The Royal College of Pathologists
Pathology: the science behind the cure

PART 2 PRACTICAL EXAMINATION IN CLINICAL BIOCHEMISTRY

Module 1 Paper 2

Spring 2024

This examination is divided into 3 parts (Parts A, B and C) and is of three hours' duration. Candidates must answer **all** questions. You are provided with all questions and it is up to the candidate how long they spend on each section.

A global mark will be awarded for the entire practical according to overall performance.

You must write your candidate number clearly on the front page and at the top of every page within the answer books and on any loose sheets of paper (e.g. graph paper), which you use.

Candidates are reminded that examiners attach great importance to the clear, legible and orderly presentation of answers.

Pay particular attention to the questions asked, you will not get marks for information that is not requested.

Tables and graphs must be presented where specifically stipulated in the question and may be used elsewhere if appropriate. They must be submitted in the answer book for the appropriate part.

Calculators but not computers or tablets are allowed. Textbooks are not allowed.

You must **not** remove the question paper from the examination hall, nor copy any question and remove it.

Your laboratory currently sends samples for plasma insulin and C-peptide to a referral laboratory, and you are now planning to bring these investigations in-house. You therefore embark on verification of the two methods. This exam paper addresses the insulin assay only. The details of the insulin method, and the manufacturer's claimed performance, are given in **Appendix 1.**

Section A: Experimental Design - Interference from triglycerides

Assume that the following materials are available, and that for these purposes supply is not limited:

All materials required to perform the insulin assay, including appropriate diluent for sample dilution.

Low internal quality control material (mean insulin concentration 60 pmol/L)

Medium internal quality control material (mean insulin concentration 275 pmol/L)

High internal quality control material (mean insulin concentration 865 pmol/L)

Human plasma pool 1 (mean insulin concentration 63 pmol/L)

Human plasma pool 2 (mean insulin concentration 1080 pmol/L)

Commercially produced lipid preparation (triglyceride concentration 226 mmol/L)

1.1 Design an experiment to verify the manufacturer's claim that there is less than 10 % interference from triglycerides up to a plasma triglyceride concentration of 34 mmol/L. You should provide sufficient experimental detail to allow a qualified Biomedical Scientist to follow the instructions and carry out the experiment.

Section B: Data Analysis – Imprecision and Method Comparison

Your Biomedical Scientist colleagues have carried out an extensive series of verification experiments to verify the manufacturer's claims and the suitability of the insulin assay for clinical use.

2.1 – Imprecision

Your laboratory has specified the following acceptance criteria for within-laboratory (total) imprecision for insulin, across the concentration range of 50 to 1170 pmol/L.

Minimum CV (%)	Desirable CV (%)	Optimum CV (%)
10.95	7.30	3.65

Two levels of internal quality control material (Level 1 and Level 3) were each analysed 20 times in one single batch on one day. The results are presented in **Appendix 2.**

Two levels of internal quality control material (Level 1 and Level 3) were each analysed 5 times in a batch on each of 5 consecutive days. The results are presented in **Appendix 3.**

Use the data in **Appendix 2** and **Appendix 3** to assess within-run and within-laboratory (total) imprecision. How do the results compare to the manufacturer's stated imprecision? Is assay performance acceptable?

2.2 – Method Comparison

You have been able to obtain some specimens from an external quality assessment (EQA) scheme and have analysed them by your method with a view to comparing your results with the EQA target values (All Laboratory Trimmed Mean, ALTM). Results are shown in **Appendix 4.**

i. Using the data in Appendix 4, use appropriate graphical technique(s) to assess how your method compares with the EQA target values. (Graph paper is provided but if you require more, ask and it will be provided.)
ii. What conclusions can you draw from this method comparison? What, if any, further method comparison work would you ideally like to do?

Section 3: Further Work

You are hoping to go live with local analysis of insulin and C-peptide within the next 3 months if possible.

What further work would you like to carry out before going live with local analysis of insulin and C-peptide? Include any further verification work you would like to carry out, and also any other steps that should be taken before going live. **NB There is no need to give detailed experimental plans in this section: an overview of the different areas to be addressed is all that is required.**

Appendix 1: Insulin method details and manufacturer's reported analytical performance

Sample requirements: Serum or plasma. 150 µL minimum sample volume. Haemolysed samples unsuitable (due to the potential for enzymatic degradation of insulin, rather than direct interference from haemoglobin).

Assay principle: One-step chemiluminescent microparticle immunoassay.

Imprecision:

Insulin mean concentration (pmol/L)	Within run CV (%)	Within lab total CV (%)
59.0	1.8	1.8
276.1	1.4	1.5
864.3	1.4	1.6

Imprecision was determined by the manufacturer using internal quality control material assayed in 5 replicates on 20 different days.

Within lab total CV includes within run and between day variability.

Lower limits of measurement:

Limit of Blank (LoB): 0.7 pmol/L (95th percentile from 60 replicates of zero-analyte samples)

Limit of Detection (LoD): 2.9 pmol/L (the lowest concentration at which the analyte can be detected with 95 % probability based on 60 replicates of low-analyte level samples)

Limit of Quantitation (LoQ): 11.5 pmol/L (the lowest concentration at which a total allowable error of 36.7 % was met)

Linearity:

Linear across the measuring interval of 11.5 to 2152.5 pmol/L.

Interference:

Less than 10 % interference at the following concentrations of interfering substances:

Bilirubin: 342 µmol/L

Haemoglobin: 5 g/L

Triglycerides: 34 mmol/L

Appendix 2: Within run imprecision

Two levels of internal quality control material (Level 1 and Level 3) were each analysed 20 times in one single batch on one day.

	Control Range
Level 1	40.2 - 74.6 pmol/L
Level 3	603 - 1119pmol/L

Date of Analysis	Level 1 (pmol/L)	Level 3 (pmol/L)
Day 1	56.1	838
Day 1	57.1	856
Day 1	57.2	851
Day 1	57.9	841
Day 1	58.3	851
Day 1	58.2	880
Day 1	58.6	857
Day 1	57.7	866
Day 1	58.4	862
Day 1	58.4	864
Day 1	59.6	849
Day 1	57.2	846
Day 1	57.6	844
Day 1	57.9	855
Day 1	58.3	858
Day 1	56.6	854
Day 1	57.8	840
Day 1	57.5	849
Day 1	57.3	867
Day 1	58.3	866

Appendix 3: Within lab total imprecision

Two levels of internal quality control material (Level 1 and Level 3) were each analysed 5 times in a batch on each of 5 consecutive days.

Date of analysis	Level 1 (pmol/L)	Level 3 (pmol/L)
Day 1	59.6 pmol/L	837.9 pmol/L
Day 1	57.2 pmol/L	855.6 pmol/L
Day 1	57.6 pmol/L	866.7 pmol/L
Day 1	57.9 pmol/L	851.4 pmol/L
Day 1	57.3 pmol/L	841.4 pmol/L
Day 2	60.0 pmol/L	883.7 pmol/L
Day 2	58.6 pmol/L	872.6 pmol/L
Day 2	58.5 pmol/L	884.8 pmol/L
Day 2	59.9 pmol/L	859.4 pmol/L
Day 2	57.9 pmol/L	848.2 pmol/L
Day 3	59.3 pmol/L	842.6 pmol/L
Day 3	60.0 pmol/L	858.5 pmol/L
Day 3	55.3 pmol/L	886.9 pmol/L
Day 3	57.1 pmol/L	869.3 pmol/L
Day 3	59.4 pmol/L	856.5 pmol/L
Day 4	59.3 pmol/L	882.8 pmol/L
Day 4	59.7 pmol/L	840.6 pmol/L
Day 4	58.5 pmol/L	846.7 pmol/L
Day 4	59.6 pmol/L	849.8 pmol/L
Day 4	57.5 pmol/L	860.8 pmol/L
Day 5	56.8 pmol/L	893.9 pmol/L
Day 5	58.3 pmol/L	877.2 pmol/L
Day 5	57.6 pmol/L	882.7 pmol/L
Day 5	54.4 pmol/L	863.5 pmol/L
Day 5	54.8 pmol/L	887.5 pmol/L

Appendix 4: Method Comparison

Specimens from an external quality assessment (EQA) scheme were analysed by your laboratory's insulin method with a view to comparing results with the EQA target values (All Laboratory Trimmed Mean, ALTM).

Specimen	EQA target value (pmol/L)	Analyser result (pmol/L)
1	10.0	11.5
2	15.0	11.5
3	23.7	19.0
4	29.9	22.1
5	35.6	31.1
6	43.0	31.3
7	64.1	51.6
8	71.2	66.7
9	72.0	62.6
10	92.0	80.0
11	121.0	106.5
12	128.2	103.0
13	138.0	120.7
14	182.0	165.1
15	215.7	185.9
16	275.6	247.2
17	363.8	327.9