Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

# Answer key: ELISA Masterclass

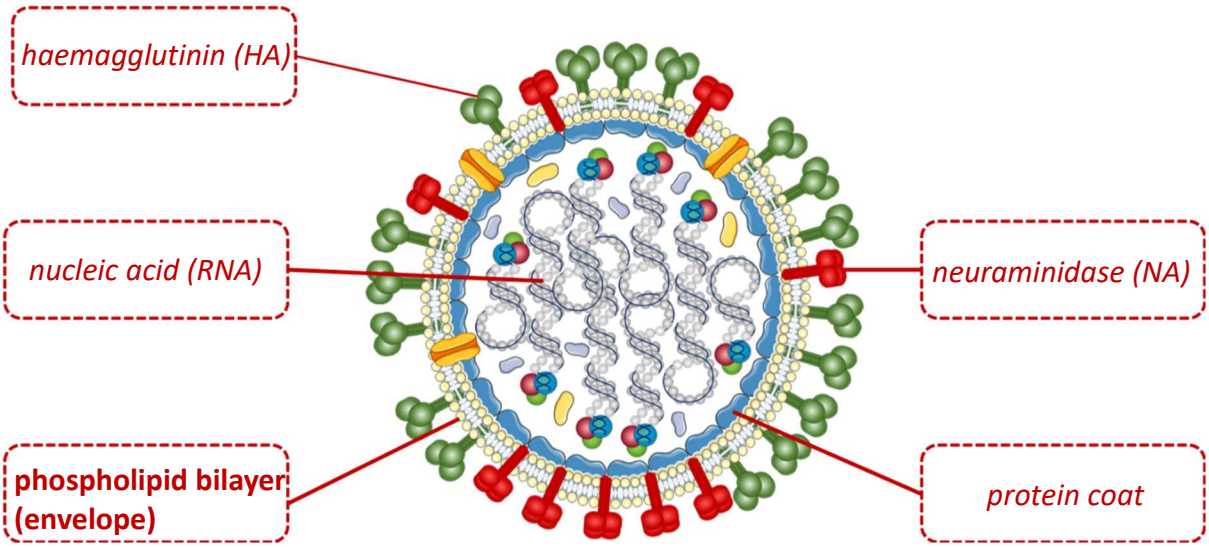
## Viruses

1. What illness does influenza virus cause?

   *Flu*

2. Label this influenza virus

nucleic acid (RNA) - protein coat - phospholipid bilayer (envelope) - antigen haemagglutinin (HA) - antigen neuraminidase (NA)




## Lab Techniques

3. What is separated by centrifugation of blood samples?

   *red blood cells, plasma, white blood cells.*

4. How does the ELISA technique work in a sandwich ELISA (the type of ELISA carried out in the workshop)?

   Students' own answers, example answer.

   *The Enzyme-Linked Immunosorbent Assay (ELISA) is a technique used to detect antibodies or infectious agents (antigens) in a sample.*

   *A capture antibody was coated on the base of the wells.*

   *A sample is added to the wells, if it has antigen it sticks to the antibody.*

   *Next, a primary antibody that matches the antigen we want to detect is added, if the antigen is present it binds.*

   *Nest, a secondary antibody is added which binds the primary antibody, if it is present. The secondary antibody is coupled with an enzyme.*

   *Finally, the substrate for the enzyme is added, if the secondary antibody is present the substrate changes from clear to blue.*

   *The reaction is stopped by adding acid.*

5. How many antibodies did we need to use in this experiment?

*3 antibodies*

1. *Capture antibody*
2. *Primary antibody*
3. *Secondary antibody*

*NOTE: the answer 2 would also be acceptable as the students did not add the capture antibody.*

6. What is an antigen?

   *Marker that is recognised by an antibody.*

7. Why did we add acid?

   *To stop the enzymatic reaction catalysed by the enzyme coupled to the secondary antibody.*

8. In the workshop you did serial dilutions to prepare your standard curve. You added 50ul PBS to the wells #2-#12. You then added 100ul of antigen to well #1 and carried out a serial dilution from well #1-#11.

A. What is the final concentration of the antigen in well #4?

*125 µg/ml*

9. We used PBS in this experiment. PBS is a very common salt solution used in labs. It stands for phosphate buffered saline. Usually, stock solutions are prepared at x10 and diluted to x1.

A. Complete the table below with the correct weights of the components for x10 PBS

| Component | Percentage | Weight |
|---|---|---|
| Di-sodium hydrogen phosphate anhydrous (Na2HPO4) | 50% | *114.9g* |
| Sodium dihydrogen orthophosphate monohydrate (NaH2PO4*H2O) | 12% | *27.6g* |
| Sodium Chloride (NaCl) | 37% | 85g |

B. Complete the table below to make up different concentrations of 1 litre of PBS using the stock solution.

| PBS Concentration | x10 PBS | Water |
|---|---|---|
| x1 | 100ml | 900ml |
| x0.25 | *25ml* | *975ml* |
| x0.1 | *10ml* | *990ml* |