. Because of these anomalies in local ranges, the Registry has shown compliance against a reference limit of 23 pmol/l (7.6 x 3) on the figures.

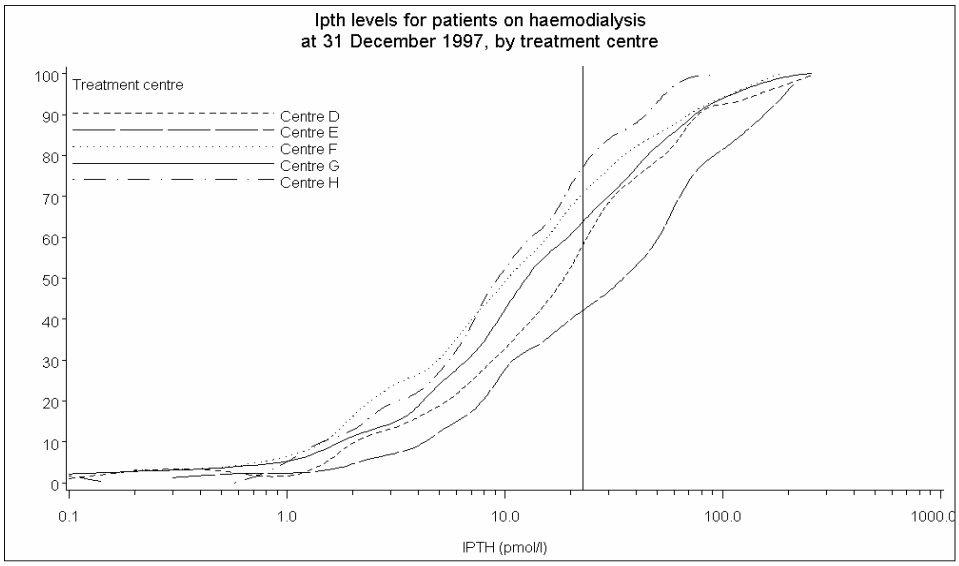

Figure 5.9 Cumulative distribution of serum iPTH for patients on haemodialysis

Unit	% <x3 local range	% <23 pmol/l	Median	Lower quartile	Upper quartile	Local range	Method
A							
B						0.9 - 5.4 pmol/l	
C						1.3 - 7.6 pmol/l	DPC
D	55	55	19	7	43	1.3 - 7.6 pmol/l	DPC
E	39	42	37	9	74	1.1 - 6.8 pmol/l	Chiron
F	54	71	10	3	28	< 4.0 pmol/l	Chiron
G	63	63	12	5	37	1.3 - 7.6 pmol/l	DPC
H	73	76	10	5	21	1.1 - 6.8 pmol/l	Nichols
						1.1 – 6.8 pmol/l	Nichols

Table 5.5 Range of iPTH for patients on haemodialysis

5:6 Discussion

This is the first time harmonisation of laboratory results has been attempted on this scale and for this purpose. The approach of taking EQA data to harmonise laboratory results from centres does appear to provide a closer agreement between centres. This harmonisation needs to be extended and monitored as more units join the Registry database. Extending this to include analytes such as PTH will be even more problematic than the albumin example discussed above. In the case of PTH discussions continue between the appropriate professional groups to develop a workable approach for use on 1998 data. In the case of albumin, and possibly other analytes, there may also be concentration-dependent biases in renal samples, which would require something other than a simple adjustment factor to correct.

Some analytes such as bicarbonate will require the co-operation of the Welsh EQAS scheme, which is currently the only scheme in the UK to offer this analyte. There may be further issues compounding the bicarbonate harmonisation due to the relative

instability of this analyte. An illustration of the difficulties for bicarbonate is shown in figure 5.10. The data represents a period from 29/12/1997 to 11/05/1998, and show the mean value for each method against the trimmed overall mean from the 200 participants analysing bicarbonate in the scheme.

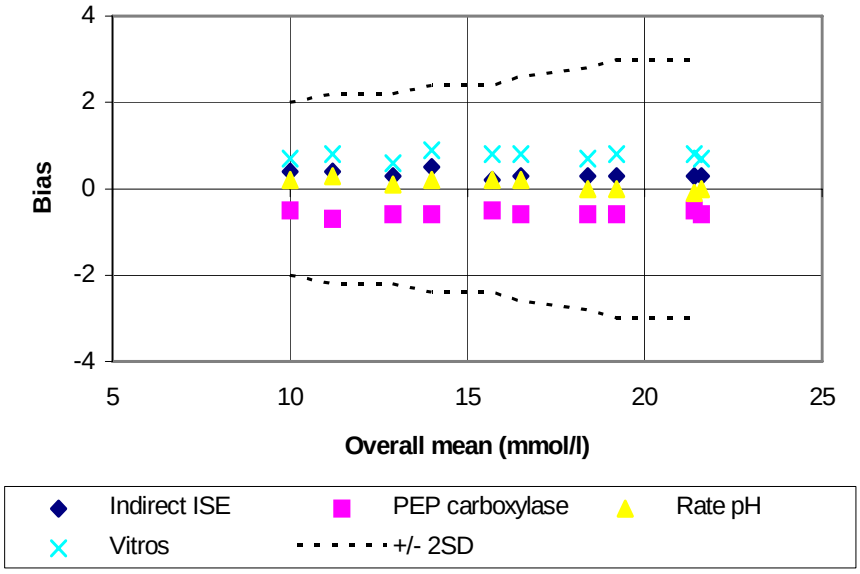

Figure 5.10 National variation in bicarbonate results according to method group from the Welsh EQAS Scheme (with permission).

This indicates a preponderance of the distributed sample concentrations lying in the acidotic range. Although this is perhaps more relevant to results from patients on haemodialysis, the Renal Standards document specifies that bicarbonate should be within the local laboratory range.

Some renal units have satellite dialysis units, from which samples are sent to laboratories other than that used by the base centre. This would require different adjustment factors to be applied to samples analysed at the different laboratories. At present there is no simple means of automatically identifying the laboratory at which a sample had been analysed. Unique laboratory identifiers may therefore need to be developed, and this issue is under national consideration.

The use of EQA data requires monitoring to ensure that the correction factors are correctly updated: this will need a continuing dialogue between the renal units and their local laboratories. Updating will be required at intervals even if the method used has not changed. This updating by use of UK NEQAS data must be with the renewed permission of the head of the laboratory, although annual renewal should not be necessary in subsequent years.

Different analytical methods have individual advantages and disadvantages. Instrument and method selection are based on the laboratory's overall role and many other practical considerations may require accepting some compromises on particular methods to

achieve an overall advantage. Different choices will continue to be appropriate for different laboratories. Limiting freedom of choice to one method is not appropriate and would limit progress.

The harmonisation of laboratory results between contributing centres is also an issue for all multi-centre clinical trials, and the Registry's collaboration with the ACB and UK NEQAS may provide answers to these not insignificant problems in the coming years. By working closely with renal units and their laboratory medicine colleagues, the Registry database will provide an invaluable audit and research resource.