Variable characteristics with insulin assays

Abstract
Factitious hypoglycaemia is a challenging diagnosis to confirm. Evaluation of surreptitious human insulin use can be distinguished from endogenous insulin excess by existing biochemical assays. However, the increasing use of insulin analogues poses a challenge because commercially available insulin assays detect these with varying accuracy and precision.

Insulin analogues are increasingly used in diabetes management and the case outlined here highlights the variations in assay. Initially, the local assay (ELISA kit – Dako, Copenhagen) failed to detect a significant concentration of insulin (<6pmol/L; range 9.6–65.4pmol/L) which an external reference laboratory subsequently detected using the Merodia Iso-insulin two-site immunoassay (Uppsala, Sweden).

The key analytical point is the recognition that different immunoassays detect insulin analogues to varying degrees. Clinical teams need to consider this if such cases are to be recognised. Following recent media reports where surreptitious insulin administration may be implicated in inpatient mortality, this knowledge is crucial to empower us to accurately diagnose all cases of unexplained hypoglycaemia. Copyright © 2013 John Wiley & Sons. Practical Diabetes 2013; 30(3): 118–120

Introduction
Hypoglycaemia is a common complication of diabetes mellitus treated with insulin or oral hypoglycaemic agents. Less frequently, it can occur in otherwise healthy individuals, secondary to hyperinsulinaemia, drugs1 (such as beta blockers, salicylates), toxins (Ackee fruit, Oriental hornet), alcohol, infection or starvation. The circumstances of the hypoglycaemic episode provide many of the clues to diagnosis. Factitious hypoglycaemia, caused by surreptitious use of insulin, is a challenging diagnosis to confirm. Consideration of this as a diagnostic possibility has the potential to impact significantly on the doctor–patient relationship, with the physician feeling deceived and the patient feeling mistrusted.

Biochemical evaluation of suspected surreptitious human insulin use is relatively straightforward – it can be distinguished from endogenous insulin excess by existing assays for insulin, C-peptide, and proinsulin. However, the increasing use of insulin analogues in lieu of human insulin has made evaluation of suspected cases of factitious hypoglycaemia more difficult to diagnose because commercially available insulin assays detect synthetic insulins with varying sensitivity and specificity. We describe a case where the diagnosis of factitious hypoglycaemia was suspected on initial tests but categorically confirmed when an alternative insulin assay was used.

Factitious hypoglycaemia
A patient presented with episodes of sweating and blurred vision. Initial investigations demonstrated no reproducible symptoms during a 72-hour fast, a negative sulphonylurea screen and unremarkable CT abdomen. Repeat clinical assessment revealed one symptomatic episode of hypoglycaemia during a prolonged fast, with a corresponding laboratory glucose of 1.0mmol/L, and undetectable insulin and low C-peptide levels. Further investigations were unreproducible. Subsequently, a supplementary report from an external reference laboratory recorded insulin levels on the same sample of 400pmol/L, with C-peptide of <94pmol/L – consistent with exogenous insulin administration.

Discussion
Insulin is a peptide hormone containing 51 amino acids. There are many compounds of a similar structure present in the circulation including proinsulin, insulin-like growth factor (IGF)-1, IGF-II, and proinsulin-related peptides. In recent years there has been a move away from...
animal insulin preparations towards recombinant insulin analogues in clinical diabetes management. These analogues display structures different from insulin which alters the rate and duration of action of glucose metabolism. Clinical laboratories use immunoassays to detect insulin and these are reliant on identifying this peptide by epitope–antibody interactions. The presence of structurally similar compounds has led to the development of highly specific assays which allow reproducible measurements of endogenous and exogenous insulin between laboratories. The variation in specificity for insulin and proinsulin, and their related peptides, represents a source of interassay variability.

Many clinical laboratories, use automated analysers for the detection and quantification of immunoreactive insulin. Table 1 illustrates the most commonly used insulin assays in the United Kingdom (from participants in the UK National External Quality Assessment Services: Guildford Peptide Hormones external quality assessment programme).

### Table 1

<table>
<thead>
<tr>
<th>Manufacturer/ method</th>
<th>Type of assay</th>
<th>Sensitivity values and manufacturer’s definition</th>
<th>Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Access</td>
<td>One-step chemiluminescent immunoenzymatic sandwich assay</td>
<td>Lowest measurable analyte concentration distinguishable from zero standard with 95% confidence 0.03mIU/L 0.21pmol/L</td>
<td>&gt;100% with Actrapid, Humulin S, NovoRapid, Novomix 30, Humalog Mix 25, Humalog Mix 50 83% with Humulin M3 40% with Detemir 100% 110% with Glargine 138% with Humalog 30% with Bovine insulin 97% with Porcine insulin</td>
</tr>
<tr>
<td>Roche Elecsys</td>
<td>Electrochemiluminescence sandwich immunoassay</td>
<td>Lowest measurable analyte concentration 2 standard deviations above zero standard 0.20mIU/L 1.39pmol/L</td>
<td>&lt;0.7% with NovoRapid, Glargine and Humalog 25% with Bovine insulin 19% with Porcine insulin</td>
</tr>
<tr>
<td>Siemens Immulite 2000</td>
<td>Enzyme labelled sandwich chemiluminescent immunometric assay</td>
<td>Calculation of lowest measurable analyte concentration not stated in package insert 2mIU/L 14.43pmol/L</td>
<td>9–28%* (mean) with NovoRapid and Humalog 4–9%* (mean) with Glargine</td>
</tr>
<tr>
<td>Siemens Centaur</td>
<td>Two-site direct chemiluminescent sandwich immunoassay</td>
<td>Lowest measurable concentration level 2 standard deviations above the mean value of 20 replicates of the zero standard 0.5mIU/L</td>
<td>&gt;100% with NovoRapid and Glargine 89% with Humalog</td>
</tr>
<tr>
<td>Abbott Architect</td>
<td>One-step sandwich chemiluminescent immunoassay</td>
<td>Lowest measurable analyte concentration at 2 standard deviations above the mean value for the zero calibrator 1mIU/L 7.18pmol/L</td>
<td>76% with NovoRapid 94% (mean) with Glargine &gt;100% with Humalog</td>
</tr>
<tr>
<td>Perkin Elmer AutoDELFIA</td>
<td>Two-site fluoroimmunometric sandwich assay</td>
<td>Lowest measurable analyte concentration at 2 standard deviations above the mean value of 20 replicates of the zero standard 0.5mIU/L 3.0pmol/L</td>
<td>0% with Actrapid</td>
</tr>
<tr>
<td>Mercodia Iso-insulin</td>
<td>Two-site enzyme immunoassay, read spectrophotometrically</td>
<td>Lowest measurable analyte concentration at 2 standard deviations above the zero calibrator 1mIU/L</td>
<td>89% with Humalog 80% with NovoRapid 22% with Detemir 44% with Glargine 306% with Porcine insulin 58% with Bovine insulin</td>
</tr>
</tbody>
</table>

All assays (aside from the Abbott Architect which was not stated on pack insert) are stated to be traceable to the WHO reference preparation 66/304. *Spread of values due to between kit lot number variation.

### Practice point

Variable characteristics with insulin assays

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**Table 1.** The common insulin assays currently used in laboratories across the UK and their characteristics.
assurance scheme); alongside is listed the cross reactivity with various types of insulin which have been published in the literature.\(^2\)\(^-\)\(^8\)

Although the majority of commercial insulin assays are currently standardised to the WHO human insulin reference material (66/304), variability of cross reactivity of the assays with different types of insulin analogues remains.

In our case, the local enzyme linked immunoassay (ELISA) kit (Dako, Copenhagen, Denmark) failed to detect a significant concentration of immunoreactive insulin (<6pmol/L; reference range 9.6–65.4pmol/L) in this patient. The C-peptide ELISA assay (DakoCytomation, Ely, Cambridgeshire) detected a very low concentration of 0.10nmol/L (reference range 0.11–0.61nmol/L), with the manufacturer stating minimal interference from human insulin but potential cross reactivity with ‘synthetic human proinsulin’ (84%) and ‘split proinsulin’ (89%).\(^3\) This sample was subsequently analysed at the Guildford laboratory using the Mercodia Iso-insulin (Uppsala, Sweden) two-site immunoassay which detected the presence of insulin (400pmol/L). No reference range is quoted as insulin concentration depends on glucose level at the time of sampling, but levels above 30pmol/L would be highlighted as ‘inappropriately high’ if the patient was hypoglycaemic at the time of sampling. The referral laboratory also performed a C-peptide test on this sample using a kit manufactured by Merckodia; the value was below their detection limit of 0.094nmol/L. Again, the manufacturers state there is minimal interference from insulin and proinsulin (<0.006% and <1.8% respectively) but variable cross reactivity with the des and split forms of proinsulin.\(^10\)

In this clinical instance where measurement of both endogenous and exogenous insulin is important, the specificity of the Dako assay meant that the administered insulin analogue was not detected. Both assays use a sandwich technique with an antibody bound to the well and an antibody linked to a marker which is measured to quantify the insulin concentration. The Dako assay contains antibodies against epitopes on the C-terminal end of the B chain of insulin and the A-loop.\(^11\) No specific details are given for the epitope recognition sites of the Mercodia Iso-insulin assay; however, these are likely to be different, explaining the variation in cross reactivities documented by the manufacturers. The Mercodia Iso-insulin assay is stated to have ≥80% cross reactivity with insulin analogues lispro (Humalog) and aspart (NovoRapid) by the manufacturers and this detection has also been shown by independent study.\(^8\)\(^,\)\(^12\) With this knowledge, the sample was sent to a reference laboratory which used the Mercodia Iso-insulin assay, specifically to look for the presence of an insulin analogue in the patient’s serum.

Insulin analogues are now increasingly used in the management of patients with diabetes. Their widespread use poses a challenge for the recognition of intentional and/or surreptitious exogenous insulin administration in patients with unexplained hypoglycaemia. Commercial immunoassays vary in their ability to detect available insulin preparations, and clinical teams should be aware of each performance characteristic of the assay used to allow them to draw appropriate conclusions from the results.\(^13\)

Guidelines have been published on the investigation of adult hypoglycaemia and, although they incorporate a pathway of investigation which includes self-administration of insulin, there is no specific reference to the variability of current assays with regard to detection of insulin analogues.\(^1\)

### Conclusion

The key analytical point is the recognition that different immunoassays may detect insulin analogues to varying degrees. This may not be entirely clear from the accompanying information from the manufacturer. Awareness of assay performance and characteristics is an important factor for clinical users to allow accurate detection of cases of inappropriate insulin analogue administration.

Initial testing in our laboratory meant we could only infer that administration of exogenous insulin would have to be an insulin analogue. In the light of recent media reports where surreptitious insulin administration may be implicated in inpatient mortality, it is important in the investigation of all suspected cases of inappropriate hypoglycaemia to know the performance capabilities of your insulin assay and seek confirmation by using an alternative insulin assay. This also illustrates the importance of communication between biochemists and clinicians, and cooperation between laboratories.

### Declaration of interests

There are no conflicts of interest declared.

### References