LONDON metropolitan university

IN CLASS TEST QUESTION PAPER: REASSESSMENT, 2014

Module code:	**BS6053**
Module title:	**Applied Immunology**
Module leader:	**Prof. Jameel Inal**

Date:	**July/August 2014**
Duration:	**1 hour**

Exam type:	**Seen, Restricted**
Materials supplied:	**Graph Paper**
Materials permitted:	rbc lysis/pellet data for all the wells used in plates 1 and 2, plus the rbc lysis/pellet data for the two control wells
Warning:	**Candidates are warned that possession of unauthorised materials in an examination is a serious assessment offence.**

Instructions to candidates:	**Write your ID number in the box provided on the following page. Answer ALL questions. Write your answers in the spaces provided.** **DO NOT TURN PAGE OVER UNTIL INSTRUCTED**

ID:

BS6053 Applied Immunology: Complement Practical

In order to carry out this assignment:

Make sure that you have the rbc lysis/pellet data for all the wells used in plates 1 and 2, plus the rbc lysis/pellet data for the two control wells

Title: You need to head your write-up with an appropriate descriptive title ***of your actual experiment.***

4 marks

As 4 marks are allocated, a simplistic, very generic title (such as: 'complement practical write-up') is unlikely to gain full marks.

Introduction, with Aims: State clearly what the aim (ie. the point) of the experiment is. It is an experiment to look at the working of the various complement activation pathways**! - So, in the Introduction you will need to provide an account of the complement activation pathways.**

BUT, the Introduction should also outline ***what <u>your</u> experiment is about***, and the basis on which the method relies.

You might also decide that diagrams will help with the descriptive outline of your experiment ***– if so, make sure they are neatly drawn and labelled.***

25 marks

As 25 marks are allocated to the 'Introduction/Aims', it is expected that this section of the write-up will be detailed, and reasonably extensive.

Methods: No detailed description of the methodology is required (this would just repeat the printed experimental protocol). Just comment on anything that needed to be varied from the printed method.

1 mark

Remember: only 1 mark is allocated.

Results: The results should be presented in tabulated form, and then expressed graphically. Remember: graphs should be drawn neatly – preferably using a PC, and a software application such as Excel.

Remember: ***draw all 4 curves (including the REA results provided – see below) together on one graph***. Do ***not*** produce separate curves on different graphs. You will also need to think about the correct way (or at least, the most appropriate way) to express the complement dilutions on the graph – ***because***, there is a problem if you just plot the straight dilution values!

In addition, you should then ***describe*** your results – describe them, but ***not*** interpret them, at this stage – interpretation is to be left for inclusion in the 'Discussion with Conclusion' section.

Tabulated data: 8 marks
Graph: 10 marks
Text-based description: 7 marks

Discussion, with Conclusion: The 'Discussion/Conclusion' section should ***interpret*** the data obtained.

You will need to discuss your interpretation of your results, ***in the context of your stated aim for the experiment. A mere description of the results, with no interpretation of what your results actually mean will not attract a high mark!***

You may need to discuss the relative merits of taking ***either*** a 50% end point ***or*** a 100% endpoint ***for your own data***.

You have been asked to interpret the data in a 'semi-quantitative' manner. What data do you have that will allow this, and how will you do this?

You are required to write a very clear conclusion to summarise your findings.

40 marks

40 marks are a substantial proportion of the total mark allocation. It is, therefore, expected that the 'Discussion/Conclusion' section is detailed and substantial, ***and addresses all the required points.***

References: References should be cited as appropriate, to indicate an understanding of complement activity, and its assessment. It is ***unlikely*** that any published reference will give an explanation of your particular experiment! - ***so it is suggested that you do not spend large amounts of time searching the internet***. ***You really need to think about your experiment and your data yourself.***

5 marks

Rabbit Ab-coated RBC (REA) results: (% lysis)

	1 in 2	4	8	16	32	64	128	256	512	1024	2048	4096
CFD	100	100	100	95	50	0	0	0	0	0	0	0
CFD + EGTA	100	50	0	0	0	0	0	0	0	0	0	0

CFD + EDTA	0	0	0	0	0	0	0	0	0	0	0	0
	Con 1	Con 2	Con 3									
	0	0	0									

BS6053 Complement Practical - Coursework Assignment

Outline Protocol:

You are provided with:
Sheep RBC, coated with anti-erythrocyte antibody (labelled SEA)
Uncoated sheep rbc (labelled SE)
Uncoated rabbit rbc (labelled RE)
We are unable to provide rabbit rbc, coated with antibody, but you are given the results.

Make serial doubling dilutions of human complement in ***two*** 96-well microtitre plates, dilutions 1 in 2 - 1 in 4096 (12 wells), in 100ml amounts, ***as below***:

Plate 1: ***8 rows***

	1	2	3	4	5	6	7	8	9	10	11	12
A	Complement diluted in CFD											
B	Complement diluted in CFD + EGTA											
C	Complement diluted in CFD + EDTA											
D	D1	D2	D3									
E	Complement diluted in CFD											
F	Complement diluted in CFD + EGTA											
G	Complement diluted in CFD + EDTA											
H	H1	H2	H3									

Controls:
D1 + H1 100ml CFD
D2 + H2 100ml CFD + EGTA
D3 + H3 100ml CFD + EDTA

Plate 2: ***4 rows only***

	1	2	3	4	5	6	7	8	9	10	11	12
A	Complement diluted in CFD											
B	Complement diluted in CFD + EGTA											
C	Complement diluted in CFD + EDTA											
D	D1	D2	D3									
E												
F												
G												
H												

Controls:
D1 100ml CFD
D2 100ml CFD + EGTA
D3 100ml CFD + EDTA

Add 50ml ***SEA*** to ***all experimental and control*** wells in rows A-D in ***plate 1***

Add 50ml ***SE*** to ***all experimental and control*** wells in rows E-H in ***plate 1***

Add 50ml ***RE*** to ***all experimental and control*** wells in rows A-D in ***plate 2***

Gently mix the wells, by ***carefully shaking*** the plate.

Cover each plate with clingfilm, and incubate 37 degrees C, 15 minutes. Remix the plate and re-incubate 37 degrees C, 15 minutes (30 minutes incubation in total).

Allow the cells in the plate to settle (requires to settle at 4 degrees C overnight).

Read (extent of lysis in each well) and intrepret the results (in terms of complement activation pathways).

Expected results:

Plate 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Complement diluted in CFD								**LYSIS**			
B	Complement diluted in CFD + EGTA								**NO LYSIS**			
C	Complement diluted in CFD + EDTA								**NO LYSIS**			
	D1	D2	D3									
D	**NO**	**NO**	**NO**									
E	Complement diluted in CFD								**NO LYSIS**			
F	Complement diluted in CFD + EGTA								**NO LYSIS**			
G	Complement diluted in CFD + EDTA								**NO LYSIS**			
	H1	H2	H3									
H	**NO**	**NO**	**NO**									

Plate2:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Complement diluted in CFD								**LYSIS**			
B	Complement diluted in CFD + EGTA								**LYSIS**			
C	Complement diluted in CFD + EDTA								**NO LYSIS**			
	D1	D2	D3									
D	**NO**	**NO**	**NO**									
E	Complement diluted in CFD								**LYSIS**			
F	Complement diluted in CFD + EGTA								**LYSIS**			
G	Complement diluted in CFD + EDTA								**NO LYSIS**			
	H1	H2	H3									
H	**NO**	**NO**	**NO**									

Grey = given results

Interpretation:
In Plate 1, using antibody-coated RBC (SEA):
Lysis occurs with complement and SEA: the most likely explanation of the lysis is antibody-dependent, complement-mediated lysis.

The EGTA (with added Mg++) will chelate the calcium present, and inhibit the classical pathway (Ca++ and Mg++ required), but not the alternative pathway (only Mg++ required).

The EDTA will chelate both the calcium and the magnesium present, and inhibit both the classical pathway, and the alternative pathway. No lysis has occurred

The results suggest antibody-dependent, complement-mediated complement activation, by the classical pathway.

In Plate 1, using <u>uncoated</u> RBC (SE):
Compared with the above result, no lysis has occurred, suggesting a requirement for anti-erythrocyte antibody.

In Plate 2, using antibody-coated RBC (REA) - <u>*GIVEN RESULTS*</u>, and uncoated RBC (REA):
The lysis results are the same for both plates, suggesting, therefore, that there is no requirement for antibody.

The EDTA will chelate both the calcium and the magnesium present, and inhibit both the classical pathway, and the alternative pathway. **No lysis occurred, suggesting that complement could be involved in the lysis, but gives no indication of pathway of activation.**

The EGTA (with added Mg++) will chelate the calcium present, and inhibit the classical pathway (Ca++ and Mg++ required), but not the alternative pathway (only Mg++ required). **Lysis occurred, suggesting antibody-independent complement-mediated lysis, by the alternative pathway.**

The results suggest antibody-independent, complement-mediated complement activation, by the classical pathway.