The Royal College of Pathologists of Australasia

Faculty of Science
Sample Biochemical Genetics Examination Questions and Model Answers

Biochemical Genetics Part 1 Written Examination

Question

Ankylosing spondylitis (AS) is a debilitating inflammatory systemic disease affecting the spine and the sacro-iliacal and peripheral joints. AS is associated with the HLA-B27 allele, with 90% of AS patients carrying this allele. HLA-B27 is typically assessed by flow cytometry using an antibody(s), directed to the HLA-B27 surface antigen. However, your laboratory has been asked to offer an in-house developed PCR-based genotyping molecular test that detects the HLA-B27 allele. Please answer the following questions, which are related to the implementation and validation of an in-house IVD for the detection of the HLA-B27 allele. Both tests provide a qualitative result (HLA-27 allele absent or present). For your answers below, you are only required to consider the test performance of the qualitative result, not the analytical factors leading to the result.

a) What factors would you consider prior to designing an assay to replace the existing tests?

b) What parameters would you assess in your strategy for validating the new HLA-B27 PCR-based assay? What controls would you include?

c) Referring to the ROC (receiver operator curves) supplied in Figure 1, explain the significance of these graphs to the performance of the PCR-based assay. What does the red line indicate in the graph?

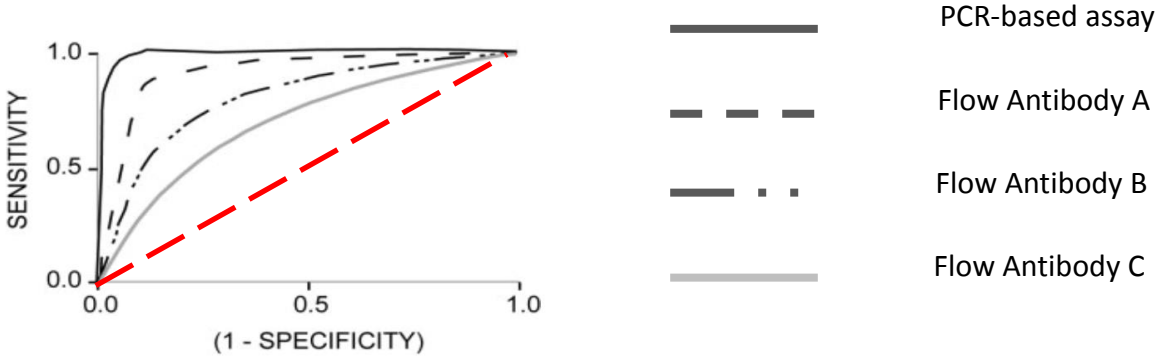

Figure 1. ROC plot for the HLA-B27 PCR-based assay and the three antibody assays for HLA-B27 as indicated by the legend

d) Clinical sensitivity and specificity are simple measures of assay performance. Using the table below, represent sensitivity and specificity as formulae. Describe how these measurements differ from the terms positive predictive value and negative predictive value.

	Condition Present	Condition Absent
Test Positive	a	b
Test Negative	c	d

Answer

a)

i. Analytical and Clinical utility – does HLA-B27 correlate with AS and does the result influence patient management? Is the TAT acceptable?
ii. Why is a replacement needed, existing assay performance
iii. What makes this approach better than alternate methods? For example, in this case DNA sequencing could be used instead of PCR genotyping
iv. Business Case: is the cost of any new method comparable to or less than existing? If not, is the expected improvement worth the extra cost? Routine operating costs including reagents consumables and labour costs.
v. Feasibility and robustness: does the lab have suitable equipment and is it adequately maintained and is back-up available? Are suitably trained staff available to perform the test? What is the failure rate of the new assay?
vi. Does the lab have access to a suitable cohort of samples from known AS patients and controls to validate the assay?

b)

i. Sensitivity & Specificity
ii. PPV and NPV; only 90% of AS patients have HLA-B27
iii. Reproducibility
iv. Wildtype and affected samples, including from patients who tested negative with the FACS assay.

c)

i. The curves indicate that the PCR-based assay is a better assay for the HLA-B27 allele than the flow cytometry antibody assays because it provides higher sensitivity at all values of specificity. The AUC is closer to 1.
ii. The red line indicates a random outcome, line of no discrimination.

d)

i. Sensitivity = a/(a+c)

ii. Specificity = d/(b+d)

iii. PPV (=a/(a+b)) and NPV (=d/(d+c)) depend on the prevalence of the disease.

Biochemical Genetics Part I Oral Examination

Question

A baby boy presented in the first week of life with severe myopathy requiring ventilator support due to breathing difficulties. Plasma lactate was elevated at 4 to 10 mmol/L (normal 1 – 2 mmol/L). Mitochondrial respiratory chain enzymes were measured in a skeletal muscle homogenate and gave the results listed below, which are diagnostic of a defect affecting respiratory chain complexes I, III and IV, each of which has subunits encoded by mitochondrial DNA.

	Activity	RefRange	%Activity	%CS Ratio	%CII Ratio
Complex I (nmol/min/mg)	**4.6**	L(19-72)	11	3	2
Complex II (nmol/min/mg)	**200.8**	H(26-63)	446	139	
(nmol/min/mg)					
Complex III (/min/mg)	**3.4**	L(12.8-50.9)	12	3	3
Complex IV (/min/mg)	**0.43**	L(3.3 - 9.1)	7	2	1
Citrate Synthase (nmol/min/mg)	**411.7**	***H**(85-179)	319		

Enzyme activities are shown as absolute values and as % residual activity relative to protein (%Activity), citrate synthase (%CS Ratio) and complex II (%CII Ratio). Complex I is NADH-coenzyme Q1 oxidoreductase. Complex II is succinate-coenzyme Q1 oxidoreductase. Complex II+III is succinate cytochrome c reductase. Complex III is decylbenzylquinol-cytochrome c oxidoreductase. Complex IV is cytochrome c oxidase.

a) What factors would have been considered in deciding to do enzyme studies on a muscle biopsy rather than a less invasive sample? What is the significance of elevated activities of complex II and citrate synthase?
b) Assume the results are caused by a mitochondrial DNA mutation. Describe what type of genes or mutations could underlie this enzyme pattern and what type or types of inheritance might be expected?
c) Assume the results are caused by a nuclear DNA mutation. Explain how a nuclear gene defect could underlie this enzyme pattern with examples of two or more general types of genes/proteins and their functions.

Answer

a) The diagnostic yield of respiratory chain enzymes is higher in skeletal muscle than in less invasive samples, particularly when muscle is a clearly affected tissue. The enzyme assays are typically not offered in blood samples so the obvious less invasive alternative would be skin fibroblasts. Turnaround time is also expected to be quicker since it may take 6 weeks or more to establish a skin fibroblast culture. This is potentially important in a ventilator-dependent child, where the condition could deteriorate or decisions may need to be made about withdrawal of care. Additional points are that it may also be ethically more justifiable to do a muscle biopsy in this situation compared to a less severely affected child e.g. fully conscious. Also, some children with these defects are more sensitive to volatile anaesthetics and at increased risk of cardiorespiratory arrest from general anaesthesia. Bonus points if they also mention that a muscle biopsy also provides the opportunity for other informative tests such as muscle histology, histochemistry and mtDNA testing.

High activity of complex II and citrate synthase reflects the fact they have no subunits encoded by mtDNA. It suggests the patient has mitochondrial proliferation and provides the opportunity to express Complex I, III and IV activities relative to these marker enzymes, which is more robust than expressing them relative to just protein.

Acceptable answer should mention higher diagnostic yield and one of turnaround time, ethics/safety or opportunity for other tests.

b) Point mutations or small indels in genes encoding tRNAs or rRNAS required for expression of multiple complexes. Mutations in protein coding genes would not typically cause this pattern since each of the 13 proteins is a subunit of only one complex, rather than three. Large scale deletions affecting multiple genes can also cause this profile since they will typically lead to loss of one or more tRNA genes. mtDNA point mutations or indels can be inherited maternally but large deletions are typically de novo/sporadic. (Bonus points if mention is made that point mutations can also be de novo)

Acceptable answer should mention mutations in tRNA and rRNA genes but not protein-encoding genes and should mention both maternal and de novo/sporadic inheritance.

c) One category is genes encoding proteins involved in mtDNA maintenance (also called homeostasis or replication) of mtDNA resulting in mtDNA depletion or multiple deletions of mtDNA. The second is genes encoding proteins involved in expression of mtDNA, i.e. in mtDNA-encoded RNA processing or translation.

For mtDNA maintenance, examples are proteins involved directly in mtDNA replication (e.g. a polymerase or helicase) versus genes involved in maintaining the mitochondrial pool of nucleotides needed for DNA synthesis.

For mtDNA expression, examples include genes involved in mtRNA transcription or processing versus mitoribosome biogenesis versus mt-tRNA processing or aminoacylation versus initiation or translation factors.

Acceptable answer should mention at least one of mtDNA maintenance and mtDNA expression with 2 examples of types of genes or proteins.