

UNIVERSITY OF CAPE TOWN



DIVISION OF CHEMICAL PATHOLOGY

REGISTRAR TRAINING MANUAL

(Dec 2010)

Contents:

Overview of specialist training in Chemical Pathology at the University of Cape Town / Groote Schuur and Red Cross Children's Hospitals

- Outcomes of the registrar training course: a list of key competencies
- Laboratory methods skills course
- Mechanisms of disease: tutorial topics
- On-call responsibilities and emergency tests

COMPONENTS OF THE SPECIALIST TRAINING PROGRAM

1. Induction period: This is a six week induction period where registrars will be introduced to the various areas in the laboratory, as well as be exposed to laboratory techniques and basic chemical pathology. They will be expected to complete and submit sections A and B in this manual by the end of the induction period. Registrars will be trained in the use of the laboratory information system. They will also receive training in the validation and monitoring of clinical results and will be expected to be competent in this function by the end of the induction period.
2. Systematic progression through each section of the Chemical Pathology diagnostic laboratories, gaining familiarity with the methodology, clinical application and interpretation of each assay. Registrars will rotate through the various benches in the Chemical Pathology division of the Consolidated GSH laboratory (C17), as well as the Molecular (C21), the Inherited Metabolic Disorders (6th Floor, Falmouth Building, UCT Medical School), and the Red Cross Children's Hospital laboratories.
 - a. C17 Pre-analytical – This rotation occurs during the Induction period of the training programme. Registrars will develop a clear understanding of the processes occurring in this important section of the laboratory. This includes flow of specimens through the lab, sendaways, procurement and stores, UCT private hospital and point of care instruments.
 - b. C17 Automation bench – registrars will become familiar with the different automated analysers in use in the laboratory, principles of the various methodologies, internal quality control and external quality assurance. This registrar is responsible for trouble-shooting any problems arising at this bench, including quality control issues. Familiarity should be gained for instruments not in use at the C17 laboratory e.g. AxSYM, Centaur, Beckman. Site visits will be arranged at other laboratories to facilitate this.
 - c. C17 - Protein electrophoresis bench – The registrar on the bench will be responsible for interpreting all cerebrospinal, serum and urine protein

- electrophoresis and immunofixation samples.
- d. C17 - Lipid electrophoresis – the registrar on this bench will attend the weekly lipid clinic meeting held at Prof D Marais's laboratory and will also be responsible for entering lipid gel results onto the Laboratory Information System (LIS). In addition, this registrar is responsible for validating and reviewing all protein electrophoresis and immunofixation results.
 - e. C17 – Manual bench – registrars will perfect their laboratory skills at this bench. They will be expected to master as many manual, semi-automated and specialist assays as possible.
 - f. C17 – Radioimmunoassays – this rotation is combined with the C17 Manual bench rotation. Registrars will familiarize themselves with the principles of immunoassay platforms, including RIA, IRMA and ELISA, etc. During this time, registrars will liaise with Dr Judy King and should aim to perform at least 1 RIA and 1 IRMA should kits permit. The registrar should also visit other laboratories such as the thyroid screening programme at Red Cross Children's Hospital.
 - g. C21- Molecular laboratory – during this rotation, registrars will be trained in the running of routine molecular tests. They will gain familiarity with the laboratory processes and set up, as well as the principles for the different tests and methodologies.
 - h. Red Cross Paediatric Laboratory – registrars will master all aspects of routine laboratory operation with a specific focus on paediatric specific and inherited metabolic disease. Registrars in this rotation will be exempted from attending most meetings at Groote Schuur Hospital (GSH) (except Chemical Pathology Journal club) and will also be exempt from routine signing out duties at GSH (excl weekends).
 - i. Inherited Metabolic Disorders laboratory – when interesting cases or test requests come up, registrars will assist in assays performed in this laboratory, learning about principles and methodology of enzyme activity assays performed on fibroblast culture as well as the disorders associated with enzymes deficiencies.
3. Structured visits to laboratories outside of Chemical Pathology (C17). This should include time spent in Porphyria, Lipid, Pharmacology (for Therapeutic Drug Monitoring – TDM) and Forensic Toxicology laboratories.
 4. Validation and monitoring of clinical results. Registrars are required to take a major role in clinical and technical validation of results, and to become actively involved in quality control procedures and meetings. Follow-up of interesting cases and liaison with clinicians on further investigations is essential. Troubleshooting discrepant results should form part of the clinical and technical validation procedure of results. Registrars will receive training during their Induction period and will be allowed to validate results unsupervised, once deemed competent.
 5. Attendance and participation in the weekly Journal Club. Current articles on a wide range of topics covering diagnostic test methodology, mechanisms of disease, and basic advances in biomedical research are presented. The candidate is helped to develop a critical approach to data interpretation in the biomedical literature, and to acquire communication and presentation skills.
 6. Participate in the Chem Path case presentations after journal club, and at local, national or international meetings and conferences, when invited.
 7. Attendance and participation in the weekly Endocrine seminars in which clinical cases of biochemical or endocrinological interest are discussed, jointly with

members of the Clinical Endocrinology Unit, Paediatric Endocrinology and Gynaecological Endocrinology departments.

8. Attendance and participation in relevant lectures at the dept of Medicine Grand Ward Round held in the GSH E-floor lecture theatre.
9. Arrange and attend relevant **clinical teaching ward rounds** and clinics including Endocrinology ward round.
10. Registrar tutorials: Registrars may arrange and attend registrar tutorials with the relevant consultants or medical scientists on topics of their choice.
11. Participation in undergraduate MBChB lectures and tutorials in Chemical Pathology.
12. All registrars are required to produce a dissertation on their research project, and to present their work at a seminar and a congress.

Typical weekly time-table at GSH and RXH:

	GSH	RXH
Monday	11: 30 Pathology Journal club	08:00 Attend Paeds Endo clinic 11:30 Journal Club
Tuesday	11:30 Endocrinology Seminar 13:00 Lipid clinic meeting 13:00 Molecular Biology Seminar	12:00 Subspeciality ward round
Wednesday	11:00 Lipidology tutorial 14:00 Journal watch	08:00 General paediatric ward round
Thursday	14:00 Biochemistry / clinic chemistry tutorial 16:00 – 18:00 Medicine Grand Ward Round	
Friday	09:00 Management tutorial 14:00 Endocrinology ward round	12:00 Registrar clinical meeting

CHEMICAL PATHOLOGY ON-CALL DUTIES FOR REGISTRARS & CONSULTANTS

1. Reviewing duties: Reviewing duties are shared by registrars and consultants. A roster has been prepared.
2. On call roster: A separate roster has been drawn up for the on call registrar and consultant. Generally consultants are on call for 2 weeks at a time, while registrars are on call for a week at a time.
 - a. On call registrar speed dial: 77 198
 - b. Registrar office number: 4135
 - c. On call consultant speed dials are available at the Modular bench.

SUGGESTED TUTORIAL TOPICS (NOT INCLUSIVE)

Undergraduate MBChB tutorials:

Registrars are expected to:

1. Give all semester 4 – 6 tutorials held during the semester after completing their first 6 months.
2. Prepare for these tutorials by reading the relevant material provided for undergraduate students and to prepare a powerpoint tutorial.

Registrar tutorials:

Registrars are expected to cover the topics in the undergraduate student notes as well as the topics listed in the Academic Programme For Specialty Training In Chemical Pathology in their tutorials with consultants and scientists. Prior preparation is encouraged (using the relevant textbooks and current literature). Some topics are listed below.

TOPICS COVERED IN UNDERGRADUATE CHEMICAL PATHOLOGY NOTES

1. Normal values, reference ranges, sensitivity & specificity. Basic statistics: normal distribution, standard error and deviation, coefficient of variation, correlation coefficient. Quality control, use of controls, internal and external. Sample collection & anticoagulants.
2. Kidney disorders
3. Acid/base disorders
4. Disorders of sodium and water metabolism.
5. Disorders of potassium metabolism.
6. Disorders of carbohydrate metabolism
7. Diagnostic enzymology
8. Intestinal absorption and pancreatic function.
9. Endocrine testing I
10. Thyroid disorders
11. Liver disorders
12. Inborn Errors of Metabolism
13. Ca²⁺ and phosphate metabolism
14. Plasma lipids
15. Iron metabolism
16. Plasma proteins
17. Vitamins
18. Porphyrrias
19. Purine & uric acid metabolism
20. Biochemical effects of tumors

BASIC SCIENCE TOPICS

21. Carbohydrates: structure & function
22. Proteins: structure and function
23. Lipids: structure and function
24. Nucleic acids: structure and function
25. Cell structure and function: organelles
26. Membrane structure and function: membrane transporters
27. Receptors and cell signalling
28. Gene expression

ADDITIONAL TUTORIAL TOPICS :

29. Principles of Dynamic testing in Endocrinology
30. Serum and urinary amino acids & their interpretation

RECOMMENDED TEXT BOOKS

See Academic Programme For Specialty Training In Chemical Pathology for recommended text books and Journals to review.

LABORATORY METHODS IN CHEMICAL PATHOLOGY

A theory-and-practice course for Chemical Pathology Registrars and Clinical Pathology Registrars at the University of Cape Town and Groote Schuur Hospital

Required knowledge and skills content are listed, followed by exercises designed to help achieve the competence in each area.

Section A and B must be completed within the first 6 weeks and must be submitted in hard copy for assessment. The rest of the sections must be submitted before the Part I examination, in the same format. Material covered in these exercises may be asked in written or oral examinations.

SECTION A

- **Patient data capture, sample collection, anticoagulants and storage.**
- **Centrifugation**

EXERCISES

1. Compare the relative stability of peptides, steroids, inorganic ions and small organic molecules in plasma.
2. List the commonly used anticoagulants and describe their mechanism of action
3. What incorrect procedures can cause dangerous accidents with centrifuges?
4. Investigate the stability of glucose in blood samples left without enzyme inhibition (Na fluoride)
5. Examine the effect of EDTA contamination of a specimen on routine laboratory results
6. Examine the effect of haemolysis on routine laboratory parameters
7. How will an icteric specimen influence the routine laboratory measurements

SECTION B

- **Concepts and definitions of molarity and normality.**
- **Interconversion of mass units and molar units.**
- **Acids, bases and buffers.**
- **The Henderson -Hasselbach equation.**
- **Titration curves and the meaning of pH and pK.**
- **Other units: kPa, osmolality.**
- **Preparation of solutions: use of pipettes, volumetric flasks, measuring cylinders, balance and pH meter.**
- **Concepts of polar and nonpolar compounds. Partitioning of compounds into solvents, and principle of solvent extraction.**

EXERCISES:

1. Convert a serum glucose level of 250 mg/dl to mmol/l. (MW of glucose = 180)
2. Convert a serum Fe level from 30 μM to $\mu\text{g}/100\text{ml}$. (MW of Fe = 56)
3. Work out the components of 550ml wash solution that is 1300 μM NaCL, 55 mM KOH and 0.55% (w/v) SDS. Use solid NaCL and SDS, and a 4M KOH stock solution to make up this wash solution. (FWs: KCl = 74.56; Tris = 121; SDS = 288.4; NaCL = 58.44)
4. The OD₂₆₀ of a 1/40 dilution of a DNA sample you have to pipette reads 0.096. How many microlitres (μl) would you need to pipette if you wanted to deliver 850ng of a dsDNA (double stranded DNA) to a new tube? (1xOD₂₆₀ dsDNA = 50 $\mu\text{g}/\text{ml}$)
5. What volume of 23.5% NaCL (w/v) would you dilute up to 500ml to give a final concentration of 300mM.
(FW: NaCL = 58.4)
6. A patient's plasma ACTH level is 33 pg/ml. Convert this to pmol/l. The MW of ACTH is 2900.
7. What is the definition of pH?
8. What is the pH of a 10 mM solution of HCl, assuming its complete dissociation?
9. What is the pH of a solution in which the [H⁺] concentration is 2.5 nmol/L?
10. What is the [H⁺] in human plasma?
11. Make up 100 ml of a 20 mM tris-Cl buffer, pH 8.2. Write up what you did in your lab book.
12. Determine the pK of Tris, by titrating Tris base with HCl and plotting the titration curve. Plot the curve both on graph paper AND using a computer program such as Excel, Prism or Sigmaplot.
13. Why does a buffer work best at a pH near its pK?

SECTION C

- **Basic statistics: normal distribution, SD and SEM, coefficient of variation.**
- **Precision and accuracy in clinical chemistry tests.**
- **Calibration and use of internal controls**
- **Analytical sensitivity and detection limits**
- **Comparing two methods: correlation coefficient, difference plots.**
- **Reference ranges: how are they chosen?**
- **Diagnostic sensitivity, specificity and predictive value.**
- **Quality control, internal and external.**
- **Westgard rules and action limits**

EXERCISES

1. Work through the numerical exercises in the tutorial.
2. Obtain an estimate of the precision (% CV) of any one routine assay of your choice, at low, normal and elevated levels relative to the reference range. Try to identify the sources of imprecision in this assay.
3. List all the possible things contributing to **Imprecision** in an assay
4. List all the possible things contributing to **inaccuracy** in an assay
5. Determine the correlation between serum urea and creatinine, using 30 randomly chosen results from the routine lab. Use any standard software package (Prism, Sigmaplot).
6. Repeat the above correlation after adding another 30 results to the sample. How do the results differ?

SECTION D

- **Principles of the spectrophotometer.**
- **Filters and monochromators**
- **Beer's Law, absorbance and % transmission, molar absorptivity**
- **Absorption spectra.**
- **Dual- and single-beam spectrophotometers**
- **Principles of fluorescence and fluorimetry: excitation spectra, emission spectra.**
- **Principles of Nephelometry**
- **Principles of turbidometry.**

EXERCISES

1. Write out Beers Law, defining each term in the equation. Understand the meaning of **absorption spectra** and **molar absorptivity**.
2. What is the relation between **absorbance** and **% transmission** ?
3. Over what range of absorbance is the average spectrophotometer accurate? (explain why).
4. Using a manual UV-VIS spectrophotometer, scan the absorbance spectra of 0.2 mM solutions of NAD and NADH over the range of the instrument.
5. Determine the molar absorptivity of NADH at 340 nm.
6. Determine the best wavelength to use for the measurement of ATP concentration. Then determine the molar absorptivity of ATP at this wavelength.
7. When is it necessary to use quartz cuvettes?
8. Using a good quality quartz cuvette (empty) as a blank, scan (a) an empty glass cuvette and (b) the quartz cuvette filled with water
9. Briefly summarize, with diagrams, the basic principles of: (a) fluorimetry.
 - a. nephelometry
 - b. turbidometry

SECTION E

- **Principles of ion-selective electrodes**
- **Blood gas measurements**
- **Flame photometry and atomic absorption spectrophotometry.**

EXERCISES:

1. Briefly describe, with diagrams, the principle of an ion-selective electrode. Which ions are measured this way?
2. Summarize the principles of the flame photometer and atomic absorption spectrometer. What analytes are measured using these methods in clinical practice?
3. Explain why pseudohyponatremia is found in cases of severe hyperlipidaemia, when using an ion-sensitive electrode to measure Na^+ . What can be done to obtain a true serum Na^+ level in these cases?
4. Test the effect on blood gases (using excess routine blood samples) of allowing the blood to equilibrate with air. Plot your data on a graph. How can this artefact be distinguished from a respiratory alkalosis?
5. Divide a fresh heparinized venous blood sample (without fluoride) into two tubes. Allow one to stand overnight at room temperature, before separating the plasma. Spin the other and store the separated plasma refrigerated. The next day, perform on both samples
 - (a) the necessary measurements to calculate the anion gap,
 - (b) as well as the following: glucose, lactate, pH, pCO_2 , Pi, LDH.
6. Write up your results and explain the differences observed between the samples.
7. Explain the concept of “corrected” Ca^{2+} . Give a formula for its calculation. What factors affect the concentration of ionized Ca^{2+} in plasma? What precautions are necessary when collecting a blood sample for ionized Ca^{2+} ?
8. Obtain a fresh blood sample and divide it into 2 aliquots. Treat one aliquot with all the precautions for ionized Ca^{2+} , and let the other stand exposed to the air for several hours. Measure both total Ca^{2+} and ionized Ca^{2+} on both samples. Discuss your results.
9. Divide a plasma sample into 2 aliquots. Add 20 mg/ml BSA (bovine serum albumin) to one sample, and measure ionized Ca^{2+} on the samples. Discuss your results.
10. Investigate the effect of changing the pH of plasma (by adding acid or alkali) on ionized and total Ca^{2+}

SECTION F

- **Chromatographic techniques**

EXERCISES:

1. Understand the concepts of polar and nonpolar compounds, partitioning of compounds into solvents, and principle of solvent extraction.
2. Perform the qualitative Ehrlich's test on,
 - a. a sample of urine,
 - b. a solution of urobilinogen,
 - c. a solution of porphobilinogen.Explain how the solvent extraction step works.
3. Briefly summarize the principles of the following types of chromatography:
 - a. Ion exchange chromatography.
 - b. paper chromatography
 - c. TLC
 - d. HPLC
 - e. gas-liquid chromatography
 - f. Size exclusion chromatography (gel filtration).
 - f. Electrophoresis.
4. Identify the unknown sample of a sugar supplied using the standard TLC method for urinary sugars.
5. What type of Sephadex is suitable for desalting a solution of albumin? Pour a 10 cm x 1 cm (approx) column of this sephadex. Make up a solution (10 ml) of BSA (1 mg/ml) in 200 mM NaCl. Separate the protein from the salt using the column. (Assay the fractions for albumin using the manual BCG method, and for Na⁺).
6. Describe the operation of the amino acid analyser. Divide a fresh serum sample into two aliquots. Freeze one and incubate the other at 37°C overnight. Perform amino acid analyses on both samples & discuss your results. Which amino acids changed in concentration ?

SECTION G

- **Enzymology: enzyme kinetics, MM equation, K_m and V_{max}**
- **Rate assays vs. end-point assays.**
- **Primary and indicator reactions.**
- **Measurement of enzyme activity and substrate concentration.**

EXERCISES:

1. Write down the Michaelis-Menten equation. Draw the shape of the curve when reaction rate V is plotted against substrate concentration $[s]$.
2. What is the reaction velocity at a substrate concentration equal to the K_m ?
3. Draw the shape of the V against $[s]$ curve in the following situations: (a) in the case of substrate
 - a. inhibition
 - b. in the presence of a competitive inhibitor
 - c. in the presence of a non-competitive inhibitor.
4. Plot the following data as rate V against $[s]$, and then do the Lineweaver-Burke plot. From the latter determine the K_m and V_{max} of the enzyme.

<u>$[s](\mu M)$</u>	<u>V (nmol/min/mg)</u>
0	0
0.03	4.0
0.1	12.9
0.3	36.2
1	92.0
3	182
10	252
20	285
30	295

How would the Lineweaver-Burke plot differ in the presence of (a) a competitive inhibitor (b) a non-competitive inhibitor (c) substrate inhibition?

5. (**Chemical Pathology major registrars only**) Determine the best estimates of K_m and V_{max} for the above data using non-linear regression. Use a software program such as Prism or Sigmaplot .
6. Describe the differences between "rate" and "endpoint" enzymatic methods for determining the concentration of a compound.
7. Describe in principle
 - a. a rate method and
 - b. an endpoint method for the determination of serum pyruvate.
8. Using a MANUAL spectrophotometer, determine the K_m of LDH for pyruvate.
9. Write down possible coupled reaction systems which could be used for the measurement of
 - a. creatine kinase activity,
 - b. creatine phosphate concentration,
 - c. plasma ammonia,
 - d. pyruvate kinase activity.

SECTION H

- **Radio-immunoassay and related techniques:**
- **Radioactive decay.**
- **β - and gamma-counters.**
- **Principle of competitive RIA**
- **Types of radioligand. Binding equation, non-specific binding and Scatchard plots**
- **Principles of IRMA and similar assays.**
- **ELISA assays.**
- **Chemiluminescence detection**

EXERCISES: (these should be done during rotation through the Endocrine section of the laboratory)

1. What types of particles are emitted during radioactive decay?
2. List the radioisotopes commonly used in biomedical work. Draw up a table showing the half-life and type of particle emitted for each isotope.
3. Write down the equation for radioactive decay which gives the fraction of undecayed atoms as a function of time
4. Calculate what percentage of radioactive (undecayed) ^3H atoms remains after 3 years, given that the $T_{1/2}$ of tritium is 12 years.
5. How is radioactivity measured? What does “specific activity” mean?
6. Units of radioactivity: What is a Ci (Curie) ? What do the abbreviations c.p.m. and d.p.m. stand for, and what is the difference?
7. The SI unit for radioactivity is the Becquerel. How many Becquerels in a Ci?
8. How much radioactivity is commonly used in a RIA (say 50 samples)? What dose of ^{131}I is usually given to patients with Graves disease?
9. Draw diagrams to illustrate the principles of
 - a. competitive RIA
 - b. double antibody (sandwich type) assays
10. What methods are available for separation of bound from free radioligand in immunoassays?
11. What does ELISA stand for? What enzymes are commonly employed in ELISA assays?
12. What factors determine the stability of hormones in plasma? Discuss the specimen handling precautions which may be necessary. What are the functions of trasylol and EDTA ?
13. Go through the laboratory protocol for one of the **kit** RIAs in the RIA Laboratory. Summarize the protocol, explaining the rationale for each step.
14. In a RIA, what is meant by the term ED50?
15. How is cross reactivity measured?
16. How much cross reactivity can be tolerated in a clinical assay?
17. Summarize the assay principles utilized by the Centaur and Elecsys Roche .
18. Summarize the principles of the assays for plasma renin activity and active renin analysers
19. Describe the terms “sensitivity” and “specificity” in the context of immunoassays.
20. Describe the terms “accuracy” and “precision” in measuring hormone levels.
21. Illustrate your understanding of a Scatchard plot.
22. Briefly describe the difference between a peptide and a steroid hormone. Measuring them by means of radioimmunoassay, indicate the type of radioactive element with which each will most likely be labelled. Provide two examples of each of these

types of hormones.

23. Briefly provide the principles of the tests performed by immunoassay and describe the differences between that performed by RIA, IRMA and ICMA. What is meant by an assay using the sandwich technique?
24. For endocrine hormones, how do you convert IU to mass or moles?
25. What are inter- and intra-assay coefficients of variation?

SECTION I

- **Proteins: structure & measurement**
 - synthesis & post-translational modification
 - measurement: colorimetric assays, electrophoresis, Western blotting, immunofixation.

EXERCISES

1. List the 20 amino acids under the following groups: acidic, basic, neutral, aromatic, sulphur-containing, branched chain, hydrophobic and hydrophilic. (some will fall into more than one group)
2. What is meant by 1°, 2° and 3° structure in proteins?
3. List, and briefly describe, the post-translational modifications of proteins
4. Explain the principle of capillary zone electrophoresis.(CZE). How does this technique differ from gel electrophoresis and which advantages and disadvantages are there to both methods
5. Summarize the method of plasma protein electrophoresis used in the clinical laboratory.
6. Obtain a normal serum protein electrophoresis densitometer trace. Label the peaks and list the major proteins in each peak.
7. Obtain and annotate examples of serum and urine protein electrophoresis traces from patients with nephrotic syndrome, cirrhosis, myeloma, hyperlipidemia & any other interesting cases you can find.
8. What is MGUS? List criteria for differentiating MGUS from myeloma.
9. Briefly explain the principle of immunofixation.
10. Familiarize yourself with the method used for concentrating urine prior to electrophoresis, and list the components of the special additive used in the urine collection jar. Why is this necessary?
11. Summarize the principles of SDS-PAGE (polyacrylamide gel electrophoresis). How do the results differ from the "standard" clinical serum protein electrophoresis?

SECTION J

- **Molecular biology techniques**
 - Nucleotides, DNA, RNA, gene structure, transcription, translation. RNA splicing and processing.

EXERCISES

1. Develop a good understanding of the central dogma of molecular biology, DNA→RNA →Protein, particularly as it applies to eukaryotes. Know how this differs from prokaryotes.
2. Describe the meaning of the terms:
Nucleotide, nucleoside, base, promoter, transcription factors, intron, exon, primary transcript, mRNA splicing, cDNA, hybridisation, ORF, pseudogene, nonsense mutation, missense mutation, frameshift mutation
3. What is a Southern blot? A Northern blot? A Western blot?
4. What is a restriction enzyme?
5. What does RFLP stand for?
6. Briefly summarize the principle of the polymerase chain reaction (PCR). Why are extensive measures undertaken to avoid amplicon contamination?
7. What is meant by autosomal dominant, autosomal recessive, X-linked recessive inheritance.
8. Give two examples of disorders which show each of these patterns of inheritance.
9. Draw typical family trees illustrating these inheritance patterns.
10. Discuss the genetics (mode of inheritance) of mitochondrial DNA mutations.

Molecular Biology Practical (1)

Isolation of DNA. Get 10ml of your own anticoagulated blood. Use 0.5ml to prepare blood spots and use the remainder to isolate DNA by three differing methods using equal amounts of blood (Consult the SOPs for the methods). These being a crude “salting-out method, and two kit methods, one of which uses a column and one not. Dilute the DNA appropriately with water and measure the OD₂₆₀ and OD₂₈₀ absorbances in a UV spectrophotometer. Calculate the yield of DNA and work out the OD₂₆₀/OD₂₈₀ ratios. What ratio would you expect for DNA of high purity. Check the integrity of your DNA by running 2µg from each preparation on a 1% agarose gel.

Restriction enzyme digestion.

You will be given 5µg of an unknown sample of DNA. Digest this with 10units of EcoRI according to the manufactures instructions. Run your digested sample alongside molecular weight marker DNA (Lambda phage DNA cut with Hind III) on a 1% agarose gel (refer to SOP) Place the gel on the UV transilluminator and use the gel-doc system to obtain a permanent record of the separated fragments. Use the molecular wt marker fragments to create a log-linear plot of fragment size vs distance travelled and work out the size (in bp) of each fragment from your unknown DNA. Using these data work out the original size of the unknown DNA you were given.

Molecular Biology Practical (2)

Microsatellite PCR

You have been given the nucleotide sequence of a small piece of Human DNA containing a microsatellite marker, which has been amplified by the Polymerase Chain Reaction (PCR) technique. The sequence of this microsatellite, and accompanying data was obtained from

the Genome Database.

Refer to the sequence given below and answer the following questions:

- a. What do the taxonomic terms Primates and Hominidae stand for?
- b. What is the length (in bp) of the DNA sequence you have been given?
- c. Locate a cutting sites for the restriction enzymes *Bti* *YI* (CC (N)₉ GG and highlight it in the sequence.
- d. How many times does the microsatellite repeating unit repeat itself?
- e. Use the rules relating to primer design and choose sequences for a forward and reverse primer pair that you could use to amplify this microsatellite. Position your primers such that your PCR product is >150bps (GREATER than 150bps) in length?

Please ensure: - that your forward and reverse primer Tms are between 60 and 65°C

- that their Tm's lie within 4°C of each other
- that their GC content is > 50%
- that their length lies between 18 and 28 bases

Write out the sequence of each primer in the 5'Æ3' direction and calculate the GC content and Tm for each.

- a. Use a highlighting pen and HIGHLIGHT YOUR PCR PRIMER SEQUENCES ON THE APPENDIX 1 SEQUENCE. Work out the length of the PCR product derived from your new primers and record your answer?
- b. If you cut your PCR product with the enzyme *Apa* *I* (CCC/GGG) how many fragments would you get and what would be their sizes? This enzyme gives you blunt ends.

Appendix 1

Question

DEFINITION	H.Sapiens (D19S417) DNA segment containing a microsatellite repeat
KEYWORDS	DNA microsatellite marker, repeat polymorphism
SOURCE	Human
ORGANISM	Homo sapiens; Eukaryota; Chordata; Vertebrata; Mammalia; Primates; Hominidae; Homo

Given below is the nucleotide sequence of a small piece of human DNA containing a microsatellite marker, which has been amplified by the polymerase chain reaction (PCR) technique. The sequence of the forward and reverse primers, used to amplify this piece of DNA, have been underlined. The sequence of this microsatellite marker and the accompanying data was obtained from the Genome database

Wed Jun 16 15:28 2004 c:\generunr\work\faked1~1.txt Page No. 1

```
5'          11          21          31          41          51
1  AGAAACAGAA CCAGAGCGAG ATCAAAATAG AGATGAGGAC AGGCACAGTG ACTCACACCTT
   TCTTTGTCTT GGTCTCGCTC TAGTTTTATC TCTACTCCTG TCCGTGTCCAC TGAGTGTGGAA

5'          71          81          91          1          11
61 GTAATCCCAG CACTTTGGGA GGCCAAAGCA GGTGGTTCAC CTGAGGTCAA GAGTTCGAGA
   CATTAGGGTC GTGAAACCCT CCGGTTTCGT CCACCAAGTG GACTCCAGTT CTCAAGCTCTT

5'          31          41          51          61          71
121 TCAGCCTGGC CAACATGGTG AACCCGTGTCT CTAGGGCCCA AATACATGCA CACACACACA
   AGTCGGACCG GTTGTACCAC TTGGGACAGA GATCCCGGGT TTATGTACGT GTGTGTGTCTT

5'          91          1          11          21          31
181 CACACACACA CACACACACA CACGAATTC CGGGATGCAG AGGACTTGAG GCAGAGAAAT
   GTGTGTGTGT GTGTGTGTGT GTGCTTAAGG GCCCTACGTC TCCTGAACTC CGTCTCTTTA

5'          51          61          71          81          91
241 TCAGAGCATG GCAGAGTCAT GGTGGCGGGC GCCTGTAATC CCAGCAGCTA AAGAGGAAAA
   AGTCTCGTAC CGTCTCAGTA CCACCAGCCG CGGACATTAG GGTCGTCGAT TTCTCCTTTT

5'          11          21          31          41          51
301 ATAATTTTAT GCCAGGTATT ATGTGATGGA GACAGAAATT CAAGATTGAG ACAAAATCGA
   TATTAATAA CGGTCCATAA TACACTACCT CTGTCTTTAA GTTCTAACTC TGTTTTAGCT

5'          71          81          91          1          11
361 GGTGCAT
   CCACGTA
```

Molecular Biology Practical (3)

Do either of these:

Use your own DNA to determine your carrier status for the deltaF508 mutation in the CFTR gene (The carrier frequency of cystic fibrosis is 1/25 in individuals of European ancestry). Think this through carefully; what will this mean to you if carrier status is confirmed?

Alternatively, you will be provided with blood from a patient with a request for known

mitochondrial DNA mutations, i.e. MELAS, MERRF and NARP. Note there are differences in diagnosing mutations in mitochondrial DNA compared to nuclear DNA.

1. Extract the DNA using the protocol currently in use in the laboratory.
2. Find the appropriate primers for amplification of the selected gene
3. Work out which restriction enzymes are appropriate to aid diagnosis. What fragment sizes would you expect?
4. Perform these PCR's and digestions according to the protocol. Run the products on an appropriate gel, with (where possible) a positive control, molecular weight marker and undigested DNA.