NHS Tayside: Abertay LSC303 BTS lab practical

Write Up deadline 05th December 2022

1. Sample Reception

At this station you will be following the procedures we use in the laboratory for every sample. This is to give you insight to our zero tolerance policy and the examples we come across on a day to day basis.

Minimum acceptance criteria are as follows:

- Forename match on sample and form
- Surname match on sample and form
- CHI/NHS number match on sample and form
- Date of birth match on sample and form

Please complete the following table.

Patient	Patient name	Sample accepted or rejected?	If rejected, why?
1	Elizabeth Masood	Accepted	
2	Alice Thompson	Accepted	
3	Ioana Martin	Accepted	
4	Finley Taylor	Accepted	
5	Leo Wilson/Charlotte Baker	Rejected	All personal details were mismatched.

2. a) Manual tube grouping

At this station you will be given a Standard Operating Procedure document (SOP) to follow. This contains all the information you need to process these samples.

Set up the manual tube group by following the SOP provided. The manual tube group is a forward group only.

Please fill in the following table with the appropriate reaction grades (e.g. 4+) for the tube groups.

Patient	Reaction grades- Tube Group					
	Anti-A	Anti-B	Anti-A, B	Anti-D1	Anti-D2	D Neg CONTROL
1	-	-	-	+	+	-
2	-	+4	+4	-	-	-
3	-	-	-	-	-	-
4	+4	-	+4	+4	+4	-

2. b) Manual card grouping

Pre-prepared card groups for each patient will be provided. Please fill in the following table with the appropriate reaction grades (e.g. 4+) for the card groups.

Patient	Reaction grades- Card Group					
	A	B	DVI-	Control	A1	B
1	-	-	+	-	+4	+4
2	-	+4	-	-	+4	-
3	-	-	-	-	+4	+4
4	+4	-	+4	-	-	+4

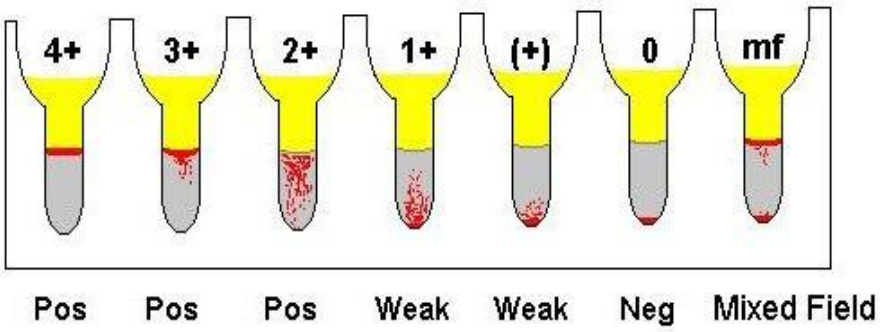
The card has a forward and a reverse group, does the reverse group match the forward group in your results? Yes, the reverse group match the forward group.

Did you get the same blood group in the card and tube group you performed for each patient? Yes, the groups were the same.

3. Antibody Screening and Panels

This station is where you will have a look at a 3 cell screen antibody panel and 11 cell antibody identification panel sheets. Using the guide below please fill in the panel sheets as appropriate to determine the antibody present. The 3 cell and 11 cell panels are both used in the laboratory to exclude and determine the antibody present. There is an explanation sheet provided to assist you.

Please fill in the table below for the initial 3 cell antibody screen with the appropriate reaction grades (e.g. 4+).



Patient	Cell 1	Cell 2	Cell 3	11 cell ID panel needed (Yes/No)
1	0	4	0	Yes
2	0	0	0	No
3	4	3	0	Yes
4	0	0	0	No

Please fill in the provided 11 cell antibody ID panel sheet with the appropriate reaction grades (e.g. 4+) for each patient that needs a further panel.

Use the panel sheet to identify the antibody specificity and complete the table below.

Patient	Antibody specificity Identified (Use N/A where not needed)
1	Anti-E/ Anti-Lu^a unable to exclude
2	N/A
3	Anti-Fy^a/ Anti-C^w Unable to exclude
4	N/A

4. Compatibility Testing

At this station you will be selecting the appropriate units for patients with red cell antibodies.
****These patients are unrelated to the previous patients in this workbook****

At your station you have a selection of 'blood packs' with different groups and phenotypes, these are labelled with letters e.g. Unit A.

Look at the table below and select suitable units for their compatibility testing.

If a patient has an antibody they must receive phenotyped blood that is antigen negative for the corresponding antibody – remember to also check expiry dates.

Also include units that could be selected if the patient's own ABO and RhD blood group is not available with compatible ABO and RhD status.

Patient blood group	Antibody detected	Units suitable for patient (List as A,B,C etc)
A RhD negative	Anti-D	I,F
O RhD positive	Anti-K	F, D, E
O RhD positive	Anti-c	D
O RhD negative	Anti-C	F
A RhD negative	Anti-E	I,F
A RhD negative	Anti-Jka	I
A RhD negative	Anti-Fya & Anti S	F
A RhD positive	Anti-S	A,F
A RhD positive	Anti-Fya	B,F

Write up

Explain in detail the reasons for zero tolerance sample acceptance criteria in blood transfusion, what patient samples you had to reject (if any) and why.

The zero tolerance acceptance criteria established in blood transfusion ensure that the patient is unambiguously identified on the laboratory database and enough patient details are shown in the sample label. This is mandatory to provide a better-quality service to the patients. The sample must be handwritten with the patients' full name, date of birth, patient identification number, ward or location, time and date of sample collection, and signature of the staff taking the specimen (NHS Manchester university). Patient 5 was the only one rejected because all personal details were mismatched.

Describe in detail how manual tube and card grouping works including what you are detecting in the forward and reverse groups. What were your patient's results and how were they confirmed?

ABO grouping is one of the main tests carried out on pre-transfusion samples. Two different methods are used for blood grouping, the reverse and forward group. The goal of this test is to find an unknown presence with a known entity. So, if an antigen wants to be detected, an antibody is used to find it. For this reason, an antigen and antibody are added to look for the absence or presence of agglutination as it is the cross linking of cells creating a clump. This test is called direct agglutination.

The forward group have preloaded monoclonal anti-B and anti-A reagents into its cells. After that, RBCs are added, and the presence of the corresponding antigens can be detected through no agglutination or agglutination. It is crucial to add RBCs to the blank control well to make sure the test is not defective, and any agglutination is generated genuinely because of an antibody-antigen reaction.

On the other side, the reverse group B and A1 reagents are put into the reverse wells. Like the forwards group, after plasma from the patient sample is added, the detection of antibodies in the patient's plasma can be done by looking at the level of agglutination. The purpose of the reverse group is to increase the confidence levels in the results and to allow the detection of unusual blood groups

The manual tube and card grouping results were the following. Patient 1 had negative results in every tube group except anti –D1 and anti-D2, meaning that the person is in the blood group of O RhD positive. Secondly, patient 2 had positive reaction of grade 4 in the anti-B and Anti-A,B tube groups, and consequently patient 2 is part of the B RhD negative blood group. Thirdly, patient 3 showed negative results in every tube, so this patient has a O RhD negative blood group. Finally patient 4 is A RhD positive as anti-A, anti-A,B, Anti-D1 and Anti-D2 were positive.

Describe the purpose of the 3-cell antibody screen and what you can determine from this. What were your patient's results, and which needed a further 11 cell identification panel.

The 3-cell antibody screen is used to identify antibodies and demonstrate their presence on the patient's sample. This is performed on samples to check if the patient's antibodies are compatible with the red blood cells of the donor. The 3-cell antibody screen must be done before the blood transfusion to avoid haemolytic transfusion reactions (Ying et al., 2020).

First, the test red cells are incubated with the patient serum. Plasma or serum taken from specific blood group O is added to RBCs to carry antigens so they can then target certain RBC antibodies. After that, the coombs reagent is added and washed afterwards to remove everything that is not bound to RBCs. This can cause two conditions, the binding between antibody in patient plasma to test cells, causing agglutination and a positive reaction or on the contrary, coombs reagent cannot bind, and test cells are not bound to antibodies (Ying et al., 2020). Patient 1 had a positive reaction of grade 4 in cell 2. Patients 2 and 4 had negative reactions in all cells. Finally, patient 3 had a positive reaction with a grade 4 observed in cell 1 and grade 3 in cell 2. The 3-cell antibody test was done, and the following antibodies were detected in patient 3: anti-D, anti-E, anti-C, anti-C^w, anti-Kp^a, anti-Fy^a, anti-Jk^a, anti-Le^a, anti-Le^b, anti-M, anti-S, anti-Lu^a. Patient 1 showed different results: anti-E, anti-Kp^a, anti-Jk^a, anti-Le^a, anti-Lu^a.

What were the results of the Identification panels for each patient that needed one?

As previously mentioned, our second and fourth patients were negative, so 11-cell antibody screen was not required. However, patient number 1 and 3 had a positive result and the 11 -cell antibody was tested. Anti-E was detected, and Anti-Lu^a was unable to be excluded in patient number 1. Anti-Fy^a was found in patient 3 and anti-Cw was unable to exclude.

For each of your patients from stations 1-3 complete the following table based on your results

Patient	ABO RhD group	3 cell Antibody screen (pos/neg)	Antibody detected	What Blood Group and phenotype of blood would you select for compatibility testing? (e.g. O RhD pos, E neg)
1	O^+	Pos	Anti-E, anti-A, anti-B, anti-Lu^a	O RhD neg, O RhD pos, E neg, and Lu^a neg
2	B^-	Neg	Anti-A, anti-Rh	O RhD neg, B RhD neg
3	O^-	Pos	Anti-Fy^a, anti-C^w, anti-A, anti-B, anti-Rh	O RhD neg, Fy^a neg and C^w neg.
4	A^+	Neg	Anti - B	O RhD neg, O RhD pos, A RhD neg, A RhD pos

Abertay Biomedical Sciences Morphology Practical

Date...1-11-2022.....

Name…Lucia Lopez Clavain…….

Slide A or B will be given out - Perform 100 cell Differential on this slide using WBC **A**- 10.1 $x10^9$/L or **B** – 30.6 $x10^9$/L – Circle A or B depending which slide was used
Calculate the absolute number of each type of white cell and fill in Table below- Remember all answers must be expressed as 10^9/L

Cell Type	Number (%)	Absolute count x 10^9/L
Neutrophils	37	3.737
Lymphocytes	35	3.535
Monocytes	16	1.616
Eosinophils	3	0.303
Basophils	9	0.909

Briefly Discuss these results

The slide chosen was letter A which had a WBC of 10.1 x109/L. The normal range of WBC is 4-11E9/L, so it can be concluded that slide A falls into the normal range.

The normal percentage of circulating neutrophils is 40-60% with a reference range of 2-8E9/L. However, the slide showed a lower level of neutrophils with 37% and a normal range of 3.74E9/L. Lymphocytes are commonly the second most common WBC subtype comprising 20-40% of total WBCs and they have a reference range of 1.5-4E9/L. For this reason, the levels of lymphocytes in slide A fall between the normal range and the total WBC. Thirdly, monocytes should have a reference range of 0.2-0.8E9/L and comprise 2-10% of WBCs. The monocytes showed in the slide were remarkably higher than the normal range. The next most abundant subtype is eosinophil with 1-4% of total WBCs and a reference range of 0-0.4E9/L. The slide showed normal levels and range of eosinophil. Finally, basophils are the least abundant subtype of WBC with less than 1% and a reference range of 0 – 0.2 E9/L. The percentage of the total basophils and the range are exceedingly higher than the references ones.

In conclusion, it was seen that the slide A comprised considerable high level of basophils, moderately higher level of monocytes, a normal level of lymphocytes and eosinophils and low level of neutrophils.

If total WBC = 35.0 $x10^9$/L- Please calculate Absolute counts

Cell Type	Number (%)	Absolute count x 10^9/L
Neutrophils	85	29.57
Lymphocytes	5	1.75
Monocytes	9	3.15
Eosinophils	0.8	0.28
Basophils	0.2	0.07

Briefly discuss these results

The normal range of WBC is 4-11E9/L, so if the total WBC is 35 $x10^9$/L, then it can be concluded that it is exceedingly higher than the common range.

The normal percentage of circulating neutrophils is 40-60% with a reference range of 2-8E9/L. However, the slide showed a higher level of neutrophils with 85% and a considerable high range of 29.57E9/L. Lymphocytes are commonly the second most common WBC subtype comprising 20-40% of total WBCs and they have a reference range of 1.5-4E9/L. For this reason, the levels of lymphocytes in slide A fell between the largely lower range and the total WBC. Thirdly, monocytes should have a reference range of 0.2-0.8E9/L and comprise 2-10% of WBCs. The range of the monocytes was remarkably higher than the normal range, however the total WBCs fells between the normal number of cells. The next most abundant subtype is eosinophil with 1-4% of total WBCs and a reference range of 0-0.4E9/L. The analysis above showed normal range of eosinophil and higher level of WBC. Finally, basophils are the least abundant subtype of WBC with less than 1% and a reference range of 0 – 0.2 E9/L. The percentage of the total basophils fell between the common levels; however, the range is slightly higher than the reference one.

In conclusion, it was seen that there was an extremely higher number of neutrophils, slightly higher number of eosinophils, and a dramatically lower number of lymphocytes. In addition, it was observed that monocytes and basophils comprised normal percentages of the total amount of WBC.

Describe each of the following **cell's function** and their **reference range** in adults.

Neutrophil

They engulf foreign bodies such as bacteria or pathogenic agents and destroy them within cell. They are the first ones to respond to inflammation and infection. In addition to phagocytosis, they can also eradicate bacteria externally due to the activation of NETs, neutrophil extracellular traps. Similarly, they stimulate the release of cytokines to induce immunology activity. The reference range in adults is 2-8E^{9}/L

Lymphocyte

There are 3 different types of lymphocytes: natural killer cells, T lymphocytes and B lymphocytes. NK cells play a role in the innate immune system by defending the body against tumour cells and viral infection.

T Lymphocytes are involved in non-antibody response and cell mediated immunity. T helper cells stimulate the activation of B cells, macrophages, and T cytotoxic. T cytotoxic lymphocytes play a role in destroy agents identified as harmful by the immune system. The main function of T suppressor cell is to shut down T cell mediated immunity at the end of an immune reaction.

B lymphocytes oversee the regulation of antibody formation and the humoral immune response. There are two types of mature B lymphocytes. The first one is plasma cells, which are responsible for cell marking, the stimulation of phagocytosis and complement activation. The second type is memory cells. They have long life expectancy and provide a quicker response to an antigen after a second exposure. The reference range in adults is 1.5 – 4E9/L

Monocyte

Monocytes have an impact in both innate and adaptive immunity. The react fast to an infection and can eliminate cells by phagocytosis. In addition, they also produce cytokines of anti-inflammatories and present the antigens to activated T cells. The reference range in adults is 0.2 – 0.8E9/L.

Eosinophil

Eosinophils regulate the immune response to infections caused by parasites. They can also influence mechanisms involved in the allergic response. The reference range in adults is 0 – 0.4E9/L.

Basophils

Basophils have a similar role as eosinophils, they impact allergic and parasitic immune responses. Nonetheless they mainly act in allergic responses. The reference range in adults is 0 – 0.2E9/L.

Examine slide C supplied concentrating on RBC features- the Hb = 91 g/L, MCV= 70.0 fl, MCH = 20.8

1. What RBC features might be reported as significant in this slide?

The red blood cells presented in the slide showed anisopoikolocytosis, differences in shape and size compared to normal RBCs. It looks like the RBCs were stuck together like a chain structure. This term is called rouleaux. However, the more expressed the more significant it is. For this reason, it cannot be confirmed the presence of rouleaux due to the minor distinction. Finally, codocytes were observed in the slide like cells with haemoglobin in the centre, an area without it surrounded by more haemoglobin on the outside (Figure 1).

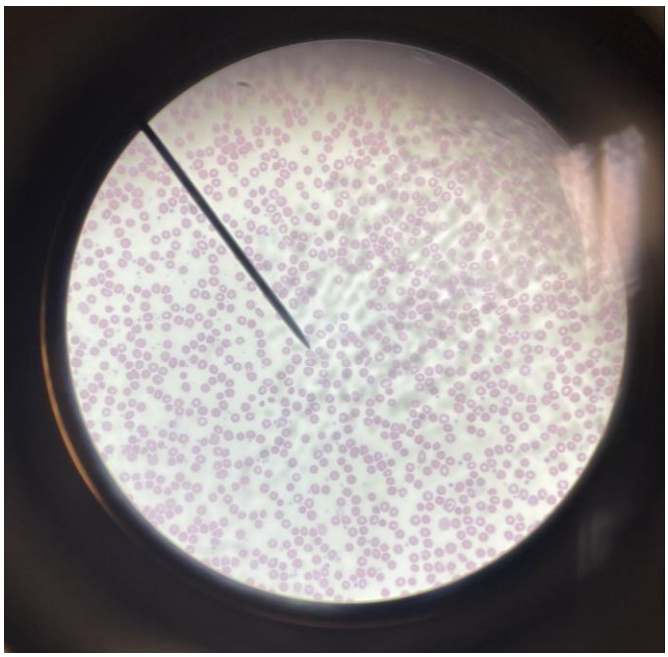


Figure 1: A picture showing abnormal red blood cells from slide C provided by the staff of Ninewells Hospital.

2. What clinical condition (s) might be associated with features apparent on the slide and from indices supplied?

The indices supplied indicated markedly low haemoglobin, MCV and MCH levels, showing moderate anaemia. In addition, target cells are associated with hemoglobinopathies like thalassemia, and splenectomy. For this reason, it can be concluded that the patient can suffer from alpha-thalassemia type HbH disease.

Explain what the following morphological features are and give an example of a condition in which they occur

Cell type	example
Target cell	They have a bullseye morphology, haemoglobin in the center and around the edge. Liver disease (Scordino, 2016).
Howell Jolly body	Nucleated smooth single red cells. Post-splenectomy (Scordino, 2016).
Pappenheimer body	Granulated RBCs due to iron granules of basophilic cells. Hemolytic anaemia (Scordino, 2016).
Rouleaux	Chain of cells stuck together. It shows high plasma viscosity. Multiple myeloma (Scordino, 2016).
Sickle cells	Classic crescent shaped or boat shaped. Sickle cell disease (HbSS) (Scordino, 2016).
Elliptocyte	Oval-shaped and elongated RBCs. They are increased in anaemia (Scordino, 2016).
Acanthocyte	They show few pointed, irregular long spikes on their circular surface. Liver disease (Scordino, 2016).
Burr cell	Small, round projections located moderately evenly over the cell surface. Myelofibrosis (Scordino, 2016).
Spherocyte	Compact RBCs in a circular shape, smaller than regular erythrocytes. In addition, they do not show central pallor. Drug induced haemolysis (Scordino, 2016).
Platelet satellitism	Platelets attached to neutrophils; they encircle the border of the RBC showing a pink appearance. It can result in pseudo-thrombocytopenia (Scordino, 2016).
Platelet clumps	Platelets are aggregated closely together causing a false platelets count (Scordino, 2016).
Teardrop poikolocytes	They have a similar shape as a tear: a rounded, non-pointed side and a tip with a leaky end. Bone marrow fibrosis (Scordino, 2016).
Schistocytes (RBC fragments)	They are short RBCs with acute angles, right edges and without central pallor. Intravascular haemolysis (Scordino, 2016).
Stomatocytes	Letterbox shaped with notch shape in the centre and central pallor. They are uniconcave and it can be observed in Rh null disease (Scordino, 2016).

References

Scordino, T. (2016) *Stomatocytes*, *ImageBank*. Available at: https://imagebank.hematology.org (Accessed: December 4, 2022).

Leppard, A. (2014) *Sample Acceptance Criteria*, *NHS choices*. NHS. Available at: https://www.barnsleyhospital.nhs.uk/pathology/blood-transfusion/sample-acceptance-criteria/ (Accessed: December 4, 2022).

Scope of Haematology service and sample acceptance (2022) *NHS choices*. NHS. Available at: https://mft.nhs.uk/the-trust/other-departments/laboratory-medicine/haematology/scope-of-haematology-service-and-sample-acceptance/ (Accessed: December 4, 2022).