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

FRCPath Immunology Part 2 practical examination

Station 4 – Data Analysis

Two example questions are given, including the images that would have been provided on a laptop as Supplementary Material, followed by the answers.

Note 1: Where data is provided that candidates may want to annotate to help calculate their answers, as in question 2 below, the data may be provided on paper to assist with this.

Note 2: Candidates are usually expected to do calculations and so should bring a basic calculator to the exam.

Example question 1

Your lab has been alerted by the manufacturer of your rheumatoid factor assay that they will be ceasing manufacture of this assay as a consequence of the new In Vitro Diagnostics Regulations (IVDR). Your lab has an alternative platform that can run this assay. Figure 1 (A,B) shows verification and comparison data for this assay.

Your laboratory's acceptance criteria for assay verification are:
Intra-assay precision <5%
Inter-assay precision <10%

Figure 1A: Precision data
Table 1: Manufacturer's data for precision

	Mean (IU/ml)	Intra-assay precision	Inter-assay precision
Control 1	27.9	2.7	5.4
Control 2	69.3	2.2	5.2
Control 3	582.7	5.1	4.8

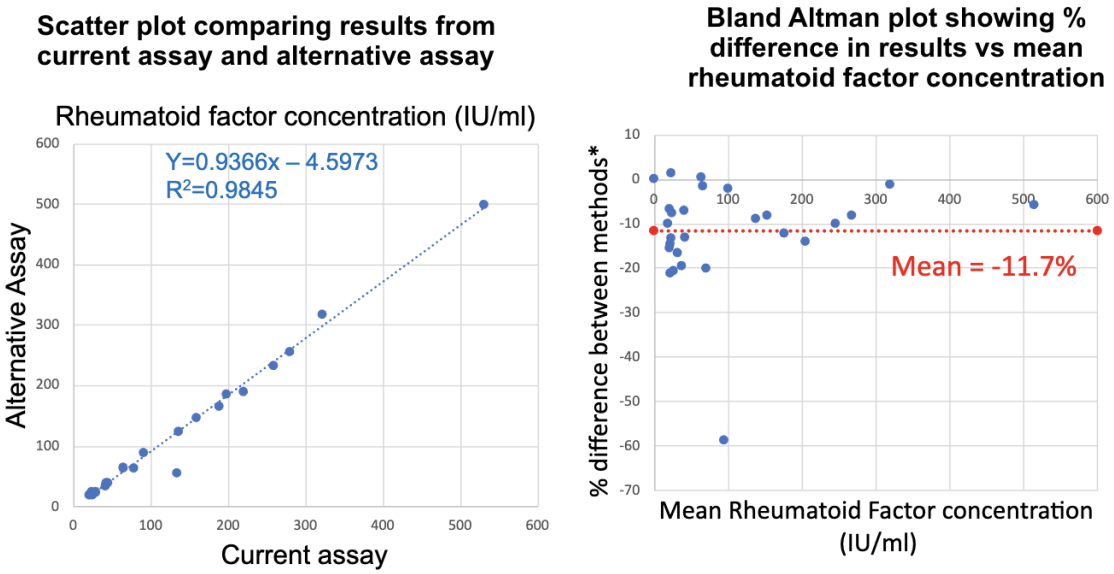
Table 2: Laboratory's verification data for intra-assay precision using the assay QCs

	Low QC	Mid QC	High QC
Mean (n=10)	18.15	26.13	33.57
sd	0.34	0.70	0.49
%CV	1.88	2.68	1.46

Table 3: Laboratory's verification data for inter-assay precision using the assay QCs

	Low QC	Mid QC	High QC
Mean (n=10)	17.33	24.21	31.63
sd	0.90	1.23	1.47
%CV	5.19	5.08	4.72

Figure 1B: Comparison data



*%difference between methods =
(alternative assay result – current assay result)/current assay result x100
Blue dotted line is line of best fit.
Red dotted line is mean % difference

(a) Based on the data shown in Figure 1A, would you consider the precision data for this assay acceptable and why (3 marks)

(b) What do you understand by the term verification? (1 mark)

(c) Based on the data shown in Figure 1B, comment on the findings of this comparison study (4 marks)

(d) Reflecting on the information in Figures 1A and 1B, and using your knowledge of this analyte, comment on the QCs used in this assay (3 marks)

(e) Give one impact of the IVDR on UK labs (1 mark)

Example question 2

Your laboratory would like to reduce the use of radioactivity in the department. You are comparing the clinical performance of an alternative ELISA-based method (Assay 1) with your existing radioimmunoassay (Assay 2) for TSH receptor antibodies. Review the data in Figure 2 and then answer the questions below:

Figure 2:

A. Assay information

Assay 1 is an ELISA for TSH receptor antibodies

Assay 2 is a radioimmunoassay for TSH receptor antibodies

B. Reference ranges:

	Assay 1	Assay 2
Negative	<0.4 U/L	<1.0 U/L
Borderline	0.4-1.0 U/L	1.0-1.5 U/L
Positive	>1.0 U/L	>1.5U/L

C. Manufacturer's information:

Assay 1: Clinical sensitivity = 95% (n=108), Clinical specificity = 100% (n=137)

Assay 2: Clinical sensitivity = 92% (n=50), Clinical specificity = 100% (n=242)

D. Laboratory Comparison data

No.	Assay 1 (U/L)	Assay 2 (U/L)	Assay 1 interpretation	Assay 2 interpretation	Diagnosis of Graves disease (Yes / No)
1	<0.40	<1.0	NEG	NEG	No
2	<0.40	<1.0	NEG	NEG	No
3	3.92	3.8	POS	POS	Yes
4	>30	>40	POS	POS	Yes
5	<0.40	<1.0	NEG	NEG	No
6	6.08	5.3	POS	POS	Yes
7	1.35	2.7	POS	POS	Yes
8	5.08	4.1	POS	POS	Yes
9	2.22	2.5	POS	POS	Yes
10	4.10	3.1	POS	POS	Yes
11	1.82	2.1	POS	POS	No
12	<0.40	<1.0	NEG	NEG	No
13	2.96	2.3	POS	POS	Yes
14	2.23	<1.0	POS	NEG	No
15	4.62	3.5	POS	POS	Yes
16	2.83	2	POS	POS	Yes
17	4.70	3.4	POS	POS	Yes
18	6.39	5.9	POS	POS	Yes
19	2.70	1.8	POS	POS	Yes
20	<0.40	<1.0	NEG	NEG	No
21	22.67	24.2	POS	POS	Yes
22	<0.40	<1.0	NEG	NEG	No
23	<0.40	<1.0	NEG	NEG	No
24	2.01	<1.0	POS	NEG	No
25	5.85	4	POS	POS	Yes
26	<0.40	<1.0	NEG	NEG	No
27	14.58	13.7	POS	POS	Yes
28	<0.40	<1.0	NEG	NEG	No

a) Comment on the qualitative agreement between the two assays (2 marks)

b) Calculate the clinical sensitivity and specificity for Graves disease for the two assays, stating the equations used and showing your working (6 marks)

c) Calculate the positive predictive value of Assay 1 and Assay 2 (2 marks)

d) Comment on the potential clinical consequence of the difference in positive predictive value between the two assays (1 mark)

e) What biochemistry test could you look at to assess or 'sense-check' TSH receptor antibody results, and what result would you expect in a patient who is strongly positive for TSH receptor antibodies for the first time? (2 marks)

ANSWERS:

Question 1

a) Yes
   Meet manufacturer's stated precision (1 mark)
   Meets lab's acceptable precision criteria (1 mark)
b) Verification CONFIRMS a manufacturer's stated performance characteristics (1 mark)
c) Good linearity (1 mark)
   Alternative assay has a negative bias compared to current assay/ -11.7% (1 mark)
   Worse at lower concentrations (1 mark)
   One outlier, or similar (1 mark)
d) Low medium and high QCs all at similar concentration (1 mark)
   QCs should cover dynamic range of assay (1 mark)
   One QC should be close to clinical cutoff (1 mark)
e) One of: (1 mark)
   Harder to do in-house assays
   Loss of repertoire - companies withdrawing kits
   Must use commercial assays where available

Question 2

a) 26/28 samples / 93% agreement / 2 discrepant samples (1 mark)
   Reasonable agreement / Would need to investigate clinical case for 2 discrepant samples (1 mark)
b) Sensitivity equation = True pos / (True pos + false neg) x100 (1 mark)
   Specificity equation = True neg /(True neg + false pos) x100 (1 mark)
   Assay 1 sensitivity = 16(16+0) x 100 =100% (1 mark)
   Assay 1 specificity = 9/(9+3) x100 = 75% (1 mark)
   Assay 2 sensitivity = 16/(16+0) x 100 = 100% (1 mark)
   Assay 2 specificity = 11/(11+1) x100 = 92% (1 mark)
c) Assay 1 = 16/(16+3) x100 = 84% (1 mark)
   Assay 2 = 16/(16+1) x100 = 94% (1 mark)

d) [The lower PPV of assay 1 (ELISA) / more false positives] could lead to unnecessary investigation for Graves disease / incorrect diagnosis of Graves disease (1 mark)

e) TSH concentration / thyroid function tests (1 mark)
Expect to see low TSH if TSH receptor antibodies are positive (1 mark)