Tips for FRCPath Practical Exam Preparation

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Part 2, Module 1: Exam Format

- **Paper 1: Objective Structured Practical Examination (OSPE)**
  - **3 hours (AM)**
  - **19 stations (9 minutes each – 3h total)**

Paper1/OSPE Preparation

- **Calculations and stats**
  - – Deacon's book/ACB News challenge
- **Cases/interpretative comments**
  - – DCB/NEQAS cases
- **Data interpretation**
  - HPLC chromatograms, xanthochromia, serum electrophoresis/CZE, ALP electrophoresis, oligoclonal bands, HbA1c, A1AT phenotyping/genotyping, HFE genotyping
- **DFT/endocrine test protocols**
  - Prolonged OGTT, ITT, STT, dex suppression tests, ARR etc.
- **Specialist test patient preparation/sample collection**
  - Aldosterone, renin, gut hormones, porphyrins
- **EQA report interpretation**
  - NEQAS, WEQAS, IMMAQS
- **Role play/communication station**
  - Staff issues, clinical/lab incidents, clinical queries

Part 2, Module 1: Exam Format

- **Paper 2**: **Bench practical**
  - 3 hours (PM)
  - Multi-part paper
  - Plan an experiment
  - Execute bench work
  - Record results
  - Analyse data
  - Draw conclusions

Paper 2/Practical Preparation

- **Get involved and become familiar with:**
  - **Setting up calibration curves**
    - Dilution of standards (sensible concentrations)
    - Minimum 3 point calibration
  - **Spectrophotometry** – determining optimal conditions, incubation times etc., enzyme inhibitor studies (Michaelis-Menton Kinetics, Lineweaver-Burke Plot).
  - **Plotting data by hand** – Calibration curves, Altman-bland plots, linear regression, precision plots, linearity data.
  - **Stats by hand/being familiar calculator** – SD, T-test (?bias), F test (varience ratio - ?difference in precision between 2 methods), calculation of r (correlation coefficient).
  - **Deriving reference ranges** (parametric and non-parametric data)

Paper 2/Potential Topics

- **Interference testing**
  - Effect of anti-coagulants
  - Effect of drugs, contrast media
  - Effect of haemolysis
  - Interference in POCT glucose method e.g. maltose, haematocrit
- **Method evaluation (quantitative method)**
  - Precision (intra-batch & inter-batch)
  - Accuracy (spike and recovery)
  - Analytical range, linearity, LOD, LOQ
  - Method comparison (bias, linear regression)
  - Carryover, drift
- **Method evaluation (qualitative/POCT method)**
  - Performance at quoted limit of detection
  - Time for result to appear/disappear
  - Cross-reactivity e.g. LH on urine HCG test
  - Ease of use, Cal and QC frequency (plus similarity to running patient)
  - QC lock-out, connectivity, audit trial, storage of results
- **Evaluate a POCT device and write a report**
- **Write a method SOP**

Paper 2/Interference Testing

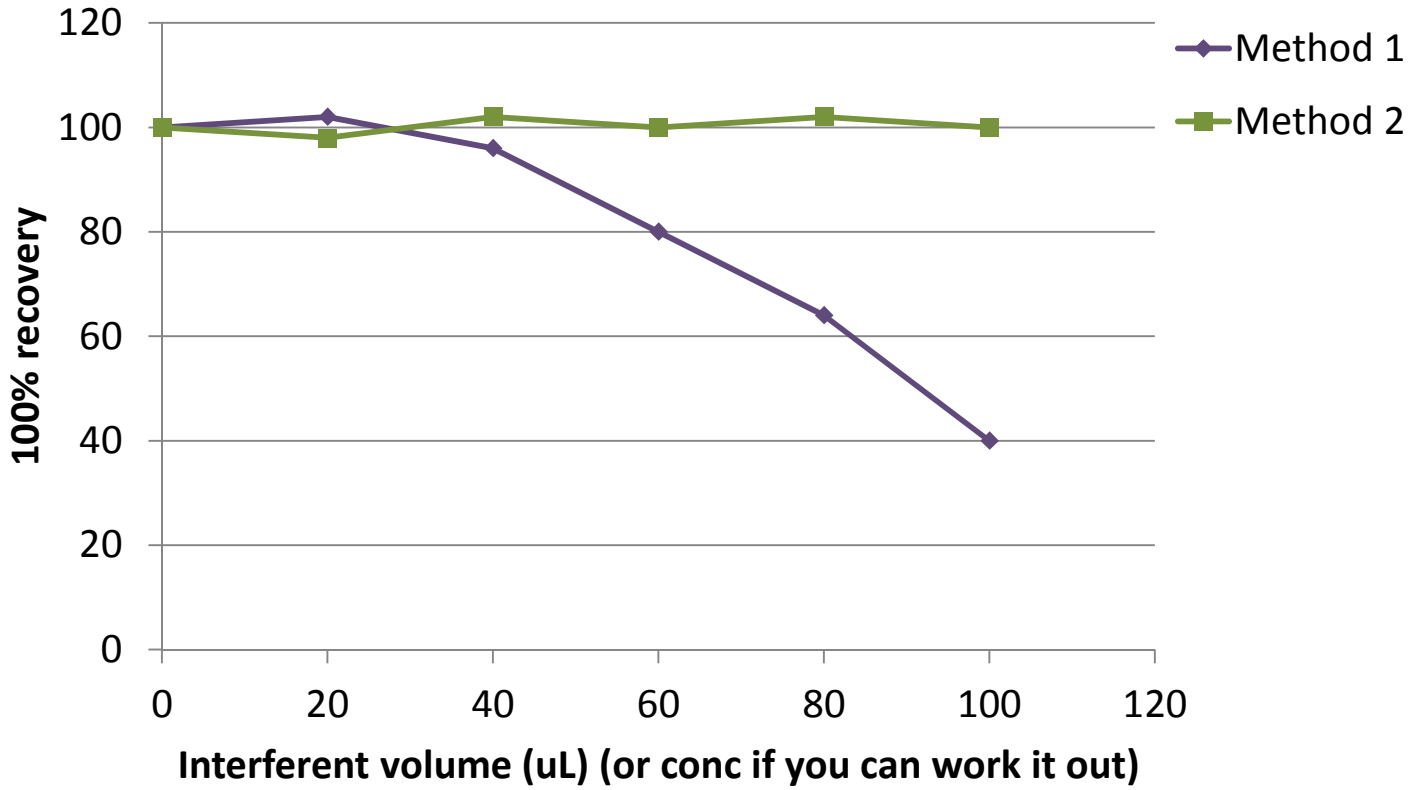
- Look at physiological concentrations of potential interfering agent if possible
- Try to avoid dilution of primary matrix by more than 10%
- Decision limits for significant effect: many people arbitrarily use >10%

Table 1 - Title

Sample name	Sample vol (uL)	Interferent vol (uL)	0.9% saline vol (uL)	Method 1 measured conc. (units)	Method 1 % recovery (compared to A)	Method 2 measured conc. (units)	Method 2 % recovery (compared to A)
A	1000	0	100	50	100	50	100
B	1000	20	80	51	102	49	98
C	1000	40	60	48	96	51	102
D	1000	60	40	40	80	50	100
E	1000	80	20	32	64	51	102
F	1000	100	0	20	40	50	100

Paper 2/Interference Testing

Fig. 1. Effect of interferent on method 1 & method 2



Also see for general practical advice:
G. Dimeski (2008) Interference Testing. Clin Biochem Rev 29 Suppl (i) S43-S48

Paper 2/Method Evaluation

- **Precision: %CV = (SD/mean) x 100**

  - Interbatch CV (over at least 5 days)
  - Intrabatch CV
  - QC material or pooled sera at least 2 levels
  - 20+ replicates ideal but 5-10 acceptable
  - Relevant/decision limits concentrations for assay of interest and ideally not around same conc. as calibrators
  - Goals for imprecision should be based on biological variation i.e. $SD_A \leq 0.5_{\text{intraindividual variation}}$

mean = $(\Sigma x)/n$

SD = $\sqrt{\Sigma(x\text{-mean})^2/n\text{-}1}$

%CV = (SD/mean) x 100

Sample/QC no.	Result (units)
1	
1	
1	
mean	
SD	
%CV	

Paper 2/Method Evaluation

- **Checking accuracy/calibration (spike and recovery)**
  - 3-4 concentrations over the claimed working range
  - Analyse in duplicate if possible
  - Use aqueous or matrix matched standards
  - If spiking serum/whole blood, spike with smallest possible vol. to avoid matrix effects (<10% of total vol.)

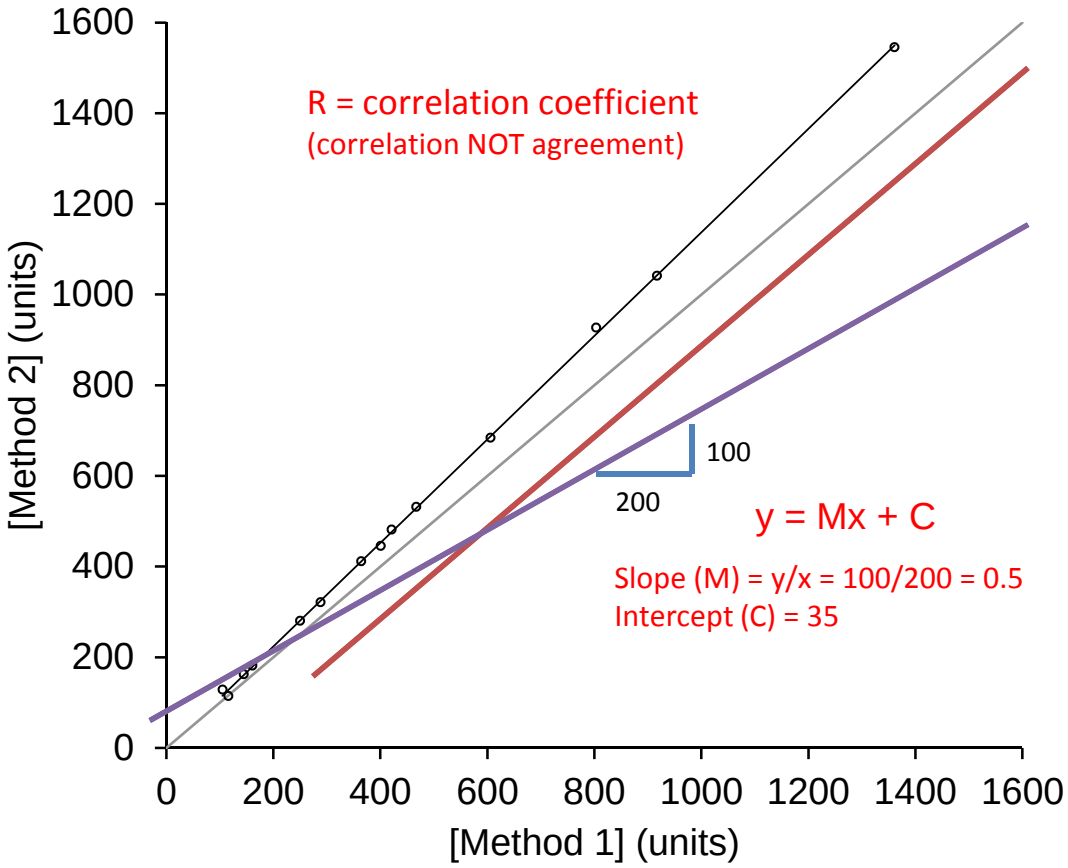
  - e.g. 100 mmol/L standard solution of glucose

Sample name	Sample vol (uL)	spike vol (uL)	0.9% saline vol (uL)	Final [Spike] in sample (mmol/L)	[measured] (mmol/L)	% spike recovery
A	900	0	100	0	4.0	/
B	900	50	50	5	9.5	(9.5 – 4.0)/5 x 100 = 110%
C	900	100	0	10	13.5	(13.5 – 4.0)/10 x 100 = 95%

Paper 2/Method Evaluation

- **Method comparison – scatter plot**

Fig. 2. Plot illustrating relationship between…

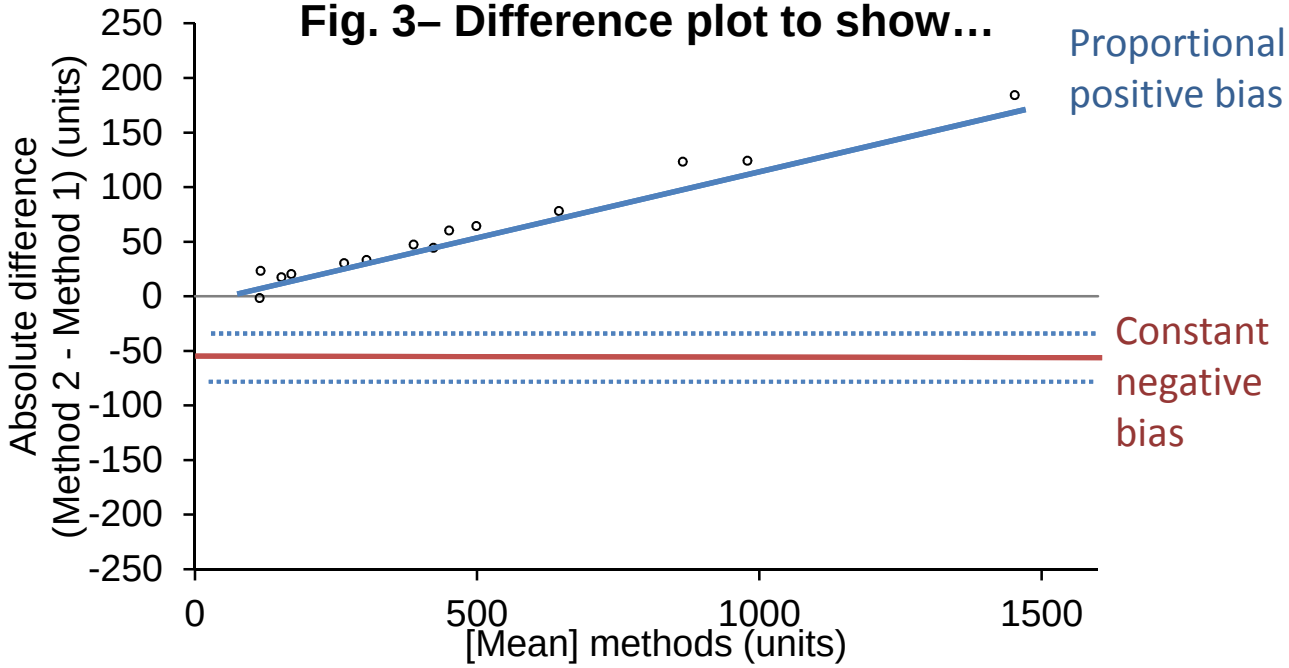


- Scatter plot:
- At least 20 samples
- Square axis – always label with units
- Include a line of equivalence and label it
- Draw line of best fit through points and calculate line equation
- Describe relationship
- Know how to calculate correlation coefficient and what this means

Paper 2/Method Evaluation

- **Method comparison - Altman-bland/difference plot**
  - At least 20 samples
  - Comparison of two routine methods: plot mean of results on x-axis.
  - Comparison of routine method with reference method: plot reference method result x-axis.
  - Difference should be calculated by new method minus old method
  - Describe any bias
  - Know how to calculate mean of difference ± 95% confidence limits (i.e. mean difference ±2 SEM)

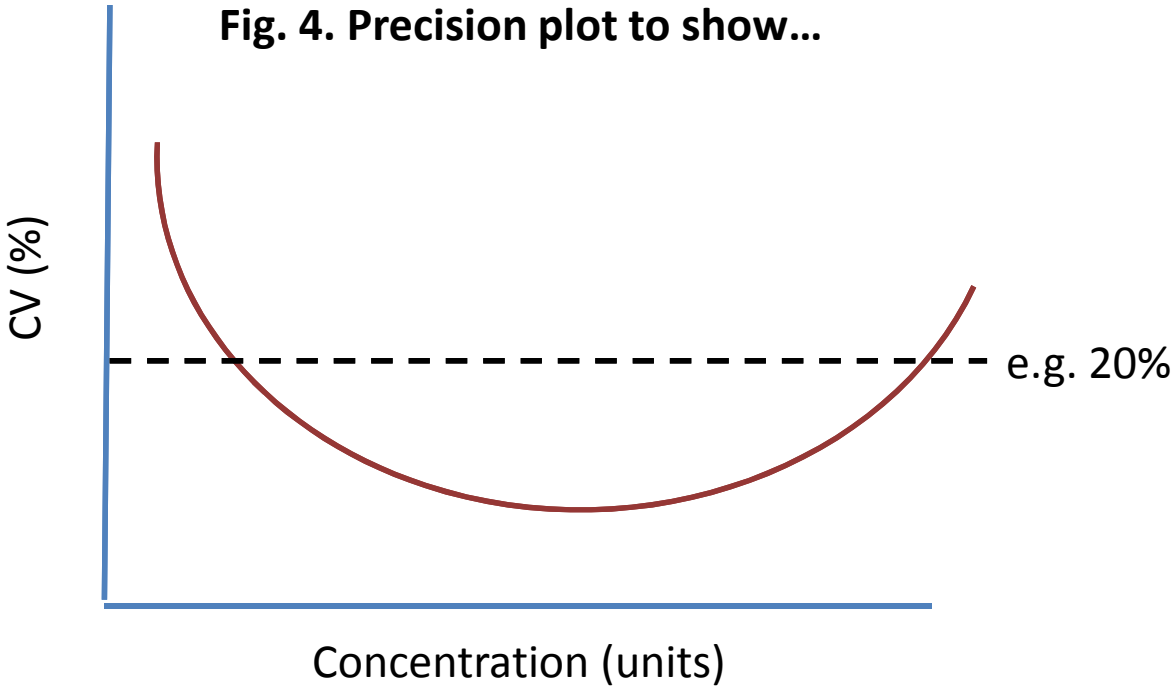
Fig. 3– Difference plot to show…



Paper 2/Method Evaluation

- **Analytical range - precision plot and limit of quantitation (LOQ)**
  - Analyte concentration on x-axis, CV or SD on y-axis
  - At least 3 concentrations – analysed at least 5-10x
  - Allows you to determine LOQ (i.e. functional sensitivity) and appropriate analytical reporting range
  - General rule: LOQ = lowest conc. where CV <20%

Fig. 4. Precision plot to show…



- **Limit of detection (LOD) – on a spectrophotometric method**
  - Analysis of a blank (ideally matrix similar to patient samples)
  - Measure blank 3-5 times (ideally 20x over different days using different blanks)
  - LOD = mean + 3SD

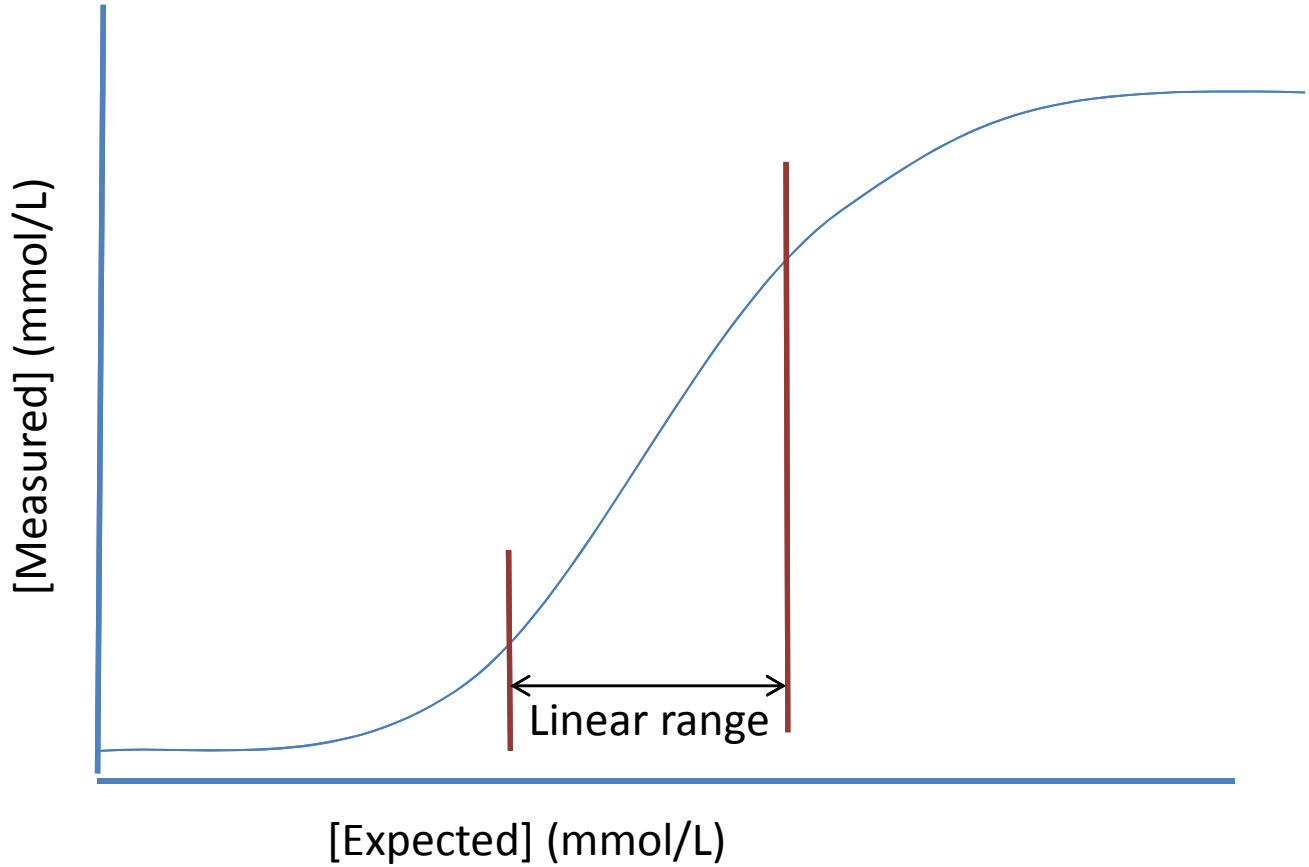
Paper 2/Method Evaluation

- **Analytical range – linearity**
  - state how dilutions will be made (doubling dilutions, 1/3 etc.)
  - state diluent matrix i.e. potential for matrix effects etc.
  - Ideally look over at least 5 concentrations
  - 2-3 replicates at each conc.
  - Plot [expected] vs [measured]

Sample name	Sample vol (uL)	Diluent vol (uL)	[expected] (mmol/L)	[measured] 1 (mmol/L)	[measured] 2 (mmol/L)	[measured] Mean (mmol/L)
A	1000	0	150			
B	500 of A	500	75			
C	500 of B	500	37.5			
D	500 of C	500	18.8			
E	500 of D	500	9.4			

Paper 2/Method Evaluation

- **Analytical range – linearity**
  - Compare with manufacturers quoted range
  - Determine reportable range
  - Determine point at which high-level samples require dilution



Paper 2/Practical Preparation

- **Think in advance about stuff YOU will need to take:**

  - Lab coat
  - Pipettes and tips (various sizes at least 5ml, P1000, P100)
  - Gloves
  - Stop-watch/timer (without loud buzzer)
  - Calculator
  - Permanent pens

  - Your own practical hints/tips note book – you may not use it on the day but putting this together is preparation in itself

  - Clin Biochem text book (may be useful as a comfort blanket or for ref ranges etc. but you probably won't open it!)

Paper 2/On The Day

- Allow yourself a **notes** page in exam booklet.

- **Check you have everything** that you're supposed to have:
  - Reagents, strips, meter, working spectrophotometer, cuvettes etc. – if you're not sure you have everything – ask!

- Take note of any stated **storage conditions/expiry dates** etc. – if QC fails this could be intentional and they may want to spot a reason for this.

- Take note of **volumes, number of reagent strips** etc. – may need to plan an experiment based on what you have.

- If **POCT** device note if it's **CE marked** or not.

- If they give you lots of data – **don't feel you have to plot it all** but make sure you plot a good number of points (e.g. 20 for scatter plot/Altman-Bland) across the concentration range given! State in your write up that not all points plotted due to time restriction.

- **Don't attempt to do too much** – make sure you can finish bench work and write it up – you only get marked on what you write down and hand-in!

GOOD LUCK!

KEEP
CALM
AND
ENJOY
YOUR EXAM