

## Marking Scheme for the MSc Exam for Grade A Clinical Biochemists.

Write short notes on each of the following:

- a) Sequencing.
- b) TaqMan assay for mutation detection.
- c) Single nucleotide polymorphism.

- A. **Sequencing.** Candidates should give an overview of the pros and cons of sequencing. Specifically it being the gold standard tests but with relatively high costs at the moment. In terms of methodology, candidates could describe the dideoxy chain termination sequencing methodology briefly mentioning how this used to be done using radioactive labels but are now performed using fluorescent tags to each of the four bases to allow the sequencing to be done in a single tube. Candidates should give an idea of clinical applications and limitations of this approach with advantages including absolute identification of single based changes and other more complex insertions and deletions but excluding larger deletions. Disadvantages might include capital costs of equipment although this is coming down. A potential disadvantage is that identified sequence variants may not be relevant clinically if there is no functional effect. Comments on the principles of pyrosequencing would be particularly impressive.
- B. **TaqMan.** Candidates should give a background around the use of TaqMan to detect mutations in real time, thereby negating the need for restriction digestion and running on electrophoresis gels. The principle of the Tack Man assay should be described with reference to reporter and quencher labels and sequence-specific probes for each allele. Diagrams would be particularly helpful in illustrating this and the comments on melting curve analysis and how this changes with mutation should be mentioned. Any comments on future developments such as microfluidic cells would also be useful. Candidates should comment on limitations and advantages of this technology; limitations including requirement for optimisation for specific mutations and again capital costs of equipment with advantages including automation compared with traditional gel based techniques. Comments on the increasing use of TaqMan technology in routine Clinical Biochemistry and molecular genetics would be welcome.
- C. **Single nucleotide polymorphism.** Candidates should describe what a SNP is (i.e. a single base pair defect in the DNA) and that this nucleotide change may be anywhere within the DNA structure. Comments on the definition of polymorphism being at least 1% of alleles would be welcome. Brief comments on types of mutations such as synonymous, non-synonymous, frameshift, splice site, etc would be useful along with comments on correlations between genotype and phenotype. Also mention of how these might be detected (such as RFLP, ARMS, TaqMan sequencing, etc) would be useful.