

2. Sample Collection

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2.1 Introduction to Chemical Pathology:

Chemical Pathology is a component of Pathology which mainly deals with chemical analysis of body fluids to identify specific molecular constituents. This component is more commonly known as 'Biochemistry'.

The most commonly received sample is blood, followed by urine and cerebrospinal fluid, and occasional requests are on other body fluids such as peritoneal fluid and pleural fluid.

The molecules of clinical interest, is wide, thus broadening the range of tests to be established in a Chemical Pathology laboratory. In addition, the concentrations of the different molecules found in health, have a wide range.

Eg. Glucose **mmol/L (10^{-3})**
Cholesterol **mmol/L (10^{-3})**

Bilirubin **$\mu\text{mol/L}$ (10^{-6})**
Iron **$\mu\text{mol/L}$ (10^{-6})**

Thyroxine **nmol/L (10^{-9})**
Cortisol **nmol/L (10^{-9})**

PTH **pmol/L (10^{-12})**

Measurement of molecules found in lower concentrations is generally more expensive as more exacting techniques have to be used.

2.2 Reporting in SI units

In order to have uniformity and easy comparability, it is recommended that all clinical laboratories of the country adopt the SI unit system (System Internationale) for reporting the results.

Eg: SI unit - mmol/L; Conventional unit – mg/dl.

(The relationship between these two, and conversion factors for commonly used tests are annexed for the user's convenience.)

2.3 Sample collection

Sample collection is often similar, requiring a minimum volume of 2 ml for a single test.

The required volume can vary depending on the facilities available in the laboratory and the method of analysis.

More sample volume is required for manual analysis and a fully automated analyzer will perform with micro volumes of sample. With automated equipment having 'random access' facility, several tests can be done on the same sample of about 3-4 ml.

2.4 Responsibilities of the clinical staff regarding Optimum utilization of Chemical Pathology services

2.4.1 Before analysis

- Filling up a Chemical Pathology Request form
- Preparation of patient for sample collection
- Instructions to patients regarding collection of urine samples
- Preparation of sample containers
- Collection of sample
- Labelling of sample
- Preparation of samples for transport
- Transport of sample to the local laboratory
- Documentation
- Other

2.4.2 After analysis

- Collection of report from the laboratory

- Scrutinization of report by the clinician and appropriate action
- Filing of report in patient's record
- Maintenance of records in the ward / clinic
- Patient's personal record of investigations, given at discharge (including negative results)

2.5 Tasks to be completed before analysis

2.5.1 Fill up a Chemical Pathology Request form:

- This is a duty of the medical officer looking after the patient. Other categories of staff are not authorized to fill pathology request forms.
- It should be **completely** filled in his / her **own handwriting**.
(Name, age, sex, ward /clinic, specimen type, date, **clinical reasons for request**, drug therapy)
- Patient's Registration no. (**BHT No.** / Clinic No.) should be **accurate**.
- It should be duly signed, indicating the designation of the medical officer.
- The requesting officer's name should be written **legibly**, under the signature.
- The name of the requested test/s should be clearly written.

- The requesting officer may write the telephone extension (or a contact number) for abnormal results to be rung through.
- Omission of destination (Ward No.) will result in loss of report.
- Deliberate substitution of samples for a genuine patient is a punishable offence.

2.5.2 Preparation of the patient for sample collection

A. fasting

Patients are kept fasting for certain tests.

Eg: Fasting plasma glucose, lipid profile, serum phosphorus.

However they should have a normal intake of drinking water to maintain hydration. (The only situation where patients are kept on an absolute fast without food or water is 'Water deprivation test' described in the guidelines on endocrinology.)

- Overnight fasting - 8–10 hours.
(A minimum of 6 hours – eg: children, and a maximum of 16 hours)
- Fasting for longer (10 – 12 hours) for lipid profile
- Investigation for hypoglycaemia – 3 day fast test (Special test – Discuss with the laboratory)

For certain tests where accuracy becomes important, it is desirable to take the sample after an overnight fast. Eg: serum calcium.

B. Drug therapy & Drug level

If certain drugs are known to interfere with the test result, it has to be omitted prior to sampling of blood. The duration depends on the half life of the drug. Seven half lives are needed to eliminate a drug from the blood.

Some tests are done to monitor the adequacy of therapy. Eg: thyroxine. Then the test has to be performed while the patient is on the medication. However, the time after the last dose becomes important in this situation.

If a drug level is being estimated for adequacy of dosage, normally trough levels are taken. Therefore take a blood sample just before the next dose. Otherwise, the requesting medical officer should advice on time of collection of sample.

2.5.3 Instructions to patients regarding collection of a sample of urine.

About 10 ml of urine is collected into a chemically clean container.

- early morning urine – collected from the first void
- Double voided urine – collected about 1-2 hours after the first void

- random urine – collected at any time of day
- spot urine - a sample of urine collected at the same time as blood.

Timed urine

All the urine passed within a stipulated time period is collected. The commonest is a 24-hour collection of urine. A prior appointment may be needed from the laboratory.

Steps.

- Obtain a container from the laboratory to collect urine. The container will have a preservative; do not wash it off. If a preservative is not recommended, the sample needs to be refrigerated during the collection period.
- Empty the bladder fully, discarding the urine – (usually at about 7 am) and note the time.
- Collect all the urine passed until the same time the next day (i.e 7 am on following day) into the container issued by the laboratory. (The patient may use a separate clean bowl to collect and transfer the urine sample in to the given container.)
- The final sample is passed at 7 am (in above example) and is collected into the container.
- Label the container (name, age sex, name of test, start time, finish time, date)

- Hand over the sample to the laboratory without delay.
- If a clearance test has been ordered (eg. Creatinine clearance), a blood sample too will be collected on submitting the urine sample.

2.5.4 Preparation of sample containers

The most commonly used sample container in Sri Lankan hospitals is a recycled injection vial ('penicillin bottle'). These are prepared by the nursing officers after washing, boiling and drying used injection bottles and washing and drying the rubber stoppers. Both urine and blood samples are sent in these bottles and the final volume therefore is < 6 ml.

In state laboratories where, evacuated blood (Vacutainer-type) tubes have been introduced, they are recycled several times by autoclaving, washing and drying in the laboratory. A tube with a rubber stopper and a sticker label is re-issued from the laboratory.

Type of anticoagulant or preservative for blood

3 types of specimen containers are used in the Chemical Pathology sections in Sri Lanka.

- Plain tube / bottle (No anticoagulant)
 - □or all estimations on serum
- □luoride-oxalate tube / bottle ('Sugar bottle')
 - □or estimation of Glucose level

- Oxalate tube / bottle
 - □or estimation of □ibrinogen level

Anticoagulated specimen containers are prepared and issued by the laboratory.

Containers for 24-hour urine collection are also supplied by the laboratory with the appropriate preservative, depending on the tests ordered. Where one preservative is not suitable for all the ordered tests, a patient may have to collect on 2 different days, using 2 different preservatives.

2.5.5 Blood collection:

Venepuncture – Collection of blood from a vein

Arterial puncture - Collect blood from an artery

Skin puncture – Collect capillary blood from the tip of the finger (or sole of the babies).

Venepuncture should be performed by a medical officer, a trained nurse or a trained blood-collector (phlebotomist). Arterial puncture can only be performed by an experienced medical officer. Skin puncture may be performed by any of the above or even the patient himself after training.

Blood collection is done as an aseptic procedure. 2 methods are used.

- Collected into a syringe and divide into the containers (tubes / bottles) in the recommended volumes.

- Collect directly into evacuated tubes with appropriate anticoagulants. If there is more than one tube to be collected, a blood collection tube holder and a separate needle for use with multiple collections can be conveniently used. □ or anticoagulated tubes, the sample volume is decided by the vacuum in the tube. Short sampling is not recommended as the ratio of blood:anticoagulant can vary.

All specimens taken into an anticoagulant, should be mixed immediately by gentle rotation or inversion.

2.5.6 Labelling of sample

The label should contain the following information

1. **Name/s of test/s**
2. **Name of patient**
3. Age
4. Sex
5. **Registration number (BHT / Clinic)**
6. **Ward No. or Clinic name**
7. **Date of collection**
8. Time of collection

2.5.7 Preparation for transport to the local laboratory

The nursing officer should prepare the specimens for dispatch to the laboratory. The samples should be placed in rows in an empty box of injection vials or in a tube rack issued by the laboratory. The relevant specimen forms too should be kept in the same order as

the samples and clipped together. They should be put inside a polythene bag and transported with the samples without leaving any room for contamination by spillage of a specimen. All breakages and spills should be attended to in the approved manner.

24-hour urine collections should be sent separately, preferably wrapped in a polythene cover, after entering the exact times on the label (start and finish).

2.5.8 Transport to the local laboratory

Specimens should be sent to the laboratory without delay. If a delay is anticipated, whole blood may be kept at room temperature for about 2 hours. If there is a longer delay, the serum or plasma should be separated from the cells and stored in a refrigerator at 4°C.

Certain molecules are heat labile and need to be kept in a constant cold chain. Eg. Parathyroid hormone (PTH), ammonia, blood gases. The sample should be kept in melting ice and delivered to the laboratory rapidly (within 10 minutes). The blood collector should communicate with the laboratory for instructions on these special tests. (See endocrine tests).

2.5.9 Documentation

The nursing staff should maintain a record of all the specimens sent to the laboratory and verify the receipt of reports.

2.5.10 Other

The staff should cancel unwanted / inappropriate tests by phone as early as possible.

Eg. In the case of death of the patient,

Cancel requests sent for serum cortisol, if the patient had been given i.v. hydrocortisone before sample collection

2.6 Tasks to be completed after analysis

- A. Collection of report from the laboratory
- B. Scrutinization of report by the clinician and appropriate action
- C. Filing of report in patient's record
- D. Maintenance of records in the ward / clinic
- E. Patient's personal record of investigations given at discharge (including negative results)

A. **Collection of report from the laboratory**

Reports may be obtained in 2 ways.

- Delivered by the laboratory
Some hospitals have a report delivery system where the laboratory reports are delivered about 3 times a day at regular intervals. Urgent reports are collected by sending a ward labourer.
- Reports collected by the ward
A labourer will visit the laboratory to collect the reports. It is the duty of the laboratory to sort out the reports by ward number, for collection by the

ward labourer. They should be collected at least twice a day.

Stapling or clipping the reports together would prevent loss of reports.

B. **Scrutinization of report by the clinician and appropriate action**

The reports should be initially scrutinized by a medical officer or nursing officer for grave abnormalities and appropriate action taken.

The rest should be available when the patient is seen next.

C. **Filing of report in patient's record**

Reports should be filed in the patient's record in a chronological manner. Separate filing of reports from different sections of the laboratory too is a practical way of filing. If any test result is being monitored regularly, the nursing staff should maintain a separate chart for same.

If the patient is discharged by the time the report is received, the report should be filed in such a manner that it could be retrieved easily when the patient arrives at the follow-up clinic. If not they should be informed to come and collect the report.

D. **Maintenance of records in the ward / clinic**

The nursing staff should ensure that reports are received in time. They should check with the laboratory for delayed reports, before collecting fresh samples for

the tests where reports are not available. This will prevent unnecessary duplication of work for the ward and the laboratory.

E. Patient's personal record of investigations given at discharge

All patients on whom laboratory investigations were done, should be given a record of the test. This can be entered in the Diagnosis card issued at discharge.

It is important to give the patient a record of even the negative results of screening tests done and when they should be screened again. Eg. Plasma glucose is commonly done to screen for diabetes mellitus. Failure to give a report to the patient will result in repeated screening for same, each time the patient gets admitted to the hospital.

2.7 Annexure 1

GUIDELINES FOR SPECIMEN COLLECTION

| TEST | COLLECTION DETAILS |
|---|---|
| Acid phosphatase | 2 ml clotted blood |
| Alanine transaminase | 2 ml clotted blood |
| Albumin | 2 ml clotted blood |
| Alkaline phosphatase | 2 ml clotted blood |
| Alpha fetoprotein | 4 ml clotted blood / 1 ml serum |
| Ammonia | 2 ml into EDTA vacuum tube. Deliver within 10 min |
| Amylase | 2 ml clotted blood |
| Arterial blood gases | 1 ml heparinized arterial blood. Transport immediately in a sealed syringe in melting ice. |
| Aspartate transaminase | 2 ml clotted blood |
| Bicarbonate (venous)= TCO ₂ | Plain vacuum tube filled with blood. Deliver within 10 min. |
| Bilirubin (Total / Direct) | 2 ml into plain bottle. Protect from light. |
| CA 125 | 4 ml clotted blood / 1 ml serum |
| CA 15 . 3 | 4 ml clotted blood/ 1 ml serum |
| Calcium (Total) | 2 ml clotted blood taken without tourniquet into acid-washed tube or send in sealed syringe itself. |

| TEST | COLLECTION DETAILS |
|----------------------------|--|
| Calcium (Ionized) | Plain acid-washed vacuum tube filled with blood and transported in melting ice. Deliver within 10 min. |
| Calculi (renal) | Send dry calculus. No preservative. |
| Carcinoembryonic antigen | 4 ml clotted blood / 1 ml serum |
| Ceruloplasmin | 2 ml clotted blood |
| Chloride | Plain vacuum tube filled with whole blood |
| Cholesterol (Total) | 2 ml clotted blood |
| Cholesterol HDL | 2 ml clotted blood |
| Creatine kinase | 2 ml clotted blood |
| Creatine kinase MB | 2 ml clotted blood |
| Cortisol | Please see Endocrine |
| C-peptide | 2 ml EDTA blood + 2 ml fluoride oxalate blood (for glucose) Deliver within 10 min. |
| C-reactive protein | 3 ml clotted blood / 1 ml serum |
| Ferritin | 3 ml clotted blood / 1 ml serum |
| Fibrinogen | 2 ml oxalated blood |
| FSH | 3ml clotted blood |
| Free thyroxine | Please see Endocrine tests |
| Gamma glutamyl transferase | 2 ml clotted blood |

| TEST | COLLECTION DETAILS |
|--|---|
| Glucose (fasting / random) | 2 ml in fluoride-oxalate bottle |
| Glucose tolerance test | Please see Pathology Diabetes guideline |
| Glycosylated Hb | 2 ml EDTA blood into each of 2 tubes. |
| Growth hormone | Please see Endocrine tests |
| Beta human chorionic gonadotrophin (b-hCG) | Please see Endocrine tests |
| Hydroxy-methoxy mandelic acid (HMMA) | Please see Vanillyl mandelic acid (VMA) |
| Insulin | 4 ml clotted blood + 2 ml fluoride-oxalate blood |
| Iron/ TIBC(Total Iron Binding Capacity) | 4 ml clotted blood into acid washed tube or send in the syringe itself. Collect in the morning. |
| Lactate dehydrogenase | 2 ml unhaemolysed clotted blood |
| Lithium | 2 ml clotted blood |
| Luteinizing hormone(LH) | 3 ml clotted blood |
| Magnesium | 2 ml unhaemolysed blood into acid-washed tube. |
| Myoglobin | 3 ml clotted blood |
| Oestradiol | 3 ml clotted blood |
| Osmolality | 2 ml clotted blood |
| Paracetamol | 5 ml blood into heparin bottle |
| Phosphate | 3 ml clotted blood (Ideally fasting, unhaemolysed sample) |

| TEST | COLLECTION DETAILS |
|---------------------------------|---|
| Potassium | 2 ml clotted blood(unhaemolysed sample) |
| Progesterone | 3 ml clotted blood |
| Prolactin | 3 ml clotted blood |
| Protein | 2 ml clotted blood |
| Protein electrophoresis | 2 ml clotted blood (Plasma is not suitable) |
| PSA (prostate specific antigen) | 3 ml clotted blood |
| PTH (parathyroid hormone) | 5 ml clotted blood send within 10mts or rapidly centrifuged and serum frozen. |
| Sodium | 2 ml clotted blood |
| Testosterone | 3 ml clotted blood |
| Thyroxin (T4) | 3 ml clotted blood |
| Triglyceride | 3ml clotted blood (12hr fasting) |
| Tri iodothyronine | 3 ml clotted blood |
| Troponin I | 2ml clotted blood |
| Troponin T | 2ml clotted blood |
| TSH | 3ml clotted blood |
| Urea | 2 ml clotted blood |
| Uric acid | 2 ml clotted blood |

2.8 Annexure 2

Conversion Factors.

| Test | SI unit | Conversion Factor | Conventional unit |
|-----------------------|---------|-------------------|-------------------|
| Albumin | g/L | 0. 1 | g/dl |
| Bicarbonate | mmol/L | 1 | mEq/L |
| Bilirubin | µmol/L | 0. 058 | mg/dl |
| Calcium | mmol/L | 4 | mg/dl |
| Chloride | mmol/L | 1 | mEq/L |
| Cholesterol | mmol/L | 38. 6 | mg/dl |
| Cortisol | nmol/L | 0. 036 | µg/dl |
| Creatinine | mmol/L | 11. 3 | mg/dl |
| Glucose | mmol/L | 18 | mg/dl |
| Iron | µmol/L | 5. 6 | µg/dl |
| Iron Binding Capacity | µmol/L | 5. 6 | µg/dl |
| Lactate | mmol/L | 9 | mg/dl |
| Magnesium | mmol/L | 2. 4 | mg/dl |
| Oestradiol | µmol/L | 27. 24 | µg/dl |
| Osmolality | mmol/kg | 1 | mosmol/kg |
| PCO ₂ | kPa | 7. 5 | mmHg |
| PO ₂ | kPa | 7. 5 | mmHg |
| Phosphate | mmol/L | 3. 1 | mg/dl |
| Potassium | mmol/L | 1 | mEq/L |
| Protein (Total) | g/L | 0. 1 | g/dl |
| Sodium | mmol/L | 1 | mEq/L |
| Testosterone | nmol/L | 28. 8 | ng/dl |
| Thyroxine | nmol/L | 0. 078 | µg/dl |
| Triglyceride | mmol/L | 88. 5 | mg/dl |
| Tri iodo thyronine | nmol/L | 65. 1 | ng/dl |
| Urate | mmol/L | 16. 8 | mg/dl |
| Urea | mmol/L | 6 | mg/dl |

Reference: Walmsley RN,White GH.A Guide to Diagnostic Clinical Chemistry.London:Blackwell Science Ltd,1994.

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