UKNEQAS

Guildford Peptide Hormones

ANNUAL REVIEW
July 2012

Participant Version

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UKNEQAS Guildford Peptide Hormones Annual Review 2012

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1. Introduction

The Guildford Peptide Hormones scheme has been in operation for over 30 years. Initially only the analytes insulin and gastrin were offered. In 1983, the scheme was extended to include C-peptide. Insulin-like growth factor-I (IGF-I) and insulin-like growth binding protein-3 (IGFBP-3) were added to the scheme's repertoire in 1991 and 2002 respectively. Further scheme development to include the analytes proinsulin, insulin antibodies, insulin-like growth factor-II (IGF-II) and urinary C-peptide is being considered if there is sufficient participant interest.

This Annual Review is the fourteenth issued from Guildford since joining UK NEQAS in 1998. The Annual Review provides an overview of the scheme's activities over the previous year, giving participants an opportunity to critically evaluate scheme design, trends in laboratory practice and assay performance.

Comments regarding the Annual Review or any other aspect of the scheme are welcomed. The scheme is run for the benefit of participants so please tell us what you want and would like changed!

a) Scheme Design

Effective external quality assessment (EQA) requires the distribution of specimen pools that closely mimic those encountered in clinical practice. Unfortunately, the labile nature of the peptides measured in the scheme necessitates the use of lyophilised rather than liquid material. This does sometimes incur an error in sample reconstitution. Nevertheless, there is the potential for greater errors due to analyte deterioration if non-lyophilised samples were distributed.

Under consideration is the periodic distribution of frozen, liquid specimen pools rather than lyophilised material. Distribution of frozen samples to all participants would be logistically difficult and expensive, which would require a large increase in scheme subscription charges.

Therefore, it would be intended to distribute the frozen, liquid specimens only to a proportion of participants that would be representative of all the methods in use for each analyte. Such data would be useful to investigate the effect of sample matrices and transport conditions on analysis.

There has been a continued effort to distribute appropriate specimens over a broad concentration range to assess analytical performance of all five analytes. In order to distribute samples appropriate for clinical situations, the scheme would appreciate serum donations from participant laboratories from individuals with appropriate clinical disorders e.g. gastrinoma, growth hormone deficiency, acromegaly, hypoglycaemia and insulin resistance.

The Human Tissue Authority (HTA) has clarified the use of leftover clinical specimens for EQA purposes and ethical approval is not required if the samples are anonymised. Further information can be obtained from the RCPath guidance document 'Guidance on the use of clinical samples retained in the pathology laboratory' [www.rcpath.org/Resources/RCPath/Migrated%20Resources/Documents/G/G035GuidanceUseofClinicalSamplesSept07.pdf] or the HTA website [www.hta.gov.uk].

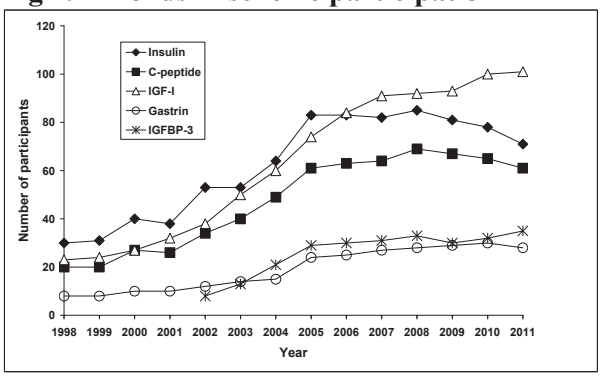
The small number of users for particular methods requires the all laboratory trimmed mean (ALTM) to be used for all analytes. Since there is still a lack of available reference methods for scheme analytes, validation of the ALTM can only be achieved by the use of stability and recovery exercises with appropriate international reference preparations (if available). Data from stability and recovery exercises are detailed in the respective analyte sections of the Annual Review. Such exercises will continue to be repeated in the future. For insulin and C-peptide, the feasibility of standardising immunoassays with isotope-dilution liquid chromatography/ tandem mass spectrometry has been reported. This will hopefully lead to the establishment of reference insulin and C-peptide methods, which can be used to obtain target values for specimen pools.

Validation of the ALTM with recovery and stability exercises has led to the National Quality Assurance Advisory Panel (NQAAP) setting acceptable BIAS and VAR performance criteria for all analytes except gastrin. Currently performance criteria for all analytes are set at ± 25% BIAS and 25% VAR.

b) Trends in Laboratory Practice

Figure 1.1 shows the trend in overall participation of the five scheme analytes since the scheme joined UK NEQAS.

Fig 1.1 Trends in scheme participation



Due to laboratory consolidation, there has been a further decline in the number of participants in the insulin and C-peptide schemes. In April 2012, there were 71 participants registered for insulin, 61 participants registered for C-peptide, 28 participants registered for gastrin, 101 participants registered for IGF-I and 35 participants registered for IGFBP-3.

c) Future Developments

Although the NQAAP has set BIAS and VAR performance criteria for scheme analytes, it is still necessary to ensure that there is continued validation of the ALTM and that the assay performance criteria are appropriate.

Assessment of analytical performance at clinical decision points and the suitability of assays for specific clinical situations (e.g. insulin and C-peptide assays for the investigation of hypoglycaemia) will continue to be examined.

INSULIN

- Further examination of assay sensitivity.
- Further recovery exercises to be undertaken.
- Investigation of assay cross-reactivity with synthetic insulin preparations.
- Investigation of the effect of haemolysis.
- Investigation of the effect of insulin antibodies on immunoassays.
- Further, sample production from serum pools containing endogenous immunoreactive peptides.
- Interpretative exercises.

C-PEPTIDE

- Further recovery exercises to be undertaken.
- Interpretative exercises.
- Examination of low-level security using serum from insulin dependent diabetics.

GASTRIN

- Sample production using serum pools collected from patients with high and low endogenous gastrin levels.
- Further recovery exercises using various forms of recombinant gastrin since the human international reference standard is no longer available.

IGF-I

- Sample production using serum pools collected from patients with high and low endogenous IGF-I levels.
- Continued examination of the security of results at the diagnostically sensitive upper and lower limits of reference ranges.
- Further recovery exercises to be undertaken.
- Examination of the effect of insulin-like growth factor binding proteins (IGFBPs) on IGF-I measurement.

IGFBP-3

- Sample production using serum pools collected from patients with high and low endogenous IGFBP-3 levels.
- Examination of the security of results at the diagnostically sensitive upper and lower limits of reference ranges.

ABBREVIATIONS

ALTM	All laboratory trimmed mean
Auto	Automated
DELFIA	Dissociation enhanced lanthanide fluorescent immunoassay
ELISA	Enzyme linked immunosorbent assay
EQA	External quality assessment
GCV	Geometric coefficient of variation
GTT	Glucose tolerance test
HTA	Human Tissue Authority
IGF-I	Insulin-like growth factor-I
IGFBP-3	Insulin-like growth factor binding protein-3
IRMA	Immunoradiometric immunoassay
IRP	International reference preparation

IRR	International reference reagent
IS	International standard
Man	Manual
MLTM	Method laboratory trimmed mean
NIBSC	National Institute for Biological Standards and Control
NQAAP	National Quality Assurance Advisory Panel
RCPath	Royal College of Pathologists
RIA	Radioimmunoassay

2. INSULIN

2.1 PARTICIPATION AND METHODS

Due to laboratory consolidation, the number of participants in the scheme has reduced from 78 to 71. There are currently eleven different insulin methods in use. The scheme is dominated by analyses on the automated Roche Elecsys and Siemens Immulite platforms. The Tosoh and Dako assays are no longer used by any scheme participant.

Table 2.1 Methods in use, April 2012
(2010/2011 in parenthesis)

Method	Type	Code	No. of labs
Abbott Architect	Auto	AB13	6 (6)
Abbott AxSYM	Auto	AB7	1 (1)
Beckman Access	Auto	SF1	4 (5)
Dako	Man	NV1	0 (1)
DiaSorin Liaison	Auto	SO4	1 (1)
Invitron	Man	IW1	1 (1)
Mercodia Iso-Insulin ELISA	Man	ME1	3 (2)
Perkin Elmer DELFIA	Man	PH2	4 (4)
Roche Elecsys	Auto	BO5	27 (22)
Siemens Centaur	Auto	CO10	3 (3)
Siemens Immulite	Auto	DC7	1 (1)
Siemens Immulite 2000 family	Auto	DC11	20 (30)
Siemens Immulite 2000	Auto		19 (25)
Siemens Immulite 2500	Auto		1 (5)
Tosoh	Auto	TO1	0 (1)
TOTAL			**71** (78)

During distributions 259–266, seventeen different specimen pools were issued as described in table 2.2 and figure 2.1. All specimens were prepared from healthy volunteers.

To assess the validity of the ALTM as a performance target, recovery exercises were undertaken by the addition of the International Reference Preparation for insulin (IRP 66/304) to

base specimen pools as indicated in table 2.2 (assuming 1 mU/L is equivalent to 6 pmol/L insulin). Insulin IRP 66/304 was kindly supplied by Dr Chris Burns at NIBSC.

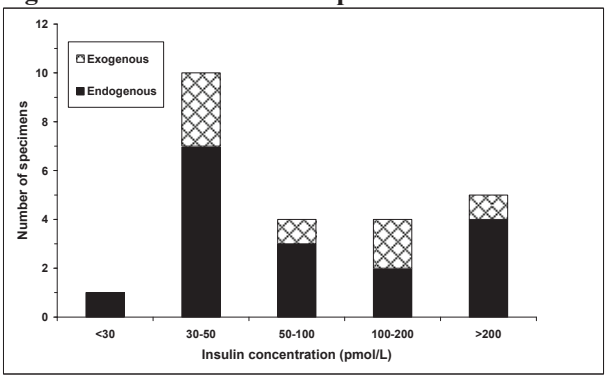
To assess insulin assay cross-reactivity, Actrapid® (Novo Nordisk) or Levemir® (insulin detemir, Novo Nordisk) were added to a base pool as described in table 2.2. Actrapid® is a neutral solution of human insulin and is a short acting insulin preparation. In contrast, Levemir® is a long-acting insulin analogue and differs from human insulin in that the amino acid threonine in position B30 has been omitted, and a C14 fatty acid chain has been attached to the amino acid B29.

Table 2.2 Serum specimens issued

Pool	Description	No. of distributions
F157	Non fasting pool	1
F158	Non fasting pool	1
N162	N161 + 15 pmol/L Insulin IRP 66/304	1
N183	Non fasting	2
N184	160 ml N183 + 160 ml N185	2
N185	Fasting	2
N186	Fasting	3
N187	N186 + 150 pmol/L Insulin IRP 66/304	1
N188	N186 + 300 pmol/L Insulin IRP 66/304	1
N189	N186 + 500 pmol/L C-peptide IRR 84/510	1
N190	N186 + 800 pmol/L C-peptide IRR 84/510	1
N191	Fasting	1
N192	Non fasting	1
N193	Fasting	2
N194	N196 + 100 pmol/L Actrapid	1
N195	N196 + 100 pmol/L Levemir	1
N196	Fasting	2

During distributions 259-266, the insulin ALTM concentrations covered the range 19 - 386 pmol/L. There is still a need to obtain specimen pools with lower insulin values to assess assay sensitivity and to issue samples that would represent the suppressed levels that would be seen as an appropriate physiological response to hypoglycaemia.

Fig 2.1 Concentrations and specimens issued



2.3 VALIDITY OF THE ALTM

Ten different sample pools have been distributed on more than one occasion. It is evident from figure 2.2 that there is no significant deterioration in the insulin ALTM. The stability of the ALTM provides further evidence that the ALTM is a valid target for scoring assay performance.

Fig 2.2 Stability of the ALTM

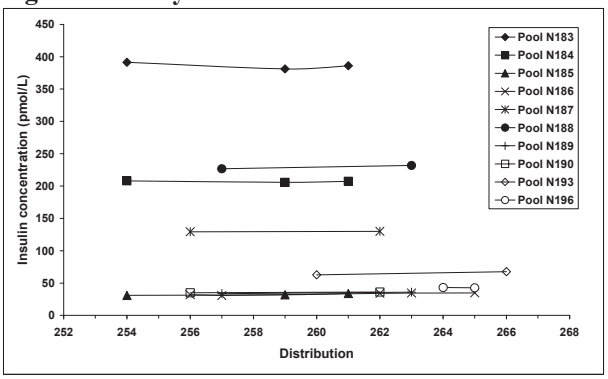


Table 2.3 Between-distribution variability of ALTMs (includes data from distributions before 259)

Pool	No. of distributions	Mean ALTM (pmol/L)	CV (%)
N183	3	386.7	1.3
N184	3	207.1	0.6
N185	3	32.2	4.6
N186	5	33.3	5.5
N187	2	129.7	0.4
N188	2	229.4	1.6
N189	2	34.8	4.5
N190	2	35.6	2.2
N193	2	65.2	5.3
N196	2	42.9	0.9

The between-distribution agreement of ALTMs shows similar variability to last year. This is due to changes in assay performance of the Siemens Immulite platform assays since December 2010.

Users who have received Immulite/ Immulite 1000 insulin kit lots 329 and above, and/or Immulite 2000/2000 XPi insulin kit lots 290 and above and/or Immulite 2500 insulin kit lot 176 and above have seen a change in assay performance. The median for the reference range has shifted downwards with the most significant deviations being at <54 pmol/L. At these levels some participants are seeing a shift of 100%.

Siemens issued a customer notification in November 2010 which 'noted that the observed median for the reference range differs from the guidelines published in the IFU. This may result in a higher frequency of fasting patient samples showing values below the assay limit of 2 uIU/ml.' These lots have been manufactured according to SOPs and meet all required product release specifications. Siemens recommend that users should re-establish their own medians and reference ranges.

In June 2012, Siemens released a customer bulletin indicating that they have corrected the low end negative bias identified in December 2010 and restored expected values to the guidelines provided in the Instructions for Use. The root cause of the bias has been identified as a processing step used in manufacturing the reagent polyclonal conjugate. Reagent kit lots 401 and above will contain the corrected conjugate and will exhibit the improved assay performance. This will be monitored in future distributions with samples containing lower insulin concentrations being issued.

RECOVERY EXERCISES

Although a pilot isotope-dilution mass spectrometry reference method for insulin is now available, this is not yet available for targeting insulin EQA sample pools. Therefore, ALTM validation has to be achieved by monitoring the ALTM stability as described above and by performing recovery exercises with appropriate reference preparations.

Insulin IRP 66/304 was established in 1974 and all the commercial insulin methods in the scheme indicate they are standardised against this material.

Recovery exercises were performed by adding known amounts of insulin IRP 66/304 to base pools as indicated in table 2.2 assuming 1 mU/L is equivalent to 6 pmol/L insulin. Similar levels of insulin IRP 66/304 (150 and 300 pmol/L) were used in previous recovery exercises and this data is shown in brackets in table 2.4 for comparative purposes.

Table 2.4 Recovery (%) of added insulin, IRP 66/304 (November 2011 & January 2012 exercises). Mean recoveries are shown. Values obtained in last years exercise are shown in brackets.

Method	+150 pmol/L IRP 66/304	+300 pmol/L IRP 66/304
All methods	63.9 (64.8)	65.7 (65.4)
Abbott Architect	64.4 (65.6)	69.9 (74.6)
Abbott AxSYM	81.2 (84.0)	61.2 (106.2)
Beckman Access	62.4 (66.1)	63.5 (66.2)
DAKO	62.4 (48.4)	60.5
DiaSorin Liaison	61.6 (63.2)	70.6
Invitron	99.4	82.0
Mercodia Iso-Insulin ELISA	71.5 (69.8)	69.3 (72.4)
Perkin Elmer DELFIA	71.1 (61.5)	65.8 (71.9)
Roche Elecsys	81.0 (73.6)	77.5 (74.4)
Siemens Centaur	98.2 (103.1)	104.8 (95.4)
Siemens Immulite 2000 family	46.7 (56.9)	49.5 (55.4)
Tosoh	70.8 (70.4)	77.4 (77.4)

In both recovery exercises with added insulin IRP 66/304, the ALTM recovery is not quantitative (± 10%). Only the Siemens Centaur assay shows quantitative recovery of added IRP 66/304 in these exercises. Although the results are similar to the previous exercises performed in 2011, these observed recoveries are lower for all assays compared to data obtained pre 2011. Samples were prepared according to protocol. However the freeze dryer did fail the day after the IRP 66/304 spiked samples were produced and therefore the lyophilisation process may not have been as efficient as usual.

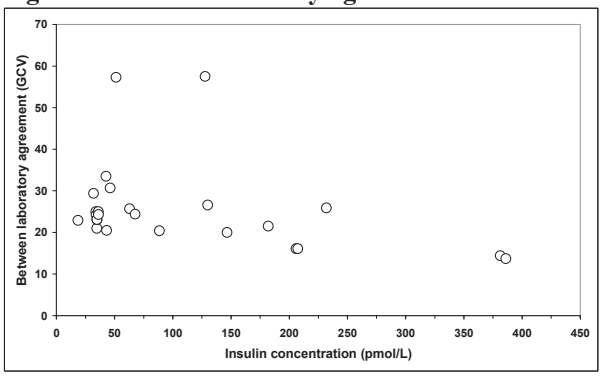
The June 2012 customer bulletin issued by Siemens acknowledged that the current Immulite insulin assay recovers approximately 20% below the IRP 66/304. Kit lots 401 and above have been recalibrated to improve alignment to IRP 66/304 and Siemens data shows an approximate 20% positive shift across the assay range.

Therefore it is a priority for the scheme to issue newly prepared IRP 66/304 spiked samples to all participants.

2.4 BETWEEN-LABORATORY AGREEMENT

The overall between-laboratory agreement for all 24 samples issued in distributions 259-266 is shown in figure 2.3.

Fig 2.3 Between-laboratory agreement



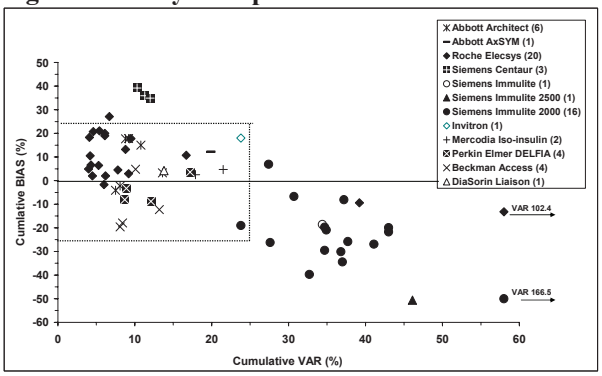
Two specimen pools showed a GCV of 57%. This is due to the specimens containing Actrapid® (Novo Nordisk) or Levemir® (insulin detemir, Novo Nordisk) and reflects assay differences in the recognition of synthetic insulin analogues.

The profile of between-laboratory agreement is similar to previous years. There is still reduced consensus at insulin concentrations below 50 pmol/L. This aspect of assay performance should be considered when participants are utilising their assays for assessing the 'appropriateness' of measured insulin levels in the investigation of hypoglycaemia. To ensure that correct diagnostic cut-offs are applied to particular assays, it is important for participants to be aware of method related differences when extrapolating data from papers and guidelines.

A snapshot of current insulin assay performance in terms of cumulative BIAS and VAR at distribution 266 is shown in figure 2.4. The dashed lines ± 25% BIAS and 25% VAR have been set by the National Quality Assurance Advisory Panel for Chemical Pathology as targets for BIAS and VAR performance.

As noted previously, the Roche Elecsys and Siemens Centaur assays are positively biased compared to the Siemens Immulite platform assays.

Fig 2.4 'Penalty box' plot of BIAS vs VAR



In the previous Annual Review, there had been a significant deterioration in assay performance as there were 18 participants (4 Roche Elecsys, 1 Abbott Architect, 3 Siemens Centaur, 1 Siemens Immulite, 2 Siemens Immulite 2500 and 7 Siemens Immulite 2000 users) outside the ± 25% BIAS limits. There has been some improvement in performance as there are currently 12 participants outside the ± 25% BIAS limits. These are 1 Roche Elecsys, 3 Siemens Centaur, 7 Siemens Immulite 2000 and 1 Siemens Immulite 2500 participants.

There has been a further deterioration in assay performance in the number of participants failing the 25% VAR limit. This has increased from 13 participants last year to 19. This reflects the change in assay performance of the Siemens Immulite assays as all participants except one have cumulative VAR >25%.

In order to implement guidelines and diagnostic clinical cut-offs, improved standardisation must be achieved between insulin methods.

Insulin assays are used in the investigation of hypoglycaemia which may arise due to the administration of insulin. There are a variety of insulin analogues which are used for the treatment of diabetes mellitus which vary in their pharmacokinetics. To assess assay cross-reactivity, a specimen pool was spiked with Actrapid® (Novo Nordisk) or Levemir® (insulin detemir, Novo Nordisk) as described in table 2.2.

Tables 2.5 and 2.6 summarise the observed Actrapid® (Novo Nordisk) or Levemir® (insulin detemir, Novo Nordisk) cross-reactivity of the insulin methods in use at distribution 264.

Table 2.5 Actrapid® cross-reactivity of assays
The insulin MLTM (pmol/L) obtained on the basal and Actrapid® spiked pool is shown for methods with 4 or more users.

Method	Basal pool	Basal pool + 100 pmol/L Actrapid®
All methods (n = 69)	43.1	127.7
Abbott Architect (n = 6)	43.2	153.8
Abbott AxSYM (n = 1)	39.6	139.2
Beckman Access (n = 4)	36.3	123.3
DAKO (n = 1)	36.6	141.6
Invitron (n = 1)	52.3	188.5
DiaSorin Liaison (n = 1)	38.4	156
Mercodia Iso-insulin ELISA (n = 2)	41.3	212.3
Roche Elecsys (n = 23)	47.7	163
Perkin Elmer DELFIA (n = 4)	38.1	150.2
Siemens Centaur (n = 3)	51.4	189.8
Siemens Immulite (n = 1)	20.0	84.4
Siemens Immulite 2000 family (n = 21)	16.6	69.46
Tosoh (n = 1)	41.4	144.6

Table 2.6 Levemir® cross-reactivity of assays
The insulin MLTM (pmol/L) obtained on the basal and Levemir® spiked pool is shown for methods with 4 or more users.

Method	Basal pool	Basal pool + 100 pmol/L Levemir®
All methods (n = 69)	43.1	51.22
Abbott Architect (n = 6)	43.2	115.9
Abbott AxSYM (n = 1)	39.6	75.6
Beckman Access (n = 4)	36.3	49.72
DAKO (n = 1)	36.6	46.8
Invitron (n = 1)	52.3	220.7
DiaSorin Liaison (n = 1)	38.4	42
Mercodia Iso-insulin ELISA (n = 2)	41.3	125.1
Roche Elecsys (n = 23)	47.7	51.11
Perkin Elmer DELFIA (n = 4)	38.1	39.45
Siemens Centaur (n = 3)	51.4	131.9
Siemens Immulite (n = 1)	20.0	56.8
Siemens Immulite 2000 family (n = 21)	16.6	35.42
Tosoh (n = 1)	41.4	115.2

It is clear from tables 2.5 and 2.6 that there are significant differences in the cross reactivity of assays for Actrapid® or Levemir®. Users of insulin assays should be aware of their assay cross-reactivity or lack of cross-reactivity with synthetic insulin analogues that can be prescribed so that cases of insulin administration are not missed. This may require in-house cross-reactivity experiments to be performed as many kit inserts do not contain such information.

3. C-PEPTIDE

3.1 PARTICIPATION AND METHODS

Scheme participation has decreased to 61 due to laboratory consolidation. Automated platforms from Siemens dominate the scheme and are in use by 54% of the participants. The Dako assay is no longer used by any scheme participant.

Table 3.1 Methods in use, April 2012
(2010/2011 in parenthesis)

Method	Type	Code	No. of labs
Abbott Architect	Auto	AB13	2 (2)
Dako	Man	NV1	0 (2)
DiaSorin Liaison	Auto	SO4	2 (1)
Invitron	Man	IW1	1 (1)
Mercodia ELISA	Man	ME1	3 (2)
Perkin Elmer DELFIA	Man	PH2	1 (3)
Roche Elecsys	Auto	BO5	17 (15)
Siemens Centaur	Auto	CO10	3 (2)
Siemens Immulite	Auto	DC7	2 (2)
Siemens Immulite 2000 family	Auto	DC11	28 (33)
Siemens Immulite 2000	Auto		26 (29)
Siemens Immulite 2500	Auto		2 (4)
Unspecified		UUU	2 (2)
TOTAL			**61** (65)

3.2 SPECIMENS ISSUED

C-peptide analysis is performed on the same lyophilised specimens issued for insulin. All specimens were prepared from healthy volunteers.

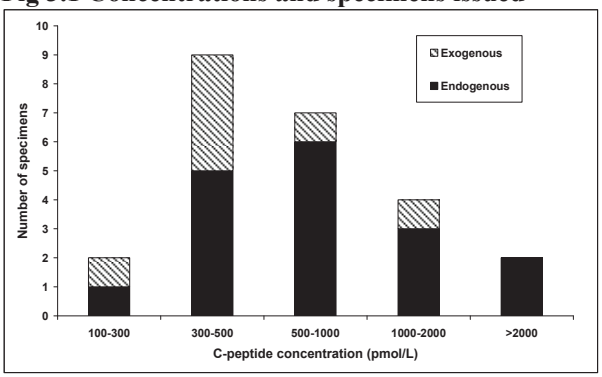
In order to assess the validity of the ALTM, two pools were distributed containing known amounts of the C-peptide international reference reagent IRR 84/510 as indicated in table 3.2. C-peptide IRR 84/510 was kindly supplied by Dr Chris Burns at NIBSC.

Table 3.2 describes the seventeen specimen pools used for distributions 259-266, their source and any modifications made.

Table 3.2 Serum specimens issued

Pool	Description	No. of distributions
F157	Non fasting pool	1
F158	Non fasting pool	1
N162	N161 + 15 pmol/L Insulin IRP 66/304	1
N183	Non fasting	2
N184	160 ml N183 + 160 ml N185	2
N185	Fasting	2
N186	Fasting	3
N187	N186 + 150 pmol/L Insulin IRP 66/304	1
N188	N186 + 300 pmol/L Insulin IRP 66/304	1
N189	N186 + 500 pmol/L C-peptide IRR 84/510	1
N190	N186 + 800 pmol/L C-peptide IRR 84/510	1
N191	Fasting	1
N192	Non fasting	1
N193	Fasting	2
N194	N196 + 100 pmol/L Actrapid	1
N195	N196 + 100 pmol/L Levemir	1
N196	Fasting	2

Fig 3.1 Concentrations and specimens issued



The C-peptide concentrations of the specimen pools covered most of the relevant clinical range. However the lowest C-peptide ALTM was 197 pmol/L. There is still a need to obtain specimen pools with C-peptide values below 100 pmol/L to assess assay sensitivity and to issue samples that would represent the suppressed levels that would be seen as an appropriate physiological response to hypoglycaemia.

3.3 VALIDITY OF THE ALTM

The long-term ALTM stability of ten specimen pools that have been issued on more than one occasion is shown in figure 3.2

Fig 3.2 Stability of the ALTM

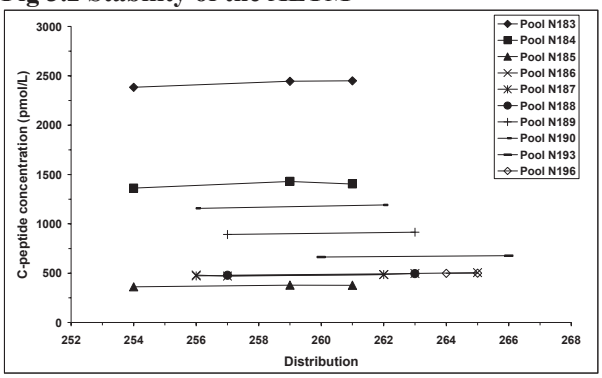


Figure 3.2 shows there is no obvious deterioration in the ALTM for the specimen pools issued. This is corroborated by the between-distribution CV of the ALTMs data for each specimen pool shown in table 3.3.

For most specimen pools, between-laboratory variability is less than that observed for insulin which is also measured on these samples.

Table 3.3 Between-distribution variability of ALTMs (includes data from distributions before 259)

Pool	No. of distributions	Mean ALTM (pmol/L)	CV (%)
N183	3	2425.8	1.5
N184	3	1398.6	2.4
N185	3	372.6	2.6
N186	5	488.5	2.8
N187	2	479.15	2.0
N188	2	487.35	2.4
N189	2	903.95	1.8
N190	2	1173.95	2.1
N193	2	670.4	1.4
N196	2	500.1	0.3

RECOVERY EXERCISES

To further validate the use of the ALTM as a target value, recovery experiments were performed as described in table 3.2. Table 3.4 shows the method related recovery data from the addition of C-peptide IRR 84/510. A similar exercise was performed last year and the data is shown in brackets for comparative purposes.

Table 3.4 Recovery (%) of added C-peptide, IRR 84/510 (November 2011 & January 2012 exercises). Mean recoveries are shown. Values obtained in last years exercise are shown in brackets.

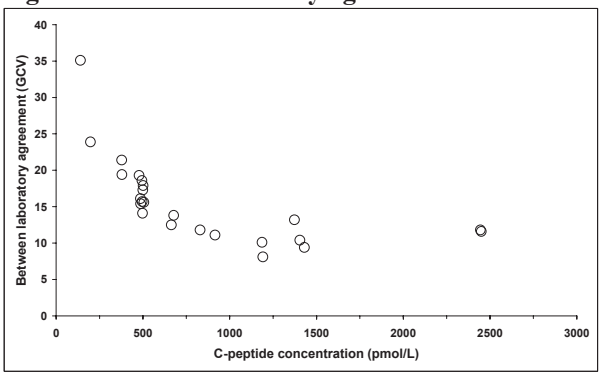
Method	+ 800 pmol/L IRR 84/510	+ 500 pmol/L IRR 84/510
All methods	88.0 (84.4)	83.6 (84.3)
Dako	103.8 (75.0)	82.9 (84.0)
DiaSorin Liaison	88.2 (62.5)	80.0
Invitron	92.0	61.4
Mercodia ELISA	94.8 (94.5)	89.6 (85.8)
Perkin Elmer DELFIA	96.9 (91.0)	89.9 (89.5)
Roche Elecsys	86.1 (83.0)	83.9 (79.7)
Siemens Centaur	72.7 (65.6)	71.4
Siemens Immulite	93.9 (85.3)	91.4 (86.1)
Siemens Immulite 2000 family	86.5 (85.0)	83.3 (85.9)

Similarly to previous exercises, the ALTM shows non quantitative recovery. The only assay showing quantitative recovery (± 10%) with both IRR 84/510 spiked samples is the Siemens Immulite.

3.4 BETWEEN-LABORATORY AGREEMENT

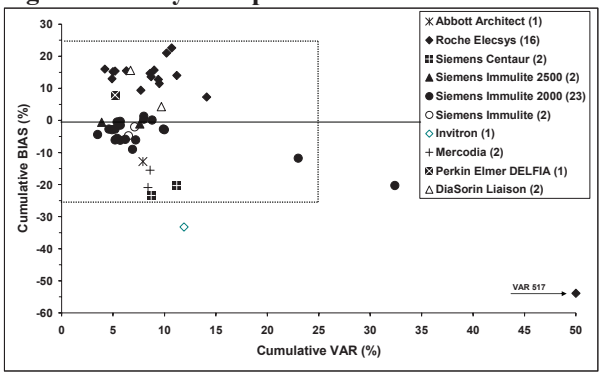
Between-laboratory agreement (figure 3.3) has a similar profile of assay performance to previous years. There is still a requirement for C-peptide assays to improve performance below 500 pmol/L.

Fig 3.3 Between-laboratory agreement



Current assay performance in terms of participant BIAS and VAR at distribution 266 is shown in figure 3.4. The NQAAP have set performance criteria for C-peptide at ± 25% for BIAS and 25% for VAR.

Fig 3.4 'Penalty box' plot of BIAS vs. VAR



As noted in previous years, the Roche Elecsys assay is positively biased compared to the Siemens Immulite platform assays. The Siemens Centaur and Invitron assays are negatively biased compared to other methods.

This year has not seen an improvement in assay performance in terms of VAR as there are five participants outside the 25% VAR limits. There has been a slight improvement in assay performance compared to last year for BIAS as only four participants are outside the BIAS limit.

4. INSULIN INTERPRETATIVE EXERCISE

Distribution 264 included an interpretative exercise where participants were invited to interpret their insulin results obtained for samples 793 and 794.

To date completed surveys have been received from 17 participants and 52% of respondents indicated that they do not routinely put interpretative comments on their insulin and C-peptide reports.

Sample 793

Participants were asked to provide interpretation on their results for sample 793 based on the clinical scenario:

'A 21-year-old woman admitted to A&E with hypoglycaemia. Measured glucose was 5.0 mmol/L.'

The insulin ALTM was 128 pmol/L (n=69) and survey respondents' insulin results ranged from 45 - 265 pmol/L. Table 2.5 shows the spread of results obtained for this specimen which had been spiked with 100 pmol/L Actrapid® (Novo Nordisk).

Table 4.1 includes all the interpretative comments returned by survey respondents. The comments and suggestions for further investigations made by participants that do not routinely provide interpretative comments are shown in italics in tables 4.1 and 4.2.

Table 4.1 Comments on sample 793 results

Suggest repeat when patient hypoglycaemic.
Unable to interpret insulin in the presence of normoglycaemia.
In the investigation of hypoglycaemia a sample for insulin + C-peptide must be taken simultaneously with a sample giving a laboratory glucose result of <2.5 mmol/L.
Patient not hypoglycaemic at time of sampling therefore difficult to interpret insulin. Suggest repeat insulin if further hypoglycaemic episode.
Inappropriate insulin if taken when hypoglycaemic - 5.0 mM not hypoglycaemia, was insulin taken post treatment ?
Patient is not hypoglycaemic for this sample, insulin & C-peptide are appropriate for this level of glucose.
Patient not hypoglycaemic at this time. Cannot interpret results.
Patient not hypoglycaemic at time of sampling, cannot interpret results as it appears sample has been taken after start of treatment. However high insulin compared to C-peptide please contact the laboratory to discuss these results.
Patient was not hypoglycaemic at time of sampling, therefore insulin result cannot be interpreted.
Hypoglycaemia not demonstrated, please repeat when hypoglycaemic. Note low IGF1 ?nutritional status/pituitary function
Results do not reflect biochemical hypoglycaemia. Results within reference range.
Insulin much higher than expected for corresponding C-peptide.
Glucose 5.0 mmol/L, not in keeping with hypoglycaemia. Insulin & C-peptide results/levels appropriate.
Insulin = 172.2 pmol/L. Normal fasting range 17.8 - 173 pmol/L; results show an insulin within this range with a normal glucose.
Insulin 24.65 mU/L (0-12) and C-peptide 1.7 ug/L (0.2-3.2). Glucose of 5.0 suggests patient has been treated for hypoglycaemia. Query timing of insulin and C-peptide sampling. Suggest repeat fasting glucose and insulin/c-peptide during hypoglycaemic episode. Check hydroxybutyrate levels.
Please repeat insulin measurement when patient is hypoglycaemic.
Record states that the patient was admitted with hypoglycaemia but the specimen submitted is euglycemic. For investigation of hypoglycemia, insulin and C-peptide analysis is only useful on the hypoglycemic specimen.

There is a consensus that the insulin result is difficult/impossible to interpret and that a sample collected during a hypoglycaemic episode is required.

Table 4.2 Further investigations for sample 793

Consider a 72 hour fast to replicate hypoglycaemia, measure insulin, C-peptide + proinsulin when hypoglycaemia is achieved.
Suggest send further specimen for insulin and C-peptide if hypoglycaemia recurs. Consider a 72 hour fast to try and provoke hypoglycaemia, measure insulin, C-peptide when hypoglycaemia is achieved.
Please repeat when patient is hypoglycaemic.
Has hypoglycaemia been confirmed by laboratory measurement of glucose. Query patient taken/prescribed insulin. Query renal function. Requires discussion with clinician.
C-peptide, oral hypoglycaemic agents.
Insulin when patient is hypoglycaemic.
If an earlier hypoglycemic specimen is available and is suitable for analysis, c-peptide and insulin could be performed. If there is a possibility of oral hypoglycemic agents as the cause, urinary screen for insulin secretagogues by LCMS could be arranged. 72 hour fast if insulinoma is being considered.

Sample 794

Participants were asked to provide interpretation on their results for sample 794 based on the clinical scenario:

'A 19 year old diabetic patient, ?any endogenous pancreatic function. Measured fasting glucose was 7.6 mmol/L.'

The insulin ALTM was 51 pmol/L (n=69) and survey respondents' insulin results ranged from 19 - 170 pmol/L. Table 2.6 shows the spread of results obtained for this specimen which had been spiked with 100 pmol/L Levemir® (Novo Nordisk).

Tables 4.3 and 4.4 describe the interpretative comments received and any suggested further investigations for sample 794. The comments and suggestions for further investigations made by participants that do not routinely provide interpretative comments are shown in italics in tables 4.3 and 4.4.

Table 4.3 Comments on sample 794 results

Consistent with endogenous pancreatic function, suggestive of type 2 diabetes.
Slightly raised insulin ?endogenous ?exogenous. Assuming type 1 DM, suggest fasting C-peptide ± response to a mixed meal test or glucagon.
C-peptide within reference range (fasting) of 400 - 800 pmol/L, inconsistent with type I IDDM.
Presence of C-peptide a degree of endogenous insulin production.
Yes because C-peptide present.
Insulin and C-peptide are measurable, indicating there is some residual pancreatic function, however raised glucose suggests it is inadequate.
Some pancreatic function left.
Insulin result consistent with either residual pancreatic function or exogenous insulin administration. Please contact the laboratory to discuss these results.
Please note insulin results may be misleading in patients receiving insulin therapy due to the presence of anti-insulin antibodies.
Relative insulin insufficiency for degree of glycaemia.
Insulin & C-peptide results confirm a small degree of endogenous pancreatic function. They are inappropriately low for her glucose level however.
No routine comment. Measureable C-peptide.
C-peptide result indicates still producing endogenous insulin.
Insulin = 51.31 pmol/L within normal fasting range; fasting glucose in the diabetic range; insulin concentration should be higher if there is significant endogenous production.
Insulin 9.27 mU/L (0-12) and C-peptide 1.7 ug/L (0.2-3.2). Fasting glucose increased. C-peptide indicates some residual action but looks suboptimal.
The insulin level is likely to reflect cross-reactivity with exogenously administered insulin rather than residual pancreatic insulin.
The patient does have some residual pancreatic function but has an 'inappropriately normal' insulin level in the face of hyperglycaemia. This is consistent with the earlier stages of type I DM.

Table 4.4 Further investigations for sample 794

C-peptide, response to mixed meal test or glucagon.
GTT + insulins
Autoantibodies - GAD, islet cell & insulin Abs
C-peptide to distinguish between residual pancreatic function and insulin therapy. Consider auto-antibody analysis e.g. GAD, insulin antibodies and islet cell antibodies if trying to distinguish between type I and II DM.
C-peptide
C-peptide
Measure C-peptide levels as a marker of residual pancreatic function.
TSH, am cortisol if not already performed.

In this exercise, many of the participants' used their C-peptide result to assist in their result interpretation. The interpretative comments range from suggesting that pancreatic function is suboptimal, to being within the reference range or to being consistent with type 2 diabetes.

The inclusion of 'diabetic' as a clinical detail should raise suspicion of the possible presence of synthetic insulin and/or insulin antibodies being present which can cause difficulties in result interpretation.

5. GASTRIN

5.1 PARTICIPATION AND METHODS

Table 5.1 indicates that there has been little change in participation or method distribution for this analyte over the previous year. The Siemens 2000 family analysers continue to dominate the scheme and are in use by 64% of the participants.

Table 5.1 Methods in use, April 2012
(2010/2011 in parenthesis)

Method	Type	Code	No. of Labs
CIS	Man	CI3	1 (2)
DiaSorin	Man	SO3	2 (2)
Eurodiagnostica		EUR	1 (1)
In-house RIA	Man	000	2 (2)
MP Biomedicals	Man	BD8	3 (2)
Siemens Immulite 2000 family	Auto	DC11	18 (21)
Siemens Immulite 2000			17 (20)
Siemens Immulite 2500			1 (1)
Unspecified		UUU	1 (0)
TOTAL			**28** (30)

5.2 SPECIMENS ISSUED

All issued specimens were prepared from serum obtained from healthy volunteers. Table 5.2 describes the specimen pools issued and any modifications made for distributions 259-266. By using blood donations from non-fasting individuals, gastrin levels greater than 200 mU/L were obtained without the need to add recombinant gastrin (see figure 5.1).

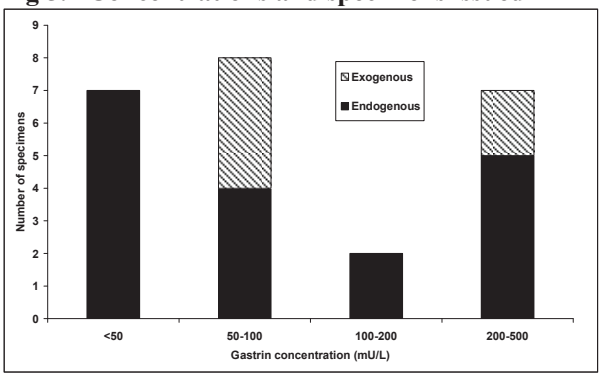
Fourteen different specimen pools have been issued during distributions 259-266. As indicated in figure 5.1 and table 5.2, most specimen pools were prepared without any modifications. The highest concentration of gastrin in an endogenous pool had an ALTM of 464 mU/L.

Since there is no human gastrin reference preparation, it is not possible to perform true recovery experiments to assess the validity of the ALTM. However to assess the closeness to expected values, sample pools were spiked with recombinant gastrin material obtained from Sigma as described in table 5.2.

Table 5.2 Serum specimens issued

Pool	Description	No. of distributions
G106	Fasting	1
G112	G111 + 125 mU/L gastrin I	1
G113	G111 + 250 mU/L gastrin I	1
G123	Fasting	2
G124	Non-fasting	1
G125	50:50 pool G123 + G124	1
G126	Fasting	1
G127	Non-fasting	3
G128	50:50 pool G126 + G127	2
G129	Non-fasting	3
G130	Fasting	2
G131	Fasting	2
G132	G131 + 13 mU/L gastrin I	2
G133	G131 + 25 mU/L gastrin I	2

Fig 5.1 Concentrations and specimens issued



Gastrin exists in 3 active forms G34, G17 and G14 which are also known as big, little and mini-gastrin respectively. Each of the gastrin forms have different potencies for stimulating gastric acid secretion. In the circulation G34 predominates but the most potent at stimulating gastric acid secretion is the heptadecapeptide, G17 (also known as gastrin I). Measurement of gastrin is used for the diagnosis of gastrinomas (Zollinger-Ellison syndrome) and recognition of the various gastrin forms is important for the diagnosis of gastrinomas.

5.3 VALIDITY OF THE ALTM

Figure 5.2 and table 5.3 indicate the stability of the ALTM of thirteen specimen pools issued on more than one occasion.

Pool G106 has been in use since March 2008 and other pools have been in use over 12 months without any apparent deterioration in the ALTM.

Fig 5.2 Stability of the ALTM

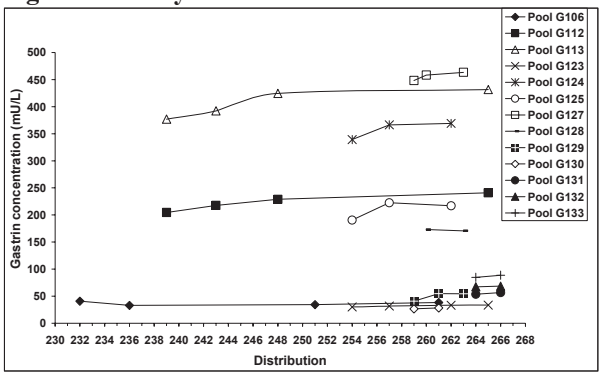


Table 5.3 Between-distribution variability of ALTMs (includes data from distributions before 259)

Pool	No. of distributions	Mean ALTM (mU/L)	CV (%)
G106	4	36.6	9.7
G112	4	223.1	7.0
G113	4	406.3	6.4
G123	4	32.1	5.0
G124	3	358.2	4.6
G125	3	210.0	8.1
G127	3	456.7	1.7
G128	2	172.0	0.9
G129	3	50.0	16.0
G130	2	27.4	3.9
G131	2	55.0	4.0
G132	2	67.9	1.4
G133	2	86.8	3.3

RECOVERY EXERCISES

True recovery exercises cannot be performed due to the lack of an international reference preparation. Since the G17 gastrin form is the most effective at simulating gastric acid secretion, a serum specimen pool was spiked with recombinant G17 (gastrin I) obtained from Sigma as shown in table 5.2. The 'spiked' material was distributed as samples in distributions 264 and 266, the 'recovery' data is presented in table 5.4 and 5.5 .

Table 5.4 Distribution 264 'Recovery' (%) of added gastrin I (G17). Mean recoveries of the method groups are shown if >1 participant.

Method	+13 mU/L	+25 mU/L
All methods (n = 26)	106.1	125.6
CIS (n = 1)	-	44.0
DiaSorin (n = 1)	30.8	44.0
Eurodiagnostica (n = 1)	76.9	56.0
In-house RIA (n = 1)	92.3	80.0
In-house RIA (n = 1)	69.2	80.0
MP Biomedicals (n = 3)	58.5	57.2
Siemens Immulite 2000 family (n = 18)	134.6	153.6

Table 5.5 Distribution 266 'Recovery' (%) of added gastrin I (G17). Mean recoveries of the method groups are shown if >1 participant.

Method	+13 mU/L	+25 mU/L
All methods (n = 23)	92.3	129.2
Eurodiagnostica (n = 1)	46.2	44.0
In-house RIA (n = 1)	53.8	60.0
MP Biomedicals (n = 3)	120.0	73.2
Siemens Immulite 2000 family (n = 17)	124.6	162.4

The ALTM 'recovery' of added 13 mU/L recombinant gastrin I (G17) is quantitative. However, the ALTM 'recovery' of 25 mU/L added recombinant gastrin I (G17) is non-quantitative as has been observed in previous years.

There are still significant method related differences. The Siemens Immulite 2000 family assays have been consistently shown to 'over-recover' added recombinant gastrin I (G17). In contrast, most of the other assays show 'under-recovery'. The observed method related variation in 'recovery' of gastrin I (G17) is due to differences in the gastrin material used for assay calibration and the use of antibodies of differing specificities for G17, G34 and G14 and other progastrin intermediates.

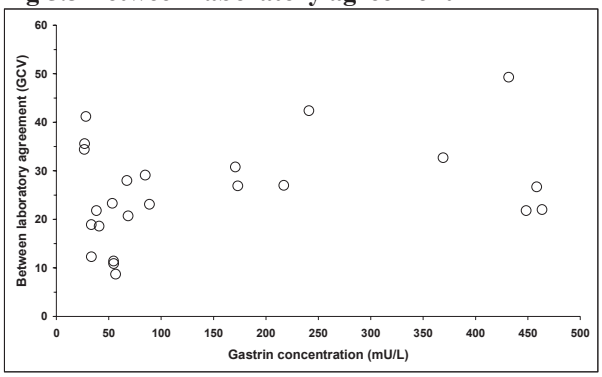
5.4 BETWEEN-LABORATORY AGREEMENT

Figure 5.3 shows a similar profile to previous years and indicates there is still poor between-laboratory consensus.

The apparent variability may be due to the distribution of fasting serum pools with lower

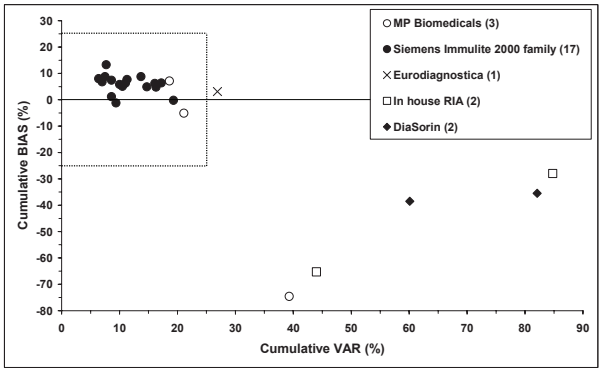
gastrin levels and the addition of synthetic G17 to specimen pools.

Fig 5.3 Between-laboratory agreement



The NQAAP for Chemical Pathology have not set any performance targets for gastrin analysis. Therefore the dotted lines shown at ± 25% BIAS and 25% VAR in figure 5.4 are arbitrary limits. Figure 5.4 is a snapshot of current gastrin assay performance in terms of cumulative BIAS and VAR at distribution 266.

Fig 5.4 'Penalty box' plot of BIAS vs. VAR



The DiaSorin and in-house RIA assays appear negatively biased compared to the Siemens Immulite 2000 platform assays.

6. IGF-I

6.1 PARTICIPATION AND METHODS

Although there are nine different assays in use by participants, the Siemens Immulite analyser platforms dominate the scheme being in use by 80% of participants.

Table 6.1 Methods in use, April 2012
(2010/2011 in parenthesis)

Method	Type	Code	No. of labs
Cisbio	Man	CI3	1 (1)
DiaSorin Liaison	Auto	SO3	4 (3)
IDS OCTEIA	Man	ID5	2 (2)
IDS IRMA	Man	ID10	1 (1)
IDS iSYS	Auto	ID9	6 (1)
In-house RIA	Man	OOO	1 (1)
Mediagnost RIA	Man	MD1	2 (1)
Siemens Immulite	Auto	DC7	13 (14)
Siemens Immulite 2000 family	Auto	DC11	68 (75)
Siemens Immulite 2000	Auto		57 (58)
Siemens Immulite 2500	Auto		11 (16)
Unspecified		UUU	3 (1)
TOTAL			**101** (100)

6.2 SPECIMENS ISSUED

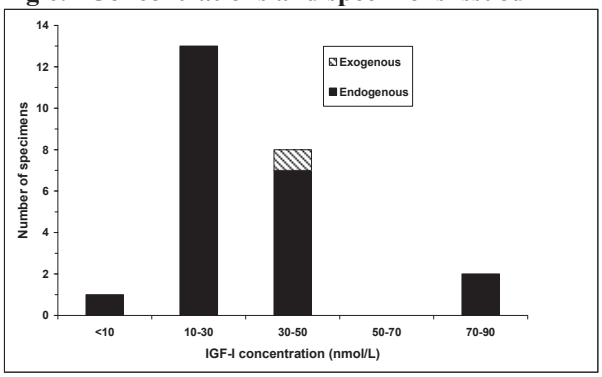
During distributions 259-266, eighteen different specimen pools were distributed including a recovery exercise. Recovery exercises were performed by the addition of IGF-I IS 02/254 to a base pool as described in table 6.2. IGF-I IS 02/254 was kindly supplied by Dr Chris Burns at NIBSC.

Table 6.2 describes the serum specimen pools issued and any modifications made for distributions 259–266.

Table 6.2 Serum specimens issued

Pool	Description	No. of distributions
F141	Random	1
F142	Random	1
F144	Random	1
F145	Random	1
F147	Random	1
F148	Random	1
F149	Random	1
F150	Random	1
F152	F150 + 26 nmol/L IS 02/254	1
F153	Random	1
F154	Random	2
F155	Random	3
F156	Random	3
F157	Random	1
F158	Random	1
F159	Random	2
F160	Random	1
N191	Fasting	1

Fig 6.1 Concentrations and specimens issued



It would be beneficial if further samples were obtained with lower IGF-I levels to assess assay performance when being used for the assessment of growth hormone deficiency. There is also a need to obtain acromegalic serum rather than using healthy volunteers to assess assay performance.

6.3 VALIDITY OF THE ALTM

The ALTM stability of thirteen specimen pools that have been distributed more than once is shown in figures 6.2 and table 6.3.

Fig 6.2 Stability of the ALTM

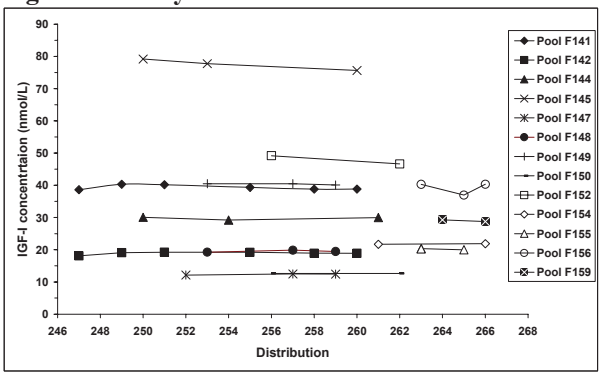


Table 6.3 Between-distribution variability of ALTMs (includes data from distributions before 259)

Pool	No. of distributions	Mean ALTM (nmol/L)	CV (%)
F141	6	39.4	1.9
F142	6	18.9	2.3
F144	3	29.8	1.6
F145	3	77.5	2.3
F147	3	12.4	1.7
F148	3	19.5	1.6
F149	3	40.3	0.6
F150	2	12.7	0.1
F152	2	47.9	3.8
F154	2	21.8	0.6
F155	3	20.2	0.9
F156	3	39.2	4.9
F159	2	29.0	1.3

Despite some specimen pools being in use for over a year the ALTM appears stable.

RECOVERY EXERCISES

Due to the lack of a reference analytical method, ALTM validation necessitates the use of ALTM stability studies and recovery exercises with appropriate reference preparations.

All commercial IGF-I assays in the scheme with the exception of the IDS iSYS assay are calibrated against IRR 87/518. Physiochemical analysis has shown IRR 87/518 to be 44% pure and predominantly methionine-IGF-I. Since stocks of IRR 87/518 are exhausted, a new recombinant IGF-I IS 02/254 has been established. The recently launched IDS iSYS IGF-I assay is calibrated against this new international standard.

Table 6.4 shows the mean method related recoveries from the addition of 26 nmol/L IGF-I IS 02/254 to a base pool.

Table 6.4 Recovery (%) of added IGF-I, IS 02/254. Mean recoveries are shown if >1 participant. Values obtained in last years exercise are shown in brackets.

METHOD	+ 26 nmol/L IS 02/254
All methods (n = 100)	131 (141)
Siemens Immulite (n = 14)	129 (137)
Siemens Immulite 2000 family (n = 71)	134 (145)
Siemens Immulite 2000 (n = 54)	135 (145)
Siemens Immulite 2500 (n = 15)	129 (145)
IDS iSYS (n = 4)	72 (71)
IDS OCTEIA (n = 2)	84 (83)
IDS IRMA (n = 1)	103
Cisbio (n = 1)	105 (98)
Mediagnost (n = 2)	141 (132)
In-house RIA (n = 1)	23 (94)
DiaSorin Liaison (n = 3)	77 (76)

The ALTM shows non-quantitative recovery of added IGF-I IS 02/254 which is expected as most of the assays are standardised against IRR 87/518.

6.4 BETWEEN-LABORATORY AGREEMENT

Figure 6.3 shows the ALTM GCV is below 14.5% for all of the specimens issued in distributions 259-266. Between-laboratory agreement is similar to that observed last year.

Fig 6.3 Between-laboratory agreement

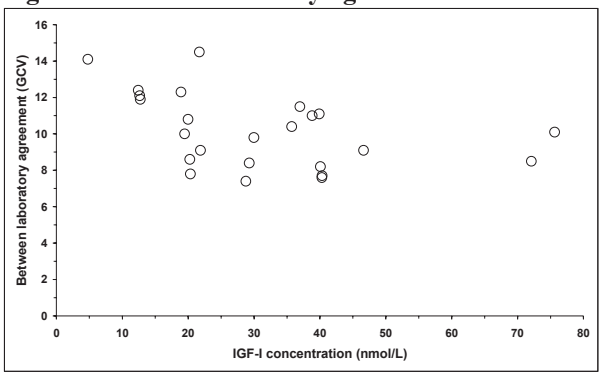
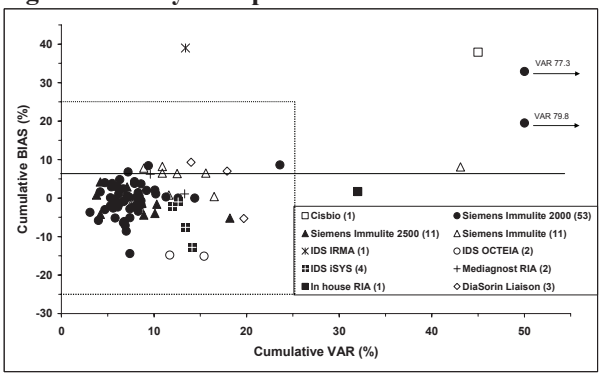


Figure 6.4 shows the status of assay performance in terms of cumulative BIAS and VAR scores of methods in use at distribution 266. Performance targets for BIAS and VAR have been set at 25% by the NQAAP for Chemical Pathology.

Fig 6.4 'Penalty box' plot of BIAS vs. VAR



In terms of VAR there are five participants outside the 25% VAR limit which is similar to last year. There are three participants outside the ± 25% BIAS criteria. This is a slight deterioration compared to last year when only two participants were outside the ± 25% BIAS limits.

As noted in previous Annual Reviews, the Siemens Immulite, IDS IRMA, DiaSorin Liaison, Mediagnost and Cisbio assays are positively biased compared to the Siemens Immulite 2000 family assay that dominates the scheme. In contrast the IDS OCTEIA and iSYS assays are negatively biased compared to the Siemens Immulite 2000 assay. Differences in assay bias has implications for ensuring IGF-I reference ranges are appropriate for the assay bias.

7. IGFBP-3

7.1 PARTICIPATION AND METHODS

Participation has marginally increased this year to 35 participants. Siemens Immulite platforms continue to dominate and are in use by 77% of participants. There is one new method in use, the IDS iSYS assay.

Table 7.1 Methods in use, April 2012
(2010/2011 in parenthesis)

Method	Type	Code	No. of labs
DiaSource RIA	Man	DE1	1 (1)
DSL IRMA	Man	DS5	1 (1)
IDS IRMA	Man	ID10	1 (1)
IDS iSYS	Auto	ID9	2 (0)
Siemens Immulite	Auto	DC7	4 (3)
Siemens Immulite 2000 family	Auto	DC11	23 (23)
Siemens Immulite 2000	Auto		19 (18)
Siemens Immulite 2500	Auto		4 (5)
Unspecified	-	UUU	3 (1)
TOTAL			**35** (30)

7.2 SPECIMENS ISSUED

IGFBP-3 analysis is performed on the same lyophilised specimens issued for IGF-I.

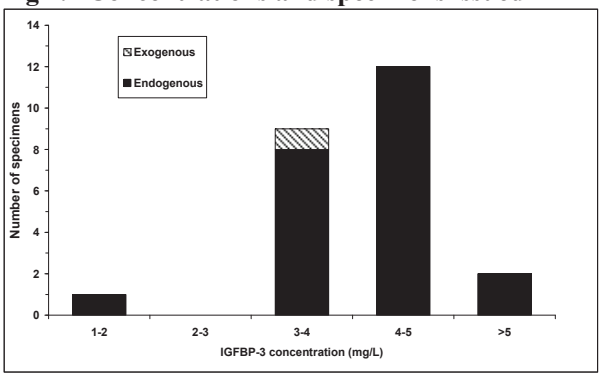
Table 7.2 describes the serum specimen pools issued and any modifications made for distributions 259–266.

Currently there is no international reference preparation available for IGFBP-3. Therefore there were no recovery exercises undertaken for IGFBP-3. It was not possible to use NIBSC reagent 93/560 which contains non-glycosylated recombinant IGFBP-3. Each ampoule of 93/560 contains 3.5 µg IGFBP-3. This concentration of IGFBP-3 is too low to be used in recovery experiments as endogenous IGFBP-3 levels are in the mg/L range. Although one of the specimen pools contained IGF-I IS 02/254, addition of the IGF-I international standard did not affect the measurement of IGFBP-3.

Table 7.2 Serum specimens issued

Pool	Description	No. of distributions
F141	Random	1
F142	Random	1
F144	Random	1
F145	Random	1
F147	Random	1
F148	Random	1
F149	Random	1
F150	Random	1
F152	F150 + 26 nmol/L IS 02/254	1
F153	Random	1
F154	Random	2
F155	Random	3
F156	Random	3
F157	Random	1
F158	Random	1
F159	Random	2
F160	Random	1
N191	Fasting	1

Fig 7.1 Concentrations and specimens issued



The specimens issued in distributions 259-266 covered the concentration range 1.9 – 5.5 mg/L. There is a lack of samples with low IGFBP-3 concentrations and the scheme would welcome blood donations from growth hormone deficient individuals.

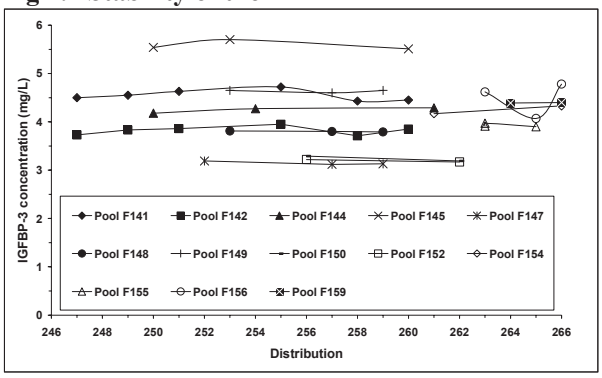
7.3 VALIDITY OF THE ALTM

The stability of the IGFBP-3 ALTM is shown in figure 7.2 and in table 7.3. Since there is no reference analytical method or an international reference preparation for IGFBP-3, validation of the ALTM has to be performed by monitoring the long-term stability of the ALTM.

Table 7.3 Between-distribution variability of ALTMs (includes data from distributions before 259)

Pool	No. of distributions	Mean ALTM (nmol/L)	CV (%)
F141	6	4.5	2.5
F142	6	3.8	2.3
F144	3	4.2	1.4
F145	3	5.6	1.8
F147	3	3.1	1.2
F148	3	3.8	0.3
F149	3	4.6	0.6
F150	2	3.2	2.2
F152	2	3.2	1.1
F154	2	4.3	2.7
F155	3	3.9	1.0
F156	3	4.5	8.3
F159	2	4.4	0.2

Fig 7.2 Stability of the ALTM

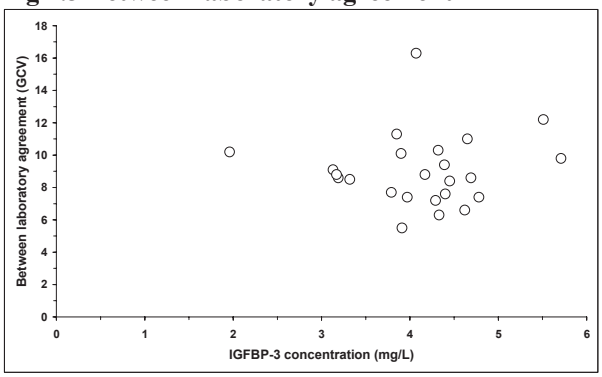


The ALTM of the twelve specimen pools that have been issued on more than one occasion appears to be stable with the exception of pool F156.

7.4 BETWEEN-LABORATORY AGREEMENT

Between-laboratory agreement is similar to last year.

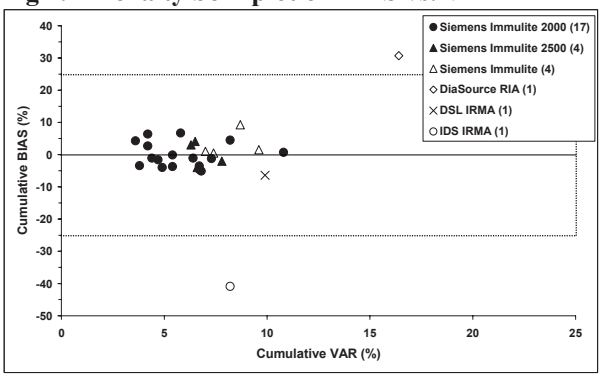
Fig 7.3 Between-laboratory agreement



Acceptable performance limits of ±25% for BIAS and 25% VAR have been set by the NQAAP.

Figure 7.4 shows a snapshot of assay performance in terms of cumulative BIAS and VAR at distribution 266.

Fig 7.4 'Penalty box' plot of BIAS vs. VAR



There are two participants outside the ± 25% BIAS performance criteria.

Currently, there is insufficient data to assess the assay performance of the new IDS iSYS assay.

The DiaSource RIA assay is positively biased compared to the Siemens platform assays. In contrast, the IDS IRMA assay is negatively biased to the other IGFBP-3 assays.

Such differences in assay bias should be reflected in the assay reference ranges used by participants.