

Overview of the Pathology department

The pathology department has many divisions that work together and complement one another, as well as having multidisciplinary connections to departments such as endocrinology. The teams meet when necessary to discuss complex patients and to gain a second opinion when necessary.

PAN – pre-analytical area

This is the first port of call for pathology samples, receiving samples from other areas of the hospital or from GP clinics in the county. This is an important area for initial sorting and prioritising of samples, and could have a large effect on outcomes if there were errors or delays in this area.

- GP samples are subject to a target of 24 hour turnaround, with results aimed to be received by GPs by 9pm the same day.
- Yellow pods are internal – received from phlebotomy, haematology or biochemistry.
- Red pods are external – received from various areas of the hospital.

Samples must be closely checked and compared to recorded data, to ensure there is never a mix up between patients. The pathology department as a whole has a strict protocol on rejecting samples which do not match exact patient details and for products related to blood transfusion, all labels must be handwritten and signed. There is a colour system in place in order to ensure samples go to appropriate departments.

Red bags – Haematology

Yellow bags -- Cytology

Blue bags – Microbiology

Green bags – Chemistry

White bags – Histology

HEALTH AND SAFETY

All labels must be checked against patient details – any errors or mismatches must be checked, and rejected if appropriate.

All samples must have a minimum number of patient details otherwise they may be rejected – this can include the patient's name, MRN number, address and date of birth.

Pre-printed labels on samples must be countersigned due to the increased likelihood of accidental mix up of patients when using stickers.

Blood transfusion related samples/products must always be hand written and signed – no pre-printed labels may be used.

BIOCHEMISTRY

The biochemistry department performs chemical analysis on various patient samples including blood, urine and faeces in order to perform, in answer to various clinical queries. They handle

Tests performed

Chemical analyser – Cobas 8000

- Cobas c 702 - performs 2000 tests per hour, for generic chemical tests.
- Performs tests such as creatine levels, liver function tests, urea tests, alkaline phosphatase, alanine aminotransferase.

Immunological analyser – Cobas e 602

- Tests for troponin, tumour markers, B12, folate, hormones, drugs and complex proteins.
- Performs immunoassays, where cross reactivity is high. Therefore, all tips are disposable rather than using steel probes, to stop cross contamination of samples.
- Nothing is reused in immunoassays.

HbA1C testing – Menarini

- Boronate affinity chromatography.
- Tests for glycated haemoglobin, in the diagnosis and monitoring of diabetes.
- Tests should not be repeated more than every 3 months due to the life span of a red blood cell being approximately 120 days.

Point of care testing

1. Glucose testing

- Used in areas such as maternity, A+E, ICU, paediatrics and DCC.
- Provide on the spot values for blood glucose – results in 6 seconds.
- Approximately 190 hand held blood glucose monitors in the trust, which need calibrating and regular monitoring of their performance.
- New electronic machines being brought in by the trust – these report to PAS directly and are locked by a username/password system, which ensures quality control is performed every day before they can be used.

2. Blood gas machines

- Can test various elements in blood, such as sodium, potassium, glucose and pH – depends on the needs of the department where it is based.
- No longer EQA by the department due to number of machines in the trust.

3. HbA1c

- ALERE machine, used in paediatric outpatients. This provides a quick and instant result for HbA1C in the blood, giving the clinician an instant idea of how well controlled the patient's blood sugar levels have been. This is important in children as diabetes tends to be less well controlled, and therefore therapies can be adjusted quickly.
- Particularly important for young patients and newly diagnosed diabetics.

Results and clinical approval queue

Results from biochemistry can have wide impacts on patient care and outcomes, and any severely abnormal results must have appropriate attention brought to them. Therefore, patients with these kinds of results are put on a telephone list, and a member of the department will contact the referring clinician as soon as possible to report the abnormality. Examples of these abnormalities can include high potassium levels, high calcium levels or extreme levels of glucose (low or high), which can all be life threatening.

Tests performed in biochemistry can be for screening, monitoring or diagnostic. It is important that only appropriate tests are performed for the appropriate clinical need, and so some tests must be clinically approved by a clinical scientist before being performed. This may be due to test irrelevance to clinical need, or inappropriate retesting within a period that will not show a difference in results (such as HbA1C testing within 3 months, due to the life span of a red blood cell).

The ICE database allows clinicians to access full pathology history for their patients, and processes requests for testing. The system prompts clinicians when the testing they have requested is inappropriate, such as in the above scenarios, and therefore should reduce the amount of inappropriate referrals that will be received by the pathology department. However, at times the testing is necessary despite guidelines and therefore it is the job of the biochemist to decide whether to override the system.

Example: Pathology has recently seen an increase in referrals for vitamin D testing. The National Osteoporosis Society guidelines (2013) state that patients should only be tested when they show musculoskeletal symptoms of vitamin D deficiency. Overall, there is debate as to the usefulness of biochemical testing for the vitamin D levels in the blood, as the outcome will be the same for most patients. Even without a formal diagnosis of deficiency, vitamin D is a harmless supplement and therefore it is proposed that patients should be provided with the supplements without undergoing testing.

HAEMATOLOGY

Haematology is responsible for the 'prescription' of blood products, which can have potentially fatal outcomes if any mistakes occur. Therefore, there are high levels of scrutiny of patient details and samples to ensure there is no chance of a mix up, through methods such as handwritten labels and signed sticker labels.

Tests performed

Blood grouping

- Checks for ABO group.
- Further screens for antibodies including Rhesus, Kell, Duffy, Kidd, Lewis, P, MN and Lutheran. This is a more comprehensive check, and delays the cross matching, meaning the blood products for transfusion may be delayed.

Crossmatching

- Choose a donor unit of blood with the same ABO and RhD type as the patient.
- Test plasma of patient with red cells from donor unit.
- See figures 1 and 2 for examples of positive and negative reactions.

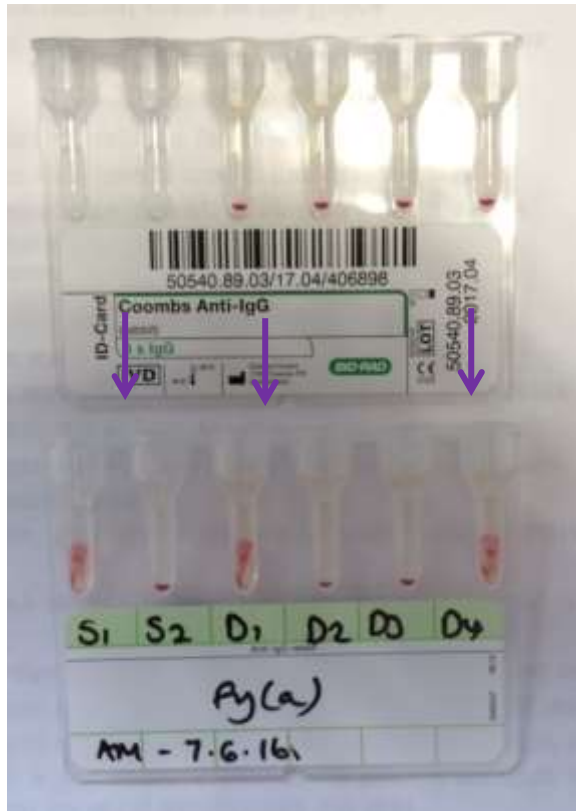


Figure 1: Photo of cross matching being performed. Purple arrows indicate positive reaction – red cells have reacted with anti-sera.

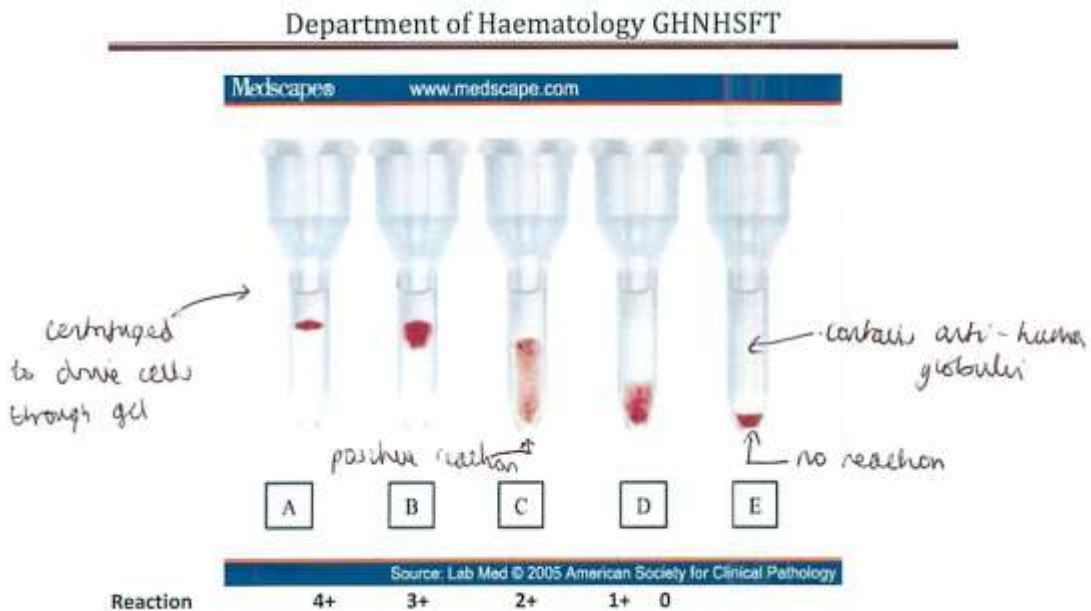


Figure 2: Scanned copy of annotated diagram demonstrating positive and negative results of cross matching.

Full blood count – Beckman Coulter DxH800

- Measures haemoglobin (Hb), red blood count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), neutrophils, lymphocytes, neutrophils, eosinophils, basophils.
- Three most important tests:
 1. Platelets – indicates bleeding problems if count is abnormal.
 2. Haemoglobin – low haemoglobin indicates the patient may be anaemic.
 3. White cells – these indicate infection and inflammation.
- See figures 3 and 4 for demonstrations of normal and abnormal FBC results.

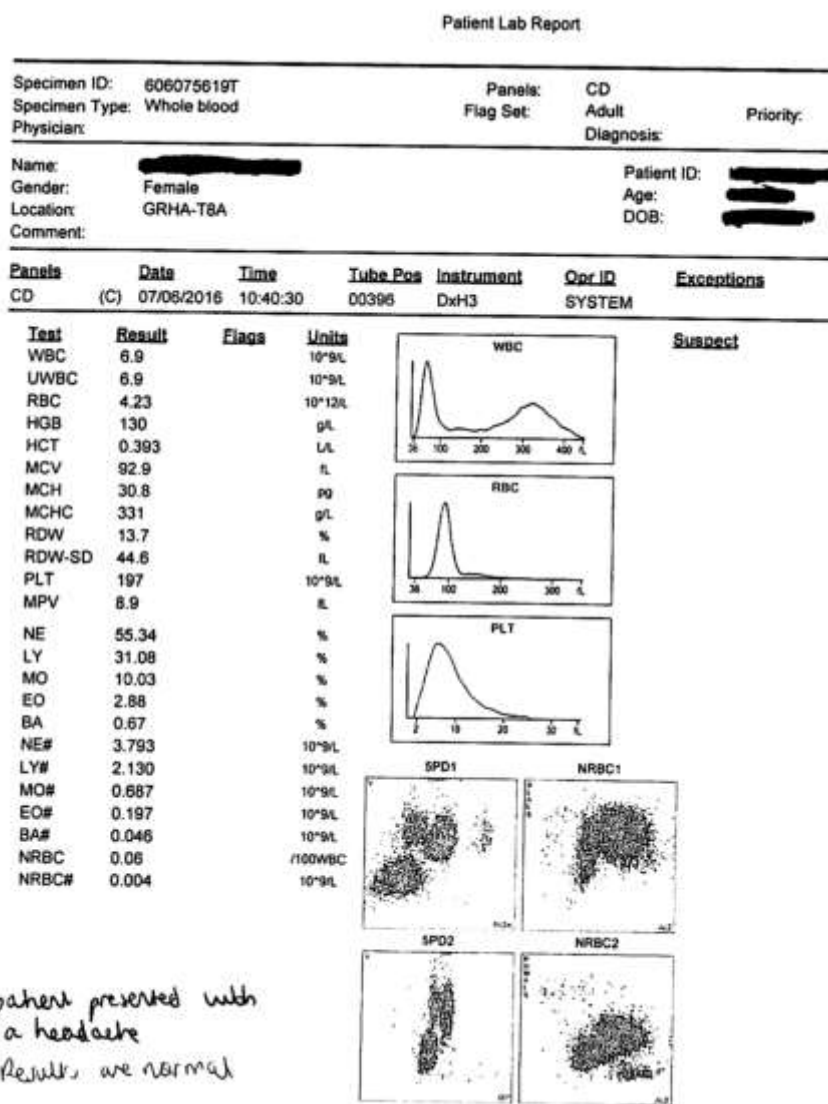


Figure 3: FBC results for a patient presenting with headaches, showing normal results

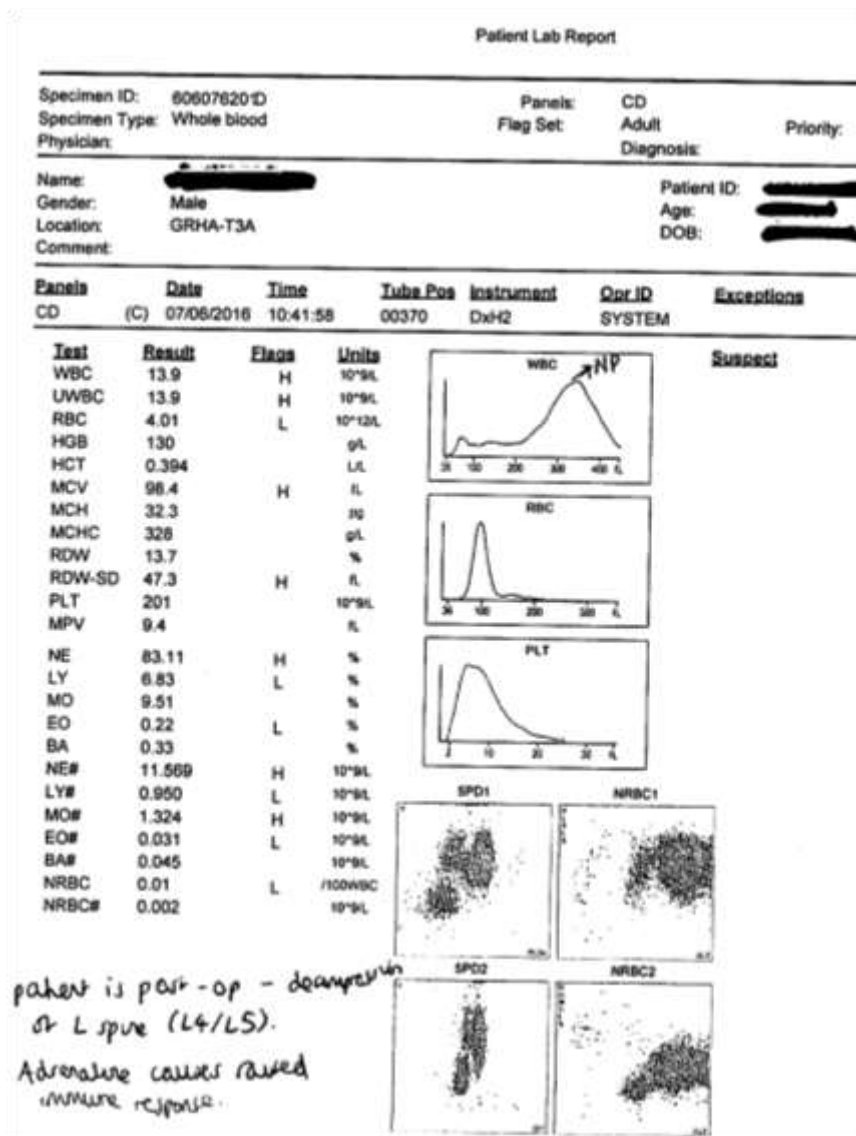


Figure 4: FBC results for a patient recovering from spinal surgery, showing abnormal results – both raised and lowered values than expected

Coagulation tests

Important to measure in order to define patients who are at risk of forming clots which can then cause myocardial infarctions or strokes, etc.

- Prothrombin time – measures coagulation factors in the extrinsic and common pathways of the clotting cascade (normal range = 11-14 seconds). Monitors the effects of long term use of anticoagulant drugs.
- Activated partial thromoplastin time - measures coagulation factors in the intrinsic and common pathways of the clotting cascade (normal range – 26-39 seconds). Used to monitor the effect of anticoagulant drugs.
- D-dimer test – negative predictor, optical or mechanical method. Produces results which indicate whether a patient has NOT had a coagulation 'event', but a positive result does not definitely mean a coagulation event has occurred.

- Wells scores are better predictors than D-Dimer tests for both pulmonary embolism and deep vein thrombosis, as D-dimers are more variable.

Blood transfusion

Hospital stocks red cells, fresh frozen plasma, platelets and cryoprecipitate. Donations are all checked for HTLV, HIV, Syphilis, Hep B and Hep C as a minimum. Those wishing to donate must not have visited a malaria affected area for the last 12 months nor had tattoo in the previous 6 months. The biggest users of blood products are cancer patients, surgery/preadmission and maternity.

Immunology

Performs tests to measure levels of immunoglobulins, such as IgG, IgA and IgM. These immunoglobulins will be raised in varying amounts in disease states, although deficiencies can also be measured.

Haemoglobinopathies

2 of the main diseases tested for are sickle cell anaemia and thalassaemia (please see other OLAT evidence for scanned copies of these tests).

Patient history is important for this testing, as both family genetics and geographic origin of the patient affect disease incidence.

MICROBIOLOGY

Microbiology is responsible for handling a wider variety of samples, including blood, sputum, cerebrospinal fluid, faeces, urine and nail, hair and skin samples. The department handles samples that are potentially infected with foreign or rare bacteria/viruses, and therefore it is even more important for precautions to be taken to reduce risk. Knowing the patient history is of particular importance in microbiology, and therefore referring clinicians are asked to provide information such as the patient's recent travel history or antibiotic use.

Tests performed

ELISA – enzyme linked immunosorbent assay

For C. difficile testing, two assays are used – one to test for the organism, and one for the toxin. The organism test is very sensitive and can sometime pick up non-toxic strains. If there is a positive result for the toxin, but a negative result for the organism, then the sample should be tested using PCR.

Lateral flow device tests

- Used in conjunction with ELISA assay.
- In ELISA tests for Giardia/Cryptosporidium, a lateral flow test is used afterwards to differentiate between which bacteria is present.

Blood culture

- Two samples taken from patient – one aerobic, one anaerobic. These samples are diluted and cultured for a number of days – positive responses normally occur between 2-5 days.

- A positive result appears as the test disk turning yellow – this indicates the presence of carbon dioxide, which alters the pH of the sample. Carbon dioxide is a product of metabolism, indicating that there is a microbe/bacteria etc. in the sample.

Gram staining

Smearing and staining of samples, and observed under microscope to check for bacteria/microbes. Samples will stain purple in the presence of Gram-positive bacteria. Samples will then go on to culture if necessary.

Plate culture

- Different agar plates used depending on clinical needs :
 - Mannitol Sugar – for pathogenic Staphylococci.
 - MacConkey's –for gram-negative bacteria and bacteria that ferment lactose.
 - Eosin Methylene Blue – for gram-negative intestinal pathogens.
 - Phenylethyl Alcohol – for gram-positive organisms.
 - Hektoen Enteric – gram-negative microorganisms.
 - Blood – for fastidious pathogens
 - Chocolate – for fastidious pathogens
- Swabs from various areas of the body, such as the mouth, ears and nose, are received and cultured to look for different bacteria, in order to guide clinicians on the appropriate antibiotics to be used to treat infections.

Serology

Serology works within microbiology to study blood serum, testing for diseases such as HIV, hepatitis, encephalitis, syphilis etc. The tests look for IgM, which indicates an acute or recent infection, and IgG, which indicates a previous infection. The department also performs TORCH screens (Toxoplasmosis, Rubella, CMV and Parvovirus) for the maternity/neonatal department. For diagnosis of major illnesses, such as HIV, double sampling is requested to be absolutely sure before providing a diagnosis.

HISTOLOGY

Histology processes 40,000 samples a year, including samples such as excised growths from GPs and whole organs from surgical teams. These samples are prepared and preserved, in order to create slides which are analysed by microbiologists, looking for any abnormalities such as cancerous cells.

Histological sample preparation

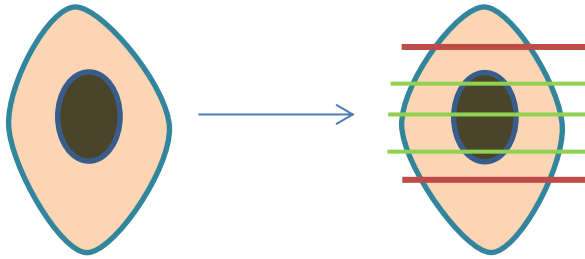
1. Samples initially arrive preserved in formalin.
2. Samples are checked and dissected to be placed into cassettes, and then preserved again in formalin.
3. Bone samples are decalcified using formic acid, otherwise sample preparation will simply cause the sample to shatter.
4. Cassettes are encased in wax, left to set, and then set in a water bath to allow the wax to melt enough to reduce creases.
5. Microtome used to create slices that are 4 microns thick.
6. Alcohol and water is used to rehydrate the sample.
7. The sample is then stained, nuclei will appear dark purple while eosin will appear pink.

8. This process takes approximately 24 hours*, and completed slides are then analysed by the consultants.

Delays in this process are caused by the need to perform decalcification for bone samples, MDT meetings to discuss complex cases and immunohistochemistry. It is important to weigh up the benefits of delaying against the consequences of waiting for results.

*Urgent samples – slides can be produced in one hour using a cryostat, for quick analysis e.g. during live surgery to check for cancerous cells.

Dissection of sample



Example of skin biopsy dissection: The red lines indicate areas that dissected off and discarded, and the green lines indicate where the cuts are made to produce slices to be placed into a cassette.

Cytology

Mostly concerned with investigating smear test samples from gynaecology, with results aiming to be processed and received by the patient in 2 weeks. The area of investigation is brushed and the fluid is collected, and the sample is processed to remove blood.

Human papillomavirus

Perform screening test – mostly automated using machines, apart from some samples, such as those with a large amount of blood in. The slides are analysed by two scientists for quality assurance, and the cells are given grades. Even if there are only minimal amounts of a grade of cell, the overall grading of the sample is based on the highest graded cell. Moderate/severe grades are suspicious and full HPV testing is performed.

No risk → normal recall	(8 week turnaround)
Low risk → HPV → colposcopy	(4 week turnaround)
High risk → colposcopy	(4 week turnaround or ASAP)

Guidelines for recall for screening is:

- 25+ years old – every 3 years
- 50 + years old – every 5 years

Bibliography

Roche Diagnostics (2013). Cobas 8000 modular analyzer series: Intelligent LabPower. Roche, Rotkreuz, Switzerland.

National Osteoporosis Society (2013). Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management. National Osteoporosis Society, Bath.