1.0 General

1.1 Introduction

The medical laboratory provides and interprets analytical and morphological information to assist in the diagnosis of clinical problems and the monitoring of disease progress and treatment.

The provision of a legible and appropriate clinical history on the request form, together with a properly collected specimen, allows the laboratory to issue relevant and accurate results, and to assist the clinician in the interpretation of these results in the clinical context.

1.2 The Request Form

1.2.1 The patient identification section of the request form must be completed with care, and the information on the specimen must correspond to that on the request form.

1.2.2 The importance of correct identification is most clearly illustrated by the potentially lethal consequences of mis-identification in the area of blood transfusion, but serious repercussions can occur in all areas of investigation.

1.2.3 DNA genetic testing (molecular genetics) is another situation where a clerical error can lead to a serious situation since a genetic disorder may be excluded or falsely associated with an individual and/or family members and this error may not be detected for many years.

1.2.4 Laboratories do (and should) reject samples where the identification data on the form and/or specimen are incomplete or inconsistent.

1.2.5 The request form must include appropriate clinical information, including medications.

1.2.6 Any difficulty in obtaining the specimen should also be noted on the request form.

1.2.7 Request forms for microbiology should include comment on antibiotic therapy.

1.2.8 The quality of the information provided to the laboratory directly affects the quality of the interpretation provided to the requesting clinician.
1.3 Timing of Collection

1.3.1. Specimens for some tests must be collected with the patient fasting, or with knowledge of when food was last taken (eg, glucose).

1.3.2. Some tests must be collected in the basal state or with due regard to diurnal variations.

1.3.3. Hormonal suppression or stimulation tests require accurately timed collections.

1.3.4. Blood for drug monitoring assays is usually collected as a 'trough' (pre-dose) sample, but in certain cases (eg, flucytosine) a 'peak' sample is required.

1.3.5. Some tests may be performed only after prior arrangement with the laboratory eg, lymphocyte function studies, platelet function tests, glucose tolerance tests.

1.4 Special Collections

1.4.1 There are specific collection requirements for some tests; where doubt exists, the laboratory should be consulted.

1.4.2 Some tests must be collected (and transported) at a specific temperature eg cold agglutinins test at 37°C; complement studies at 0°C (with the specimen container on melting ice).

1.4.3 Blood for some tests (eg, platelet function studies) should be collected by laboratory staff.

1.4.4 Blood taken for DNA diagnosis of genetic disorders (molecular genetics) requires particular care.

1.4.5 Anticoagulants (eg, EDTA or citrate) are used and the blood should be kept cool, particularly if there will be a delay in transport.

1.4.6 Some laboratories recommend that blood for DNA testing in the case of genetic disorders should be collected on two separate occasions to reduce the errors related to sample taking and laboratory processing.

1.4.7 This is particularly relevant to DNA testing for predictive (presymptomatic) or carrier testing since an individual will usually have no clinical parameters to guide the requesting doctor.

1.4.8 Thus both false negative and false positive results will have serious consequences for the person who is tested and his/her family.

1.4.9 See Annex A for discipline specific collection criteria.
1.5 **Blood Collection**

1.5.1 Blood should never be collected from a vein proximal to an infusion site.

   a) If collecting directly from an indwelling line, the first few mL of blood should be discarded and a note of the collection site made on the request form.

   b) If a heparinised syringe is used (eg, for arterial blood gases) the sample must not be submitted for coagulation studies.

1.5.2 **Technique**

   a) Venous stasis (tourniquet application) should always be minimised.

   b) Cell counts, and the levels of proteins (including enzymes) and protein bound substances (eg. calcium, cholesterol, many drugs) will be increased by prolonged or excessive venous stasis.

   c) Venepuncture should be clean and atraumatic.

   d) If difficulty is experienced, the attempt should be abandoned.

   e) A second venepuncture (preferably by a more experienced collector) should be attempted with a new needle and syringe, or evacuated container, at a different site.

1.5.3 **Tubes**

   a) Blood must be added to the tubes immediately but gently and without frothing.

   b) If a syringe with needle is used, the needle must be removed (see Safety below) before adding blood to the specimen tubes.

   c) If tubes containing anticoagulant are used, the correct amount of blood must be added to the tube (usually indicated by a mark on the label) and mixed immediately by thorough, but gentle, inversion.

   d) Tubes should never be shaken and blood should never be poured from one container to another.

   e) Blood culture specimens should, if possible, be collected from a separate venepuncture site. If a single venepuncture is necessary, the blood culture bottles must be inoculated first.

   f) The needle should then be removed for addition of blood to the remaining specimen tubes.

   g) Specimen tubes should be labeled immediately after the specimen is collected.
1.5.4 Safety

a) All blood samples must be treated as potential infection risks.

b) Care should be taken to avoid over-filling of tubes which is likely to be associated with leakage of blood and contamination of the external surface of the container.

c) Needles must be disposed of with care into a ‘sharps' container.

d) Syringes, swabs, or any other blood contaminated materials must be placed in an appropriate contaminated waste container immediately after use.

e) Evacuated collection systems are now frequently used for blood collection as there is less chance of blood spillage and thus exposure to blood-borne diseases.

1.5.5 Specimen transport

a) Blood samples should be transported to the laboratory in biohazard bags with minimum delay.

b) If delay is inevitable it is generally better to refrigerate samples in the interim.

c) However refrigeration may itself cause artefactual changes in the results.

1.5.6 Electrolytes

a) Blood for electrolytes should not be refrigerated; if delay is anticipated, plasma should be separated and stored at 4°C. Blood cultures and CSF specimens for culture must not be refrigerated.

b) Unseparated samples of blood must never be frozen.

c) Samples should not be subject to temperatures of >25°C, even for short periods.

d) Some tests involve especially labile components (eg, complement) and blood must be transported to the laboratory immediately.

e) See Test List for Special Collection Criteria for Clinical Chemistry in Annex A
1.5.7 **Microbiological examination**

a) Specimens for microbiological examination must be appropriate eg, sputum rather than saliva.

b) In general, specimens should be collected into, and transported in, a sterile container.

c) Wherever possible, samples should be collected before any anti-microbial therapy is started to avoid inhibiting the growth of the microorganisms.

d) Sterile apparatus and aseptic techniques to collect the samples. Avoid the introduction of indigenous microorganisms during the invasive procedures.

e) Aspirated pus may be transported in a syringe, which must be capped immediately the needle has been removed and disposed of safely.

f) Specimens should be delivered promptly to the laboratory, preferably within 2 hours.

g) Although many specimens will tolerate a delay of several hours if refrigerated, cerebrospinal fluid must be transported to the laboratory immediately, without refrigeration.

h) Similarly, for the detection of *Neisseria gonorrhoeae* and other fragile organisms, special arrangements may be needed: eg, express delivery, inoculation of plates at the time and place of collection, provision of special transport containers.

i) Blood for culture should not be withdrawn through an indwelling intravenous or intra-arterial catheter unless it cannot be obtained by venepuncture.

j) Special requirements, for individual tests, are noted in the Test listing.

1.6 **Histopathology & Cytopathology**

The correct handling of specimens submitted for morphological assessment is critical. When in doubt consult the pathologist. Complete patient data and pertinent clinical information must be included on the request form.

1.6.1 **Light Microscopy**

a) Formalin (10%) fixation is suitable for nearly all specimens where routine light microscopy is required. It is imperative that only fresh, neutral, buffered formalin is used otherwise fixation will be suboptimal.
b) Tissue should be completely covered by fixative and sent in a container large enough to accommodate the specimen in ten times its volume of fixative.

c) If there is likely to be delay before receipt by the laboratory large specimens should be carefully sliced and viscera should be opened.

d) Some laboratories (particularly when on site) prefer some specimens to be submitted fresh and unfixed, in tissue culture medium, to allow sampling of lesions for special procedures when appropriate.

e) This is particularly important when cell phenotyping or flow cytometry is needed for diagnosis (eg, lymph node biopsy for lymphoma) or when hormone receptor analysis is to be performed.

1.6.2 Electron Microscopy

a) The pathologist should be consulted prior to collection and submission of tissue for electron microscopy, to allow timely and appropriate processing of the specimen.

b) Specimens should either be transported rapidly and submitted fresh to the laboratory or dissected by laboratory staff at the time and place of collection eg, renal biopsies.

c) Glutaraldehyde fixative is commonly used and must be prepared fresh.

d) If special fixatives are unavailable fresh neutral buffered formalin may be substituted.

e) Rapid fixation is critical to the success of the procedure.

1.6.3 Immunohistological Examination

a) Formalin fixation is suitable for many immunohistological procedures although some are best performed using frozen sections of fresh tissue.

b) Fresh tissue can be transported to the laboratory using various transport media eg, tissue culture media.

1.6.4 Cytological Examination

a) Smears, brushings, washings, fluids, effusions and tissue imprints on glass slides may be submitted.

b) Adequate slide preparation requires experience in smearing material on to slides, and careful fixation.
c) For most specimens wet fixation is suitable. This must be immediate (within seconds) by immersion in alcohol solutions (95% ethanol is optimal) or by spraying with commercially available aerosol preparations.

d) Air-dried smears may be more satisfactory for some purposes and the pathologist will advise regarding these.

e) Fluids from serous effusions should be collected in plain sterile containers.

f) Such fluids are best sent immediately to the laboratory but may be stored refrigerated at 4°C overnight.

g) Cells in other fluids degenerate rapidly, even if refrigerated - this applies particularly to CSF, urine and gastric or bronchial washings - these specimens must reach the laboratory without delay.

h) Cells in sputum degenerate only slowly and specimens may be kept satisfactorily at 4°C for several days if necessary.

i) Fine needle aspiration biopsy should be performed only by individuals experienced in the technique.

j) Ancillary investigations such as stains for organisms, culture, biochemical tests, immunohistology and electron microscopy can be performed on some specimens.

2.0 References

2.1 RCPA Manual
2.2 SGH Service Manual
2.3 NUH Service Manual
Annex A

Guidelines on Special Instructions on Sample Collection and Handling for Patient Preparation and Time Sensitive Tests

1.0 Clinical Chemistry

Special instructions are critical to ensure patients have been well prepared prior to specimen collection. Patients’ medical history and medication status have a direct effect on the interpretation of tests results. This list of critical tests mentioned below serves as guidance and is not exhaustive.

1.1 Patient preparation prior to sample collection, such as fasting, food intake restrictions or physical exertions:
   a) Aldolase
   b) Ammonium chloride load test
   c) Beta-2-Microglobulin, Urine
   d) C-Peptide
   e) Catecholamines, urine
   f) Cortisol
   g) Creatine Kinase
   h) Dopamine, urine
   i) Ephinephrine, urine
   j) Glucose Tolerance Test
   k) Gastrin
   l) Homovanillic acid, Urine
   m) Homocysteine
   n) Insulin
   o) 4-Hydroxy-3-methoxymadelic acid, urine
   p) 5-hydroxy Indole Acetic Acid, urine
   q) Lipids
   r) Metanephrines, urine
   s) Norepinephrine, urine
   t) Normetanephrine, urine
   u) Parathyroid hormone, intact
   v) Theophylline (Aminophylline)
   w) Triglycerides
   x) Xylose Absorption test

1.2 Medication dosage timing prior to sample collection:
   a) Amikacin
   b) Cyclosporine A monoclonal blood
   c) Digoxin (Lanoxin)
   d) Gentamicin
   e) Valporic acide (Depakene)
   f) Vancomycin
1.3 Time sensitive tests:
   a) Amino acids (CSF, Plasma, Urine)
   b) Ammonia (Plasma)
   c) Beta-Crosslaps (CTx), Plasma
   d) Blood gases
   e) Calcium, Ionic
   f) Cupronic Test, urine
   g) DNPH (Dinitrophenyl Hydrazine) test, urine
   h) Galatose-1-Phosphate Urindyl Transferase (Gal-PUT), blood
   i) Glucose
   j) Homocysteine, plasma
   k) Homogentisic acid, urine
   l) Lactate (CSF, Plasma)
   m) Methaemoglobin, quantitative, blood
   n) Methylmalonic acid, urine
   o) Mucopolysaccharides, urine
   p) Nitroprusside test, urine
   q) Nitrosonapthol, urine
   r) Oligosaccharide, urine
   s) Organic acids, urine
   t) Osteocalcin, N-Mid, plasma
   u) Parathyriod hormone, intact
   v) Pyruvate (Pyruvic acid) (blood, CSF)
   w) Reducing sugar/substance, urine
   x) Silver nitroprusside test, urine
   y) Sulphite screening test
   z) Urine phase contrast microscopic examination
       aa)Vitamin E

1.4 Light sensitive samples:
   a) Bilirubin
   b) Folate
   c) Porphobilinogen, qualitative, urine
   d) Uroporphyrin, qualitative, urine
   e) Vitamin E
   f) Vitamin B12

1.5 Sample collection technique such as avoiding haemolysis:
   a) Alanine Transaminase (ALT, GPT)
   b) Aspartate Transaminase (AST, GOT)
   c) Homocysteine, plasma
   d) Lactate Dehydrogenase
1.6 Temperature sensitive samples. Following samples are required to be chilled (2°C to 8°C):
   a) Adrenocorticotropic Hormone (ACTH)
   b) Ammonia
   c) Arterial Blood Gases
   d) Gastrin
   e) Lactate
   f) Parathyroid hormone, intact
   g) Renin

2.0 **Haematology**

2.1 Patient preparation prior to sample collection, such as fasting, food intake restrictions or health status:
   a) Platelet Function Test
   b) Protein S Antigen, Total
   c) Protein S Antigen, Total
   d) Haemoglobin, Unstable
   e) Ham’s Test

2.2 Medication dosage timing prior to sample collection:
   a) Anti-thrombin III, Functional Plasma
   b) Bleeding Time
   c) Protein C Assay (Functional)

2.3 Time sensitive tests:
   a) Nitroblue Tetrazolium Test (NBT Test)
   b) Osmotic Fragility

3.0 **Immunology**

3.1 Time sensitive test:
   a) Total serum complement (CAE)