Country:

PRACTICAL EXAMINATION # 2: CELL BIOLOGY LABORATORY

This practical examination is composed of 3 Tasks:

Task 1: Differential leucocyte count **(16 points)**

Task 2: Blood group analysis **(11 points)**

Task 3: Single radial Immunodiffusion antigen analysis **(13 points)**

Total Points available: 40

Total time available: 90 minutes

GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.

TASK 1: Differential Leucocyte Count.

Requirement

In this task, you are required to perform a differential leucocyte count and answer two supplementary questions.

Material and equipment

1. Binocular microscope - with 10X, 40X, 100X (oil immersion) objective lens and 10X eyepiece lens.
2. Microscope oil immersion lens
3. Oil for oil immersion microscopy
4. Cell maturation charts (provided).
5. Stained blood smear (Wrights stain).

Procedure

You are supplied with a prepared blood smear that has been stained with Wright's stain. Wright's stain is a standard haematological stain for blood smears. This blood film was collected from a patient who has a persistent cough and fever. The patient is a male adult and had a total white cell count (WCC) of 15.0×10^{9}/L. You are required to complete a differential leucocyte count and to record your results in the table supplied. The cell maturation charts will help you identify the leucocytes.

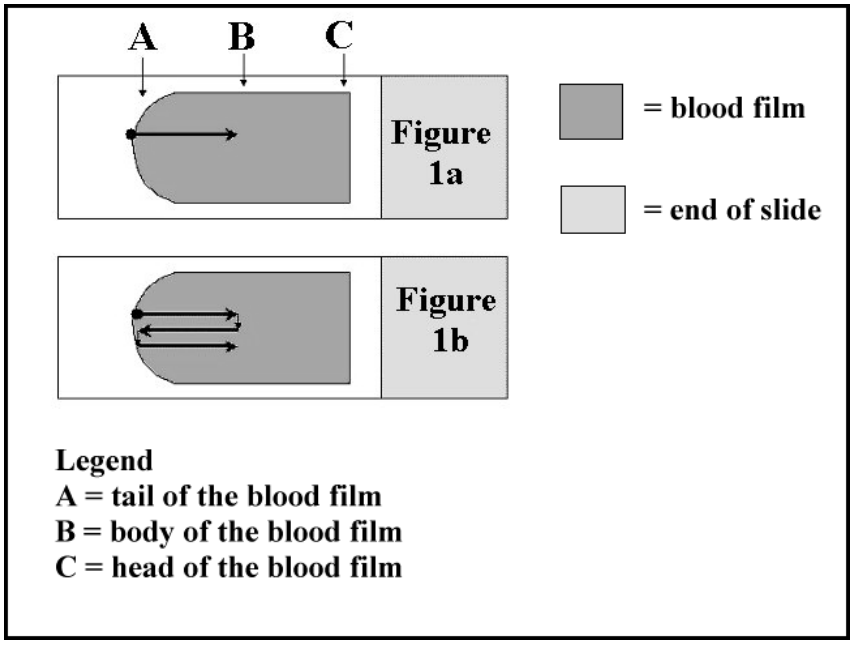
How to perform a differential leucocyte count

(i) It is **recommended** that you use a 100X oil immersion objective lens. Focus on 10X objective. Put a drop of oil on the slide. Turn the turret carefully to bring the 100X objective into the oil. Focus.

(ii) **Identify and count 100 consecutive leucocytes** in a longitudinal strip from the tail end towards the head of the smear as shown in Figure 1a, recording the occurrence of each cell.

(iii) The lateral edges of the smear must be avoided. It may not be possible to count 100 consecutive leucocytes in a single longitudinal strip due to the thickness of the smear and the subsequent difficulty in cell identification. If this situation occurs, adopt the technique of counting from the tail to the head end and back again as shown in Figure 1b.

FIGURES 1a and 1b



(iv) Record the results of the differential count on the answer sheet (Table 1), taking care that the results are expressed as percentages, and that the total recorded adds up to 100%. An example is given in Figure 1c.

FIGURE 1c: Example of a completed differential count

LEUCOCYTES	**%**	Absolute count (10^9/L)
Neutrophils (total of band form and segmented)	60	6.0
Lymphocytes	30	3.0
Monocytes	8	0.8
Eosinophils	2	0.2
Basophils	0	0
Total	100	10

TABLE 1: Results of differential leucocyte count.

Type of leucocyte	Occurrence (%)	Absolute count (10^9/L)	Reference Range (10^9/L)
Neutrophils (total of band form and segmented)			2.0 - 7.5
Lymphocytes			1.5 – 4.0
Monocytes			0.2 – 0.8
Eosinophils			0.04 - 0.4
Basophils			0.0.- 0.1
Total WCC	100	15.0	4.0 - 10.0

Enter results on answer sheet. **(14 points)**

Questions

P2.T1.1 How could you improve the accuracy of your differential leucocyte count?

A. Count 50 cells.

B. Count 200 cells.

C. Only include cells that are easily identified.

D. Only use the x40 objective.

E. Count all red blood cells in each field.

(1 point)

P2.T1.2 In the differential leucocyte count, calculation of the absolute count from the percentage of each cell type is an important step because of which of the following factors?

A. Absolute counts provide an indication of anaemia.

B. Percentage counts do not vary with the type of infection.

C. A reference range (normal range) for each cell type can be determined.

D. Leucocyte numbers cannot be validated from a blood smear.

E. All of the above.

(1 point)

TASK 2: Blood Group Analysis.

Background

Column agglutination blood grouping cards are used to determine the blood group of individuals in term of the ABO and Rhesus blood groups.

Material and equipment

1. Images of 12 column agglutination blood grouping cards. Ten are labelled with patient identification numbers.
2. Examples of two column agglutination blood group cards (provided).

Procedure and requirement

You are provided with the images of ten (10) blood group cards, each with a unique patient identification number. You are required to interpret the ABO and Rh D (Rhesus) blood group for each patient and record the results in the table provided on the answer sheet. Please refer to Figure 2 for the ABO grouping reactions table and Figure 3 for the Rhesus group reaction table. Individuals with the D-antigen are described as Rhesus positive (Rh +) and those without the D-antigen as Rhesus negative (Rh NEG).

Additional notes on column agglutination cards for blood grouping.

- The cards use the principle of column agglutination. If there is a reaction between the cells and an antibody the cells agglutinate, and become trapped in the column.
- A column may contain antisera, e.g. anti-A, anti-B or in the case of the control no added antisera.
- Columns contain micro glass spheres so they trap agglutinated but not single red blood cells.
- A **positive reaction** is indicated by trapped red blood cells at the top.
- A **negative reaction** is indicated by red blood cells not being trapped and passing completely through the column.
- The columns on the card from left to right are patient cells added to anti-A, patient cells added to anti-B, patient cells added to anti-D, control column (patient cells only), A_1 cells (strongest form of A cells) added to patient serum, B cells added to patient serum.
- The control has just cells added to allow for the detection of spontaneous autoagglutination – if this occurs, it makes the results invalid.
- For a card to be valid the control column must read negative.
- For all cards that are invalid, write INVALID in the ABO Blood Group columns.

Be sure to transfer your answers from Table 2 to the answer sheet.

Figure 2: ABO grouping reactions table

PHENOTYPE	Anti-A	Anti-B	A_1Cells	B Cells
A	POS	NEG	NEG	POS
B	NEG	POS	POS	NEG
AB	POS	POS	NEG	NEG
O	NEG	NEG	POS	POS

Figure 3: Rh D (Rhesus) grouping reactions table

Rh Phenotype	Anti-D
Rh POS	POS
Rh NEG	NEG

TABLE 2: Results of Patient Blood Grouping

Patient identification number	RESULTS – POS or NEG						ABO blood group (A,B,O or AB)	Rh D (POS or NEG)
	(Anti-) A	(Anti-) B	(Anti-) D	Control	A_1 (cells)	B (cells)		
P 942715								
P 945857								
P 942675								
P 974199								
P 926723								
P 976348								
P 923413								
P 981342								
P 917300								
P 981398								

(8 points)

Enter your results on the answer sheet.

Questions

P2.T2.1 If a person's blood group is O Rh POS which of the following ABO antigens are present on their red blood cells?

A. A antigens only.

B. B antigens only.

C. Both A and B antigens.

D. Neither A nor B antigens.

E. A_1 antigens.

(1 point)

P2.T2.2 A person who has the blood group A Rh NEG has which combination of (non red blood cell stimulated) ABO antibodies?

A. anti-B.

B. anti-A.

C. anti-A,B.

D. anti-H.

E. None.

(1 point)

P2.T2.3 On the basis of the blood grouping reactions you have recorded in Task 2, which of the patients is the **most** likely to have been transfused?

A. Patient P 942715

B. Patient P 945587

C. Patient P 942675

D. Patient P 974199

E. Patient P 926723

F. Patient P 976348

G. Patient P 923413

H. Patient P 981342

I. Patient P 917300

J. Patient P 981398

(1 point)

TASK 3: Single radial immunodiffusion antigen analysis.

Background

Single radial immunodiffusion (SRID) is used to measure the concentration of immunoglobulins in blood. It is normally carried out by incorporating an antibody in an agarose gel at a known concentration and placing samples containing an antigen into the standardised wells in the gel. At completion of immunodiffusion a stable precipitate forms, with the diameter squared (D^2) having a linear relationship with the antigen concentration.

A standard curve of diameter squared (D^2) versus antigen concentration plotted on ordinary graph paper can then be used to estimate the concentration of a number of unknowns. It is normal for just three standard points to be used to construct the standard curve.

In this task you are required to construct the standard curve for two immunoglobulins (IgG and IgA), and then determine the immunoglobulin concentration for two patients. There are also three supplementary questions on the technique.

Requirement

You are required to construct standard curves for two (2) sets of SRID reactions (IgG and IgA), and then to determine the concentration of the immunoglobulin from each patient.

Material and equipment

1. 2 x images of SRID (IgG and IgA) plates with standards and unknown.
2. Reading ruler.
3. Graph paper.

Procedure

You are provided with two SRID plates. These plates have been loaded with standards of varying immunoglobulin concentrations, a control serum and serum from a patient. The diffusion has been allowed to come to completion.

For each plate, measure the diameter (D) of the precipitation rings (standards, controls and unknowns) using the reading ruler provided. (**Hint:** place the gel over the ruler, aligning the centre of the well with the central line of the ruler). Move the gel until the outer rim of the precipitin circle **just touches** the inside of both divergent lines. Read to 0.1 mm accuracy.

Record the measurements in the table provided.

Plot the square of the diameter (D^2) of the precipitin rings against the immunoglobulin concentration of the standards. For the plot use the graph paper provided, with immunoglobulin concentration on the horizontal (x) axis and ring diameters squared (D^2) on the vertical (y) axis. A line of best fit is to be drawn through the three points. (**Hint**: The y-intercept should be in the range of 10 mm^2 to 12 mm^2).

Interpolate the IgG and IgA concentrations of the patient serum from the graphs obtained and record your results on the answer sheet.

There are three supplementary questions

TABLE 3: Results from the analysis of the IgG plate.

Well Number	Description	IgG Concentration (g/L)	Diameter (D) (mm)	D^2 ($mm^{2)}$
4	Standard 1	2.9		
3	Standard 2	9.2		
2	Standard 3	17.6		
8	Control	14.1		
10	Patient A			

The IgG concentration of Patient A is _____g/L

(5 points)

Enter this value on the answer sheet.

TABLE 4: Results from the analysis of the IgA plate.

Well Number	Description	IgA Concentration (g/L)	Diameter (D) (mm)	D^2 (mm^2)
4	Standard 1	1.20		
3	Standard 2	3.55		
2	Standard 3	5.55		
8	Control	2.85		
10	Patient B			
11	Patient B (1/4 dilution)			

The IgA concentration of Patient B is _____g/L

(5 points)

Enter this value on the answer sheet.

Questions

P2.T3.1 Why is it that the plot of the line does not pass through the origin?

A. The technique is designed for only low concentrations of antibody.

B. The technique is designed for only low concentrations of antigen.

C. The size of the well introduces a zero error.

D. The gel system expands during incubation, introducing an error.

E. Deformation of the gel due to sample application introduces an error.

(1 point)

P2.T3.2 What could cause a poor (non-linear) calibration curve in this technique?

A. Omission of the control sample.

B. Cloudy gel.

C. Patient serum too dilute.

D. Patient serum too concentrated.

E. Incomplete diffusion.

(1 point)

P2.T3.3 How could you improve the accuracy of this technique?

A. Use a thicker agarose gel.

B. Use concentrated antibodies in the wells.

C. Heat the gels in a dry oven at 37 degrees Celsius.

D. Adjust the antibody concentration in the gel.

E. None of the above.

(1 point)