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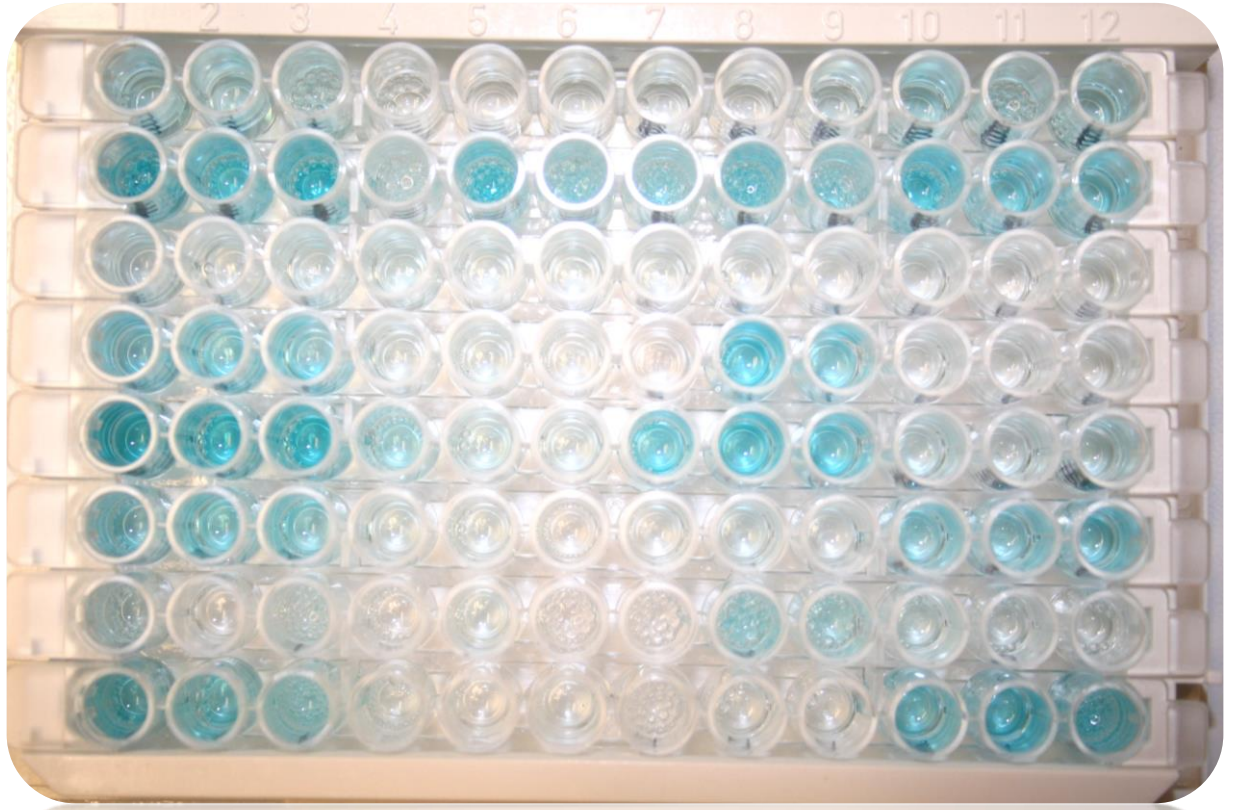

ELISA Masterclass: Flu Fighters

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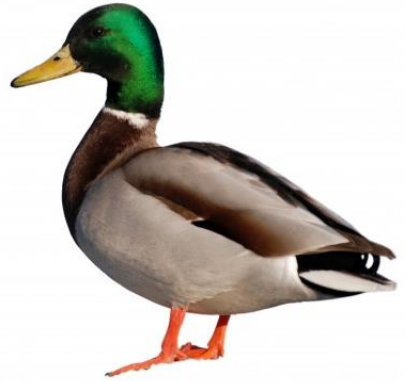
Which species does flu infect?



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Which is the natural host for flu?

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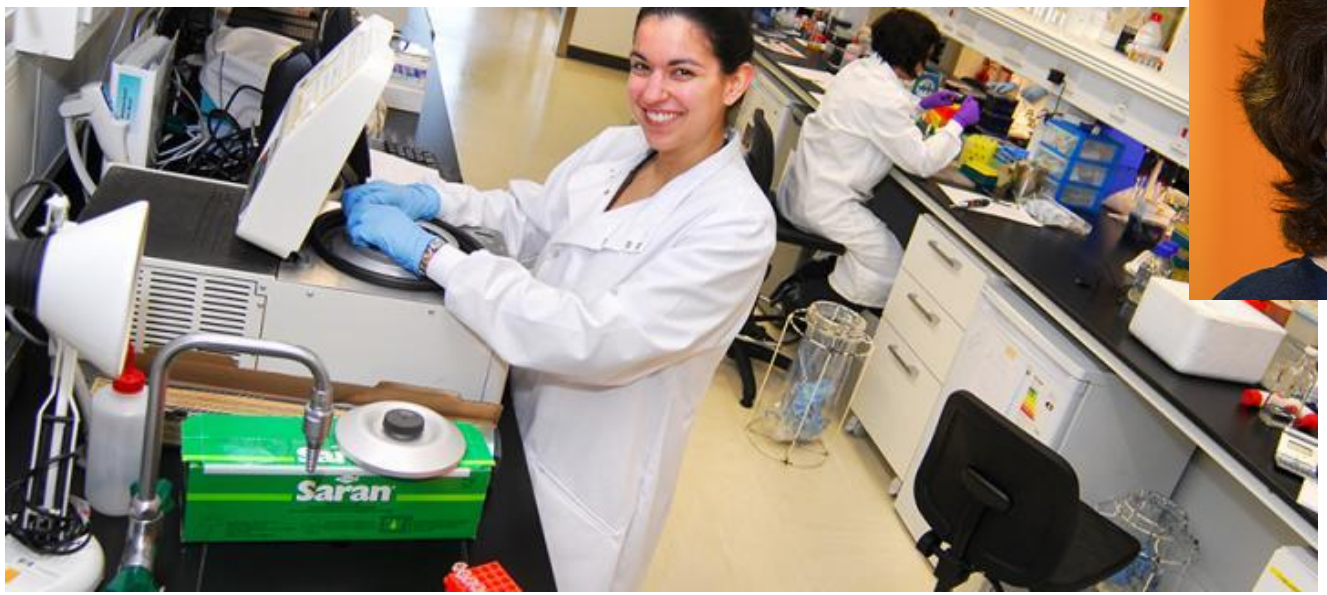
Bird Flu in the Headlines

Bird Flu Is Spreading in Asia, Experts (Quietly) Warn

Doctors attended to a H7N9 bird flu patient in Wuhan, China, in February. T
country has been experiencing a "fifth wave" of flu since October 2016. Agenc
France-Presse — Getty Images

Bird Flu in Research in Roslin



Helen Sang

GM chickens that don't transmit bird flu developed

Breakthrough could prevent future bird flu epidemics

Chickens genetically modified to prevent them spreading bird flu have been produced by researchers at The Roslin Institute of the University of Edinburgh and the University of Cambridge.

The scientists have successfully developed genetically modified (transgenic) chickens that do not transmit avian influenza virus to other chickens with which they are in contact. This genetic modification has the potential to stop bird flu outbreaks spreading within poultry flocks. This would not only protect the health of domestic poultry but could also reduce the risk of bird flu epidemics leading to new flu virus epidemics in the human population.

The study, funded by the Biotechnology and Biological Sciences Research Council (BBSRC), is published today in the journal Science. A list of questions and answers together with downloadable images is available.

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The influenza virus

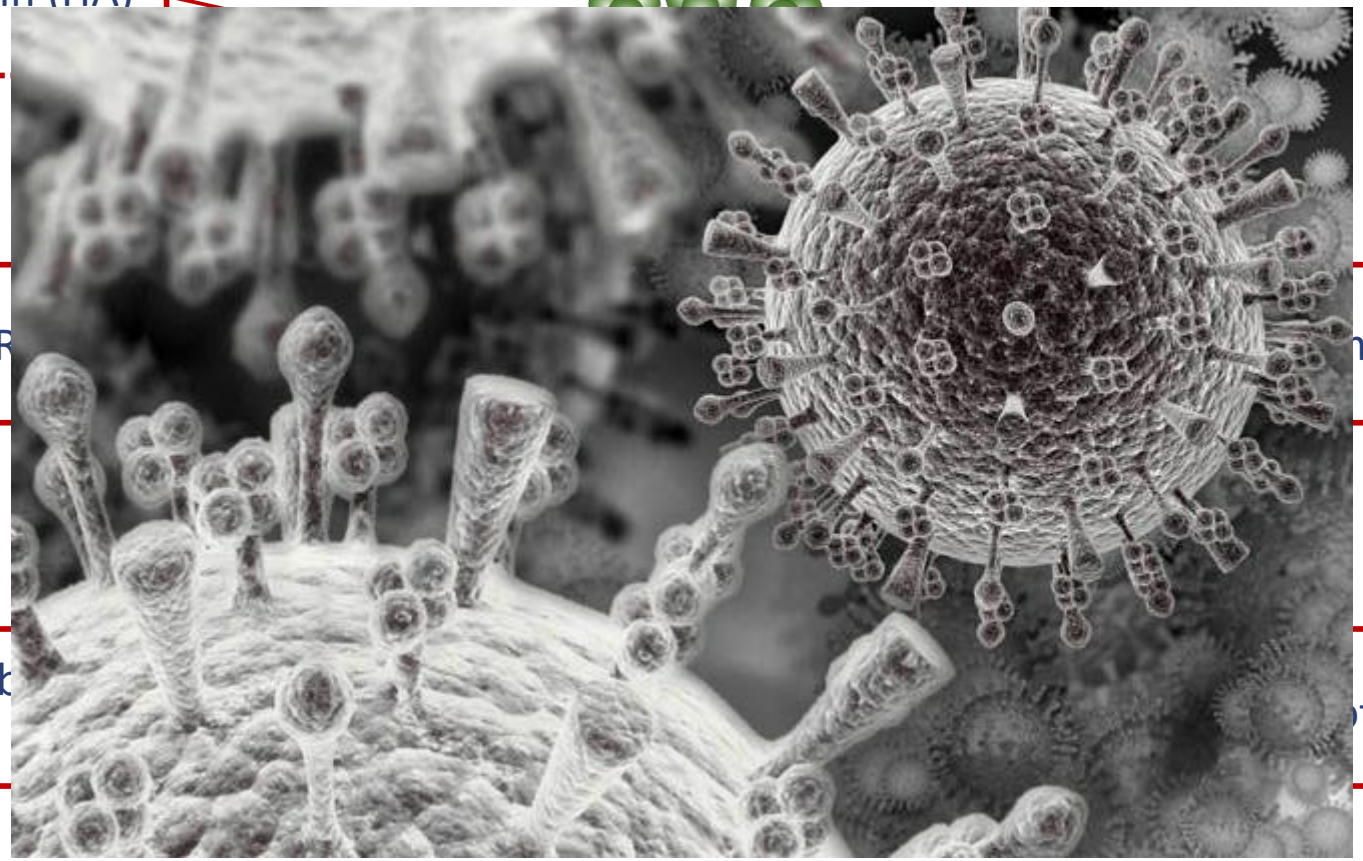
1) haemagglutinin (HA)

2) nucleic acid (R

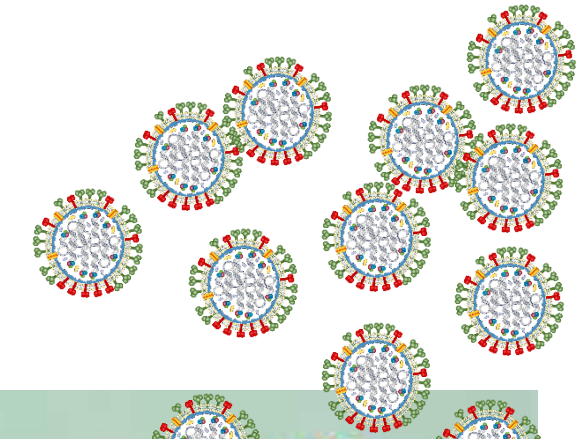
ninidase (NA)

3) phospholipid b
(envelope)

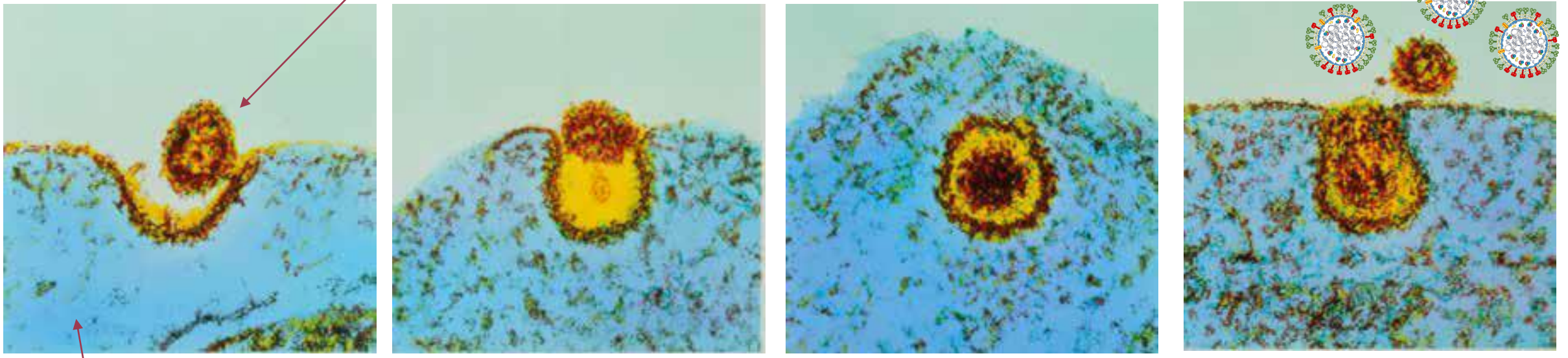
tein coat



The Flu Life Cycle- up close..



Influenza virus



Host cell cytoplasm

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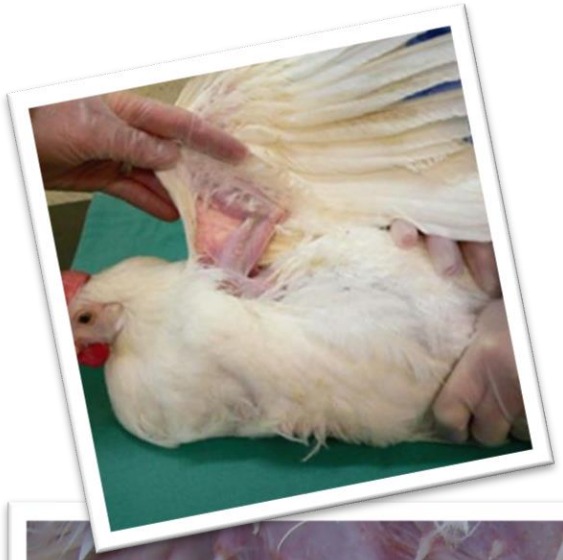
Today's workshop- Bird Flu Outbreak

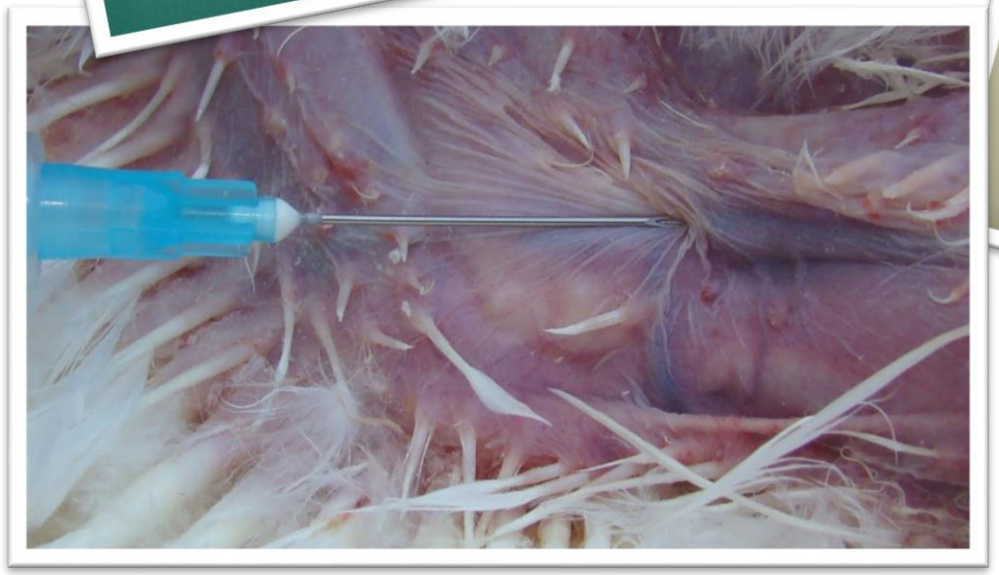
The aim of today's workshop

Analyse chicken blood samples from farms A – D to find out if they have been infected by the flu virus

Blood samples were taken from chickens on farms A - D

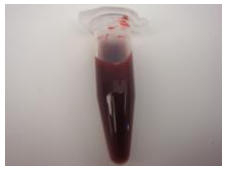
Taking a blood sample

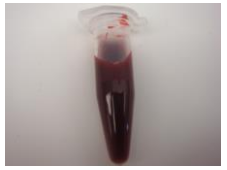


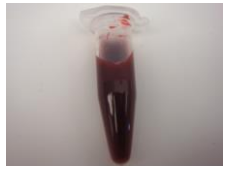


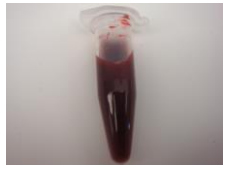
Obtain a pure serum sample from 4 chickens

Chicken blood sample from

Farm A 

Farm B 

Farm C 

Farm D 

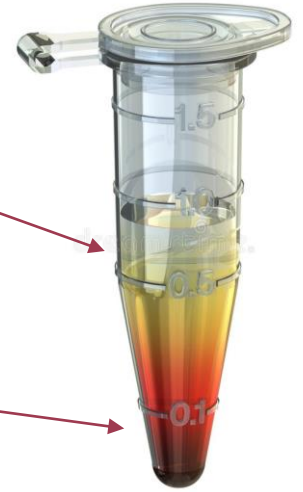
1) Spin the tubes in the centrifuge for 1 minute.

Centrifuge spins tubes at 14,000 revolutions per minute (rpm) for 2 minutes

Heavier material (the cells) is thrown outwards and collects to form a **pellet**

Liquid

Pellet



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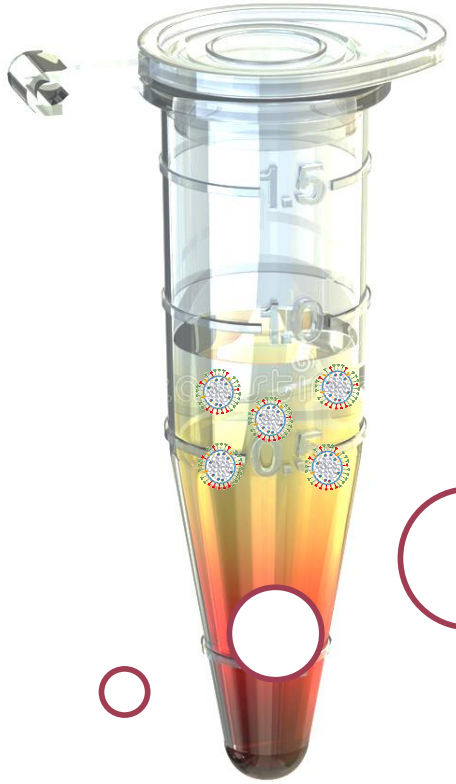
Serum- the liquid part of blood

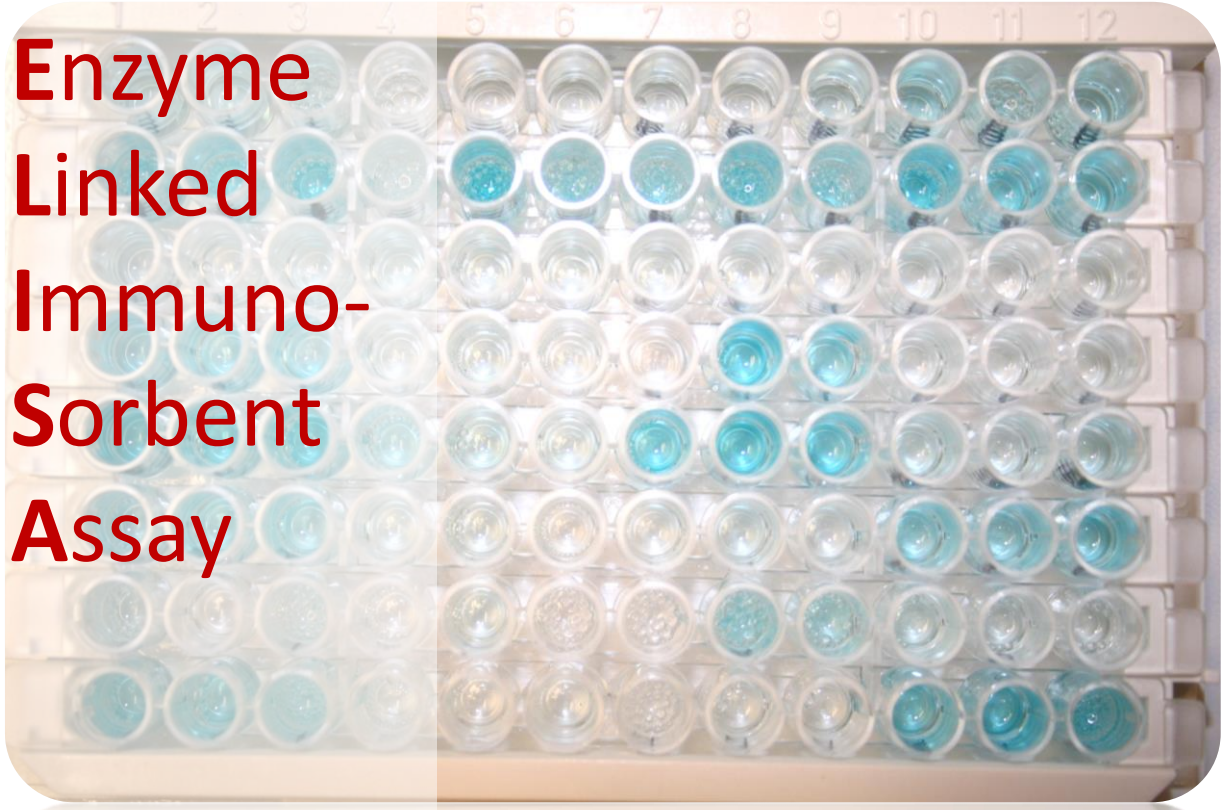


What technique can we use to detect virus in the serum?

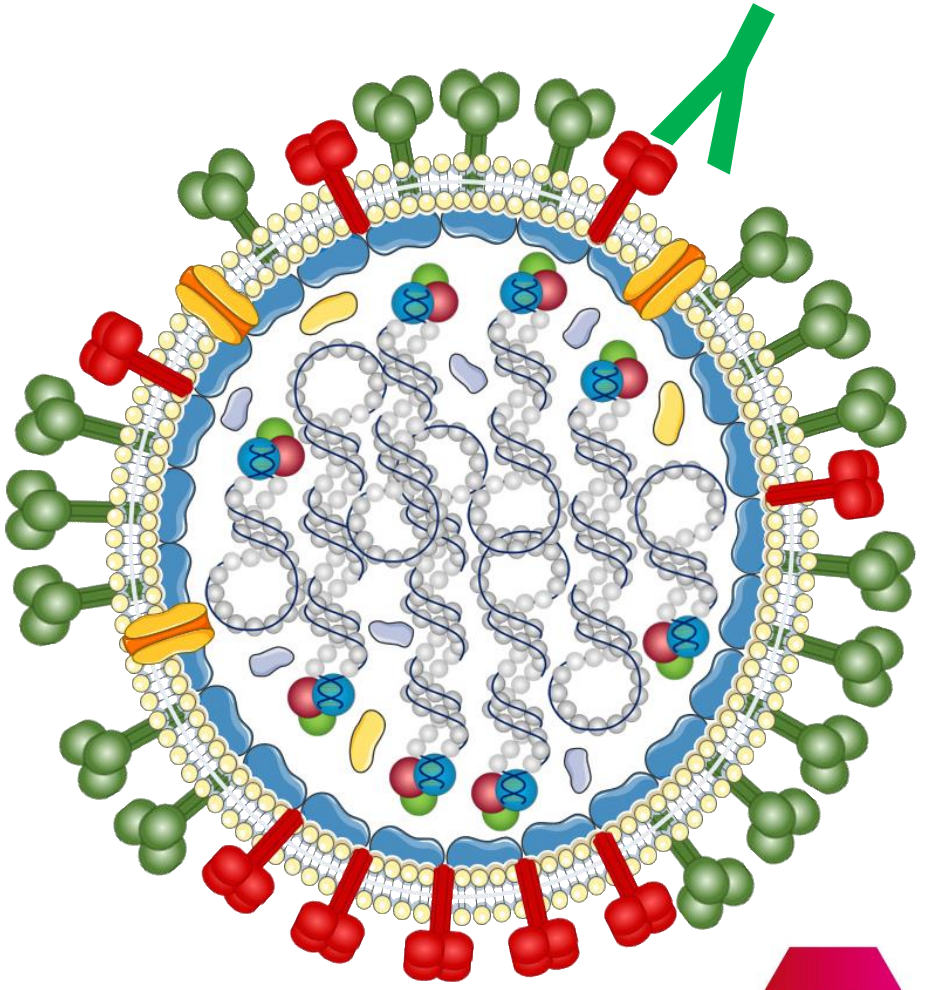
The liquid part of blood that will contain flu viruses in infected animals

The method - how do you detect influenza virus in chickens?

Use a laboratory technique called an ELISA



Enzyme
Linked
Immuno-
Sorbent
Assay



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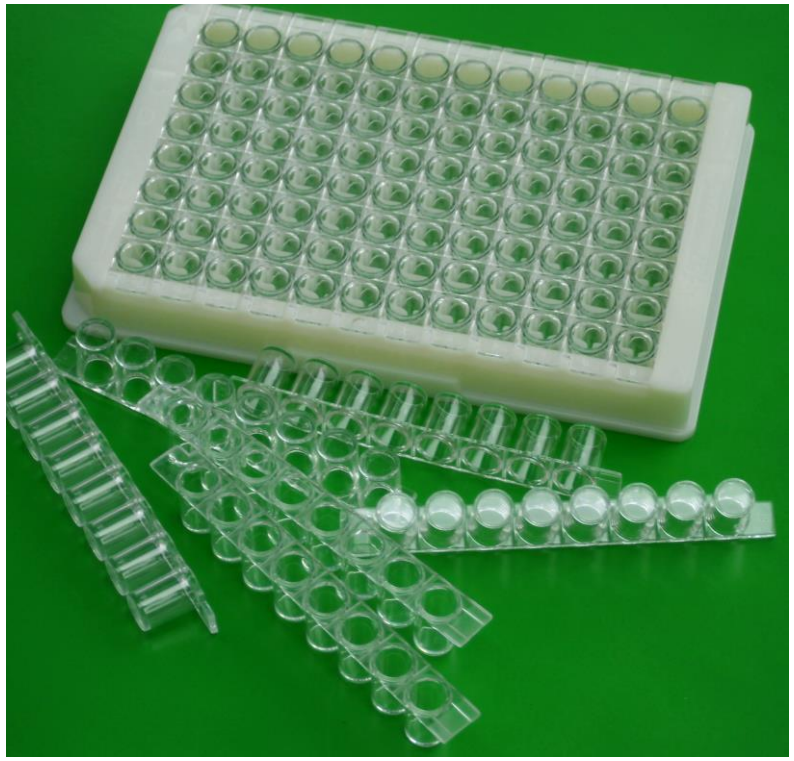
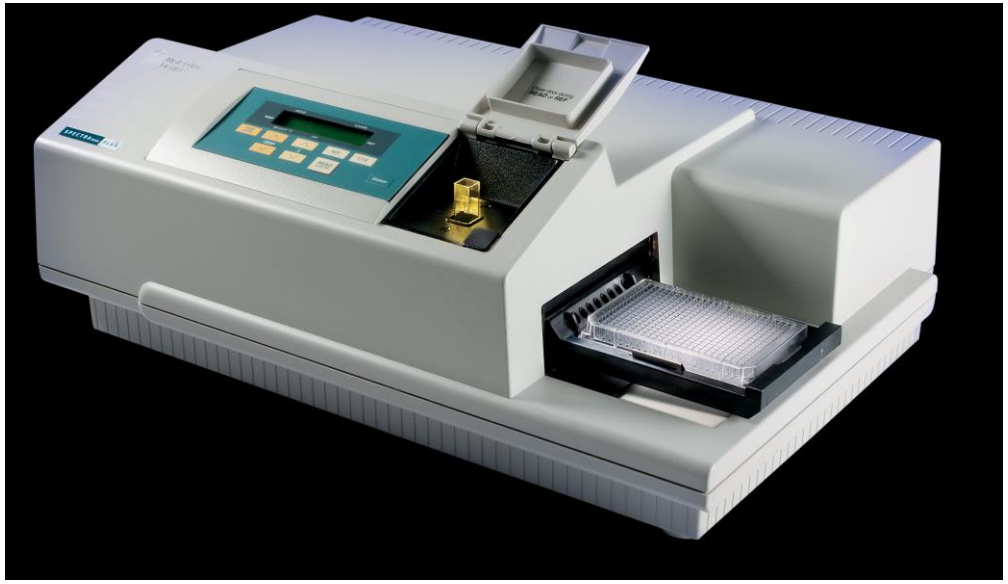
ELISA Equipment



12- well strip



microplate reader

micropipettes

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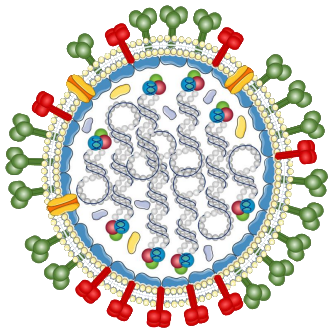
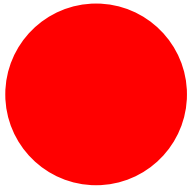
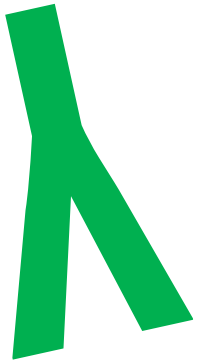
ELISA Ingredients





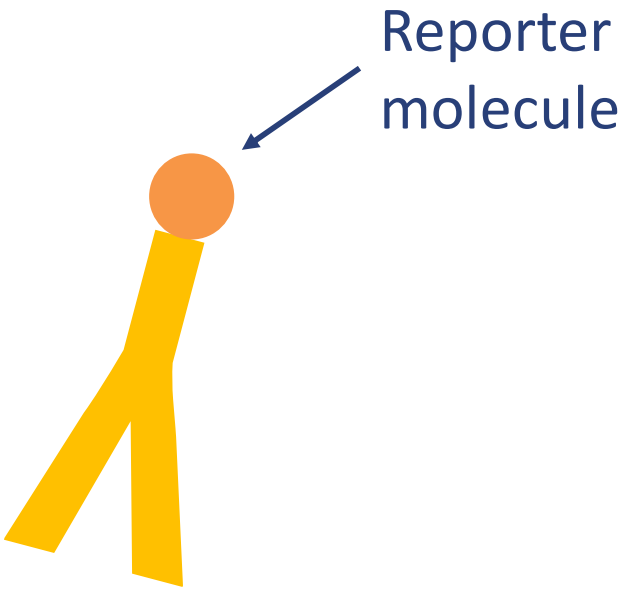
Antigen

An **NA1 protein** on the surface of the flu virus



Primary antibody

recognises the N1 protein



Secondary antibody

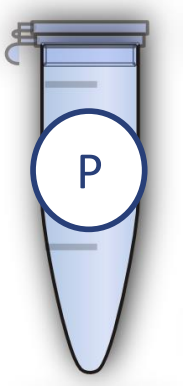
recognises primary antibody

Activity - using micropipettes

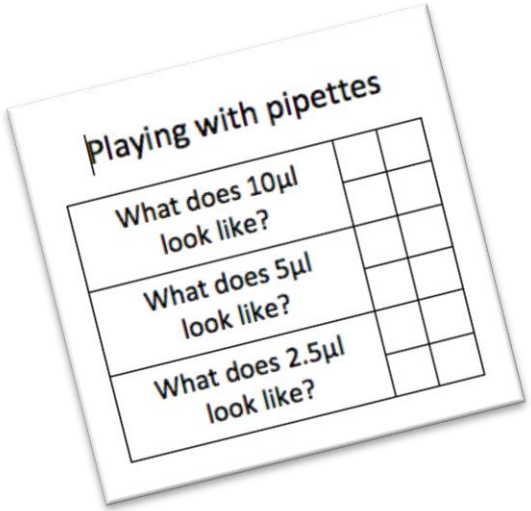
P20 pipette

P200 pipette

Tips

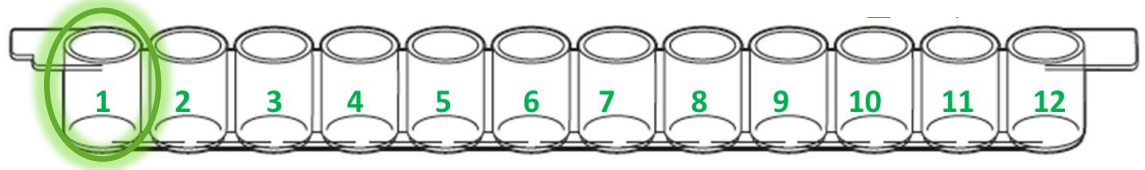


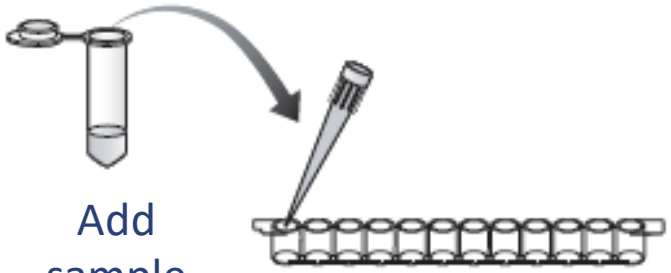
Practice dye



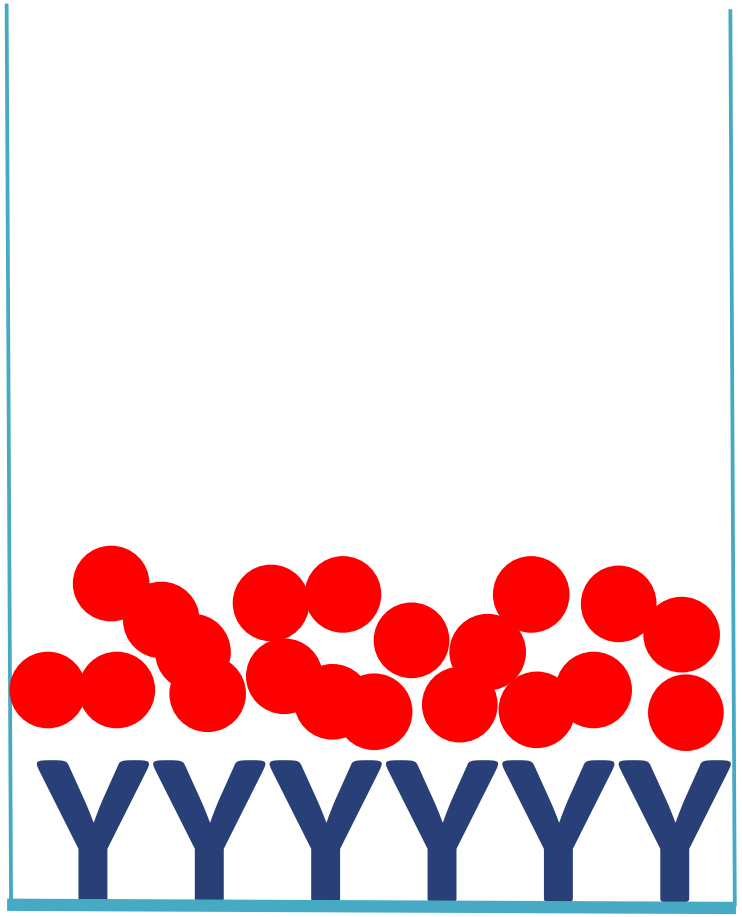
Practice card

How do ELISAs work?





Add sample



Add the serum sample to the well

antigen

Capture antibody

Primary antibody

secondary antibody

substrate

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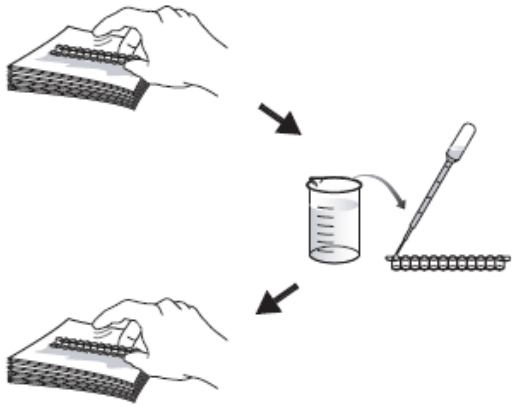
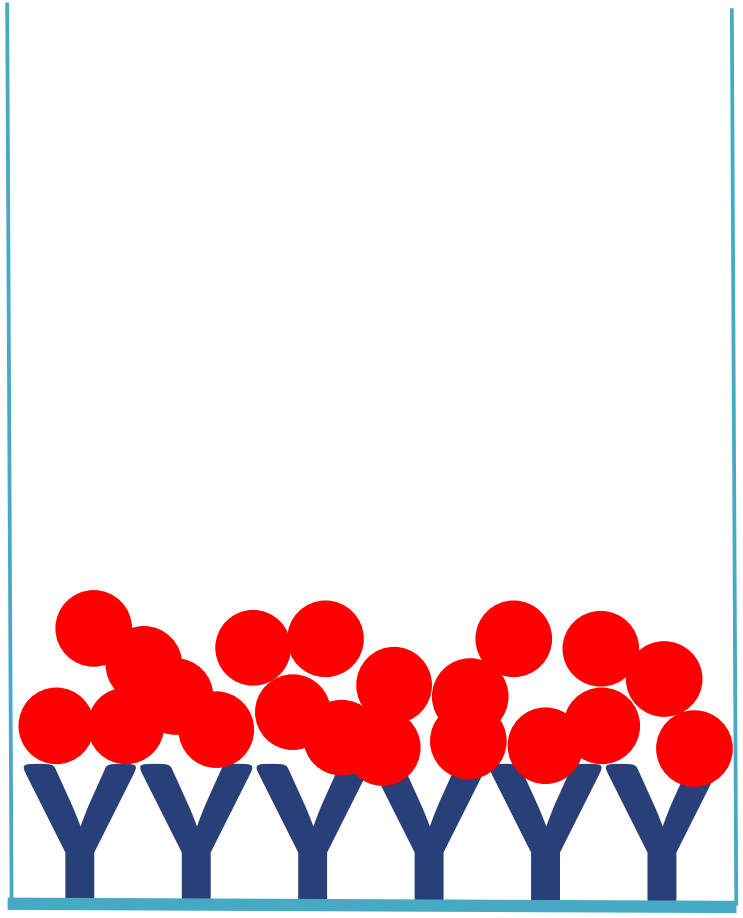




Wash the wells 2x to get rid of excess antigen

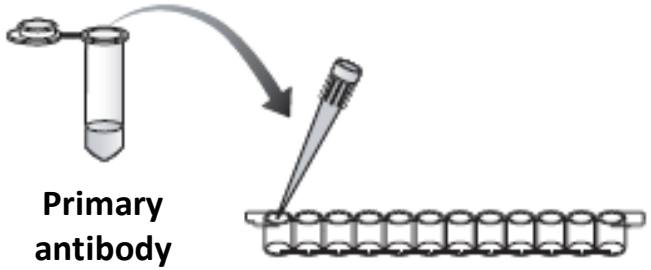
antigen

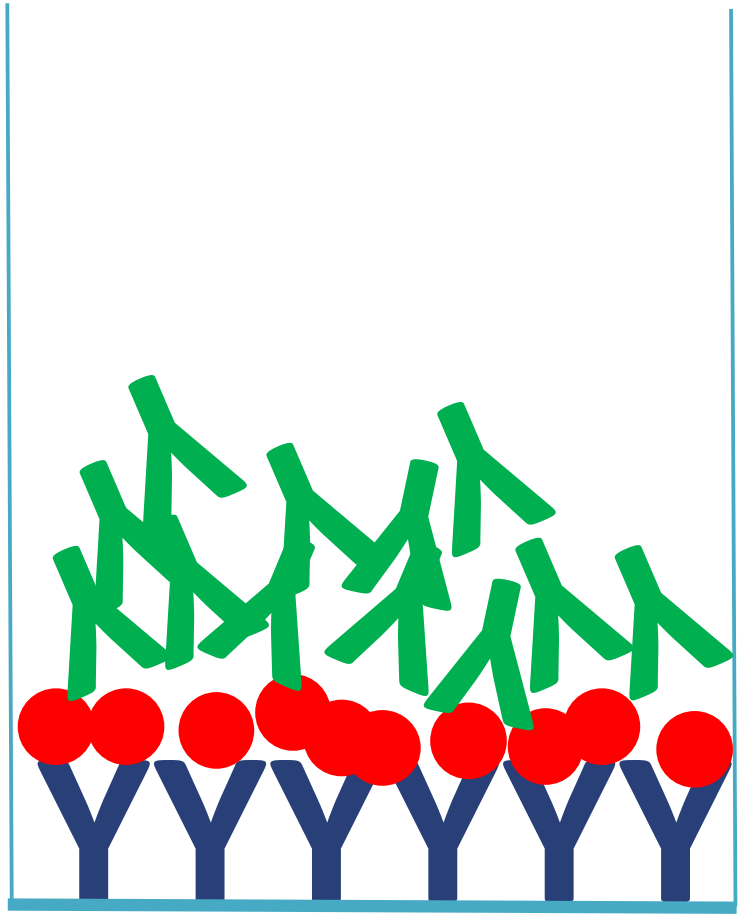
Capture antibody

Primary antibody

secondary antibody

substrate





Add the primary antibody

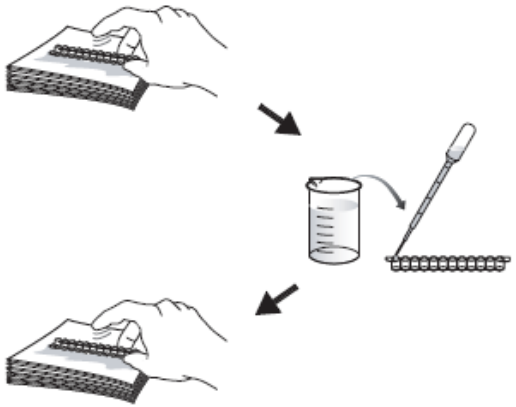
antigen

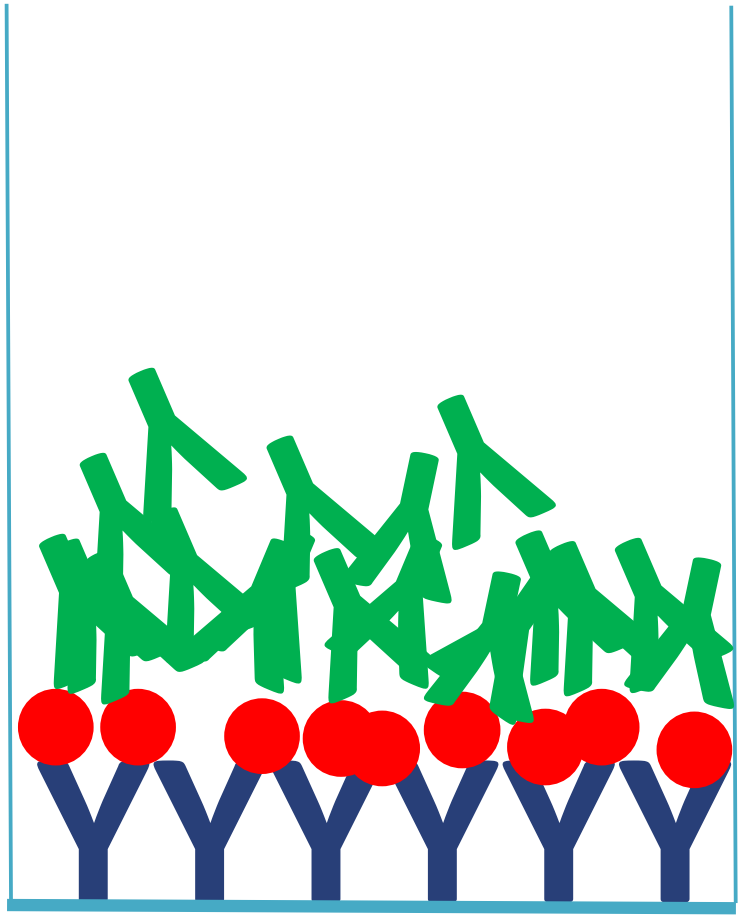
Capture antibody

Primary antibody

secondary antibody

substrate





Wash the wells 2x to get rid of the excess

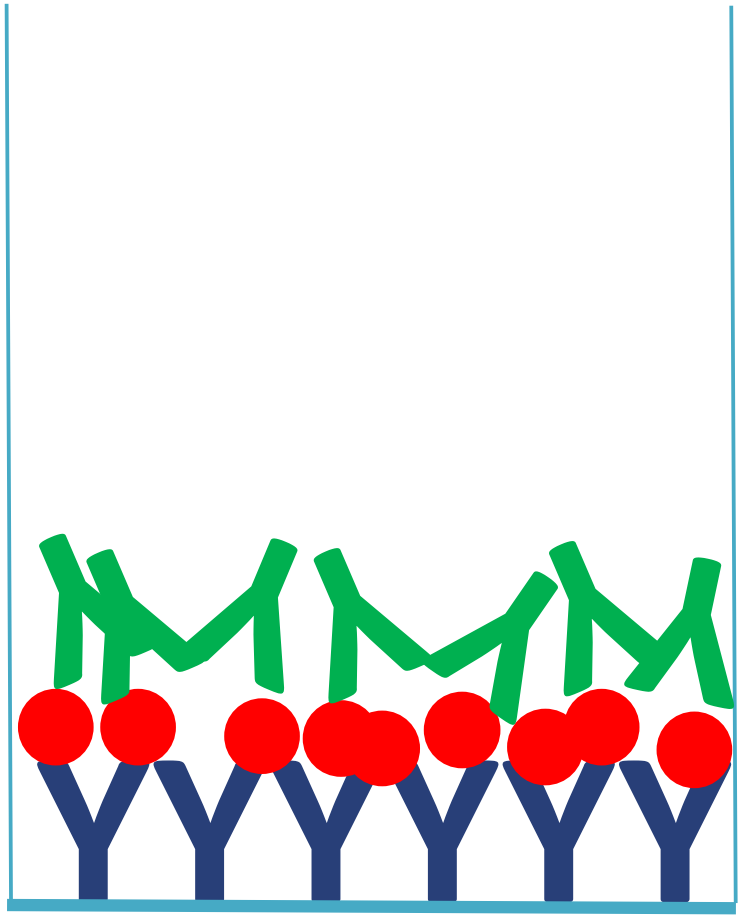
antigen

Capture antibody

Primary antibody

secondary antibody

substrate



Wash the wells 2x to get rid of the excess

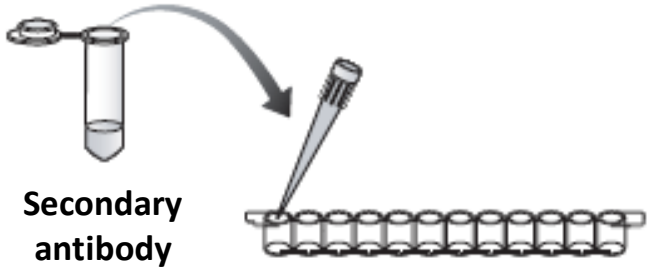
antigen

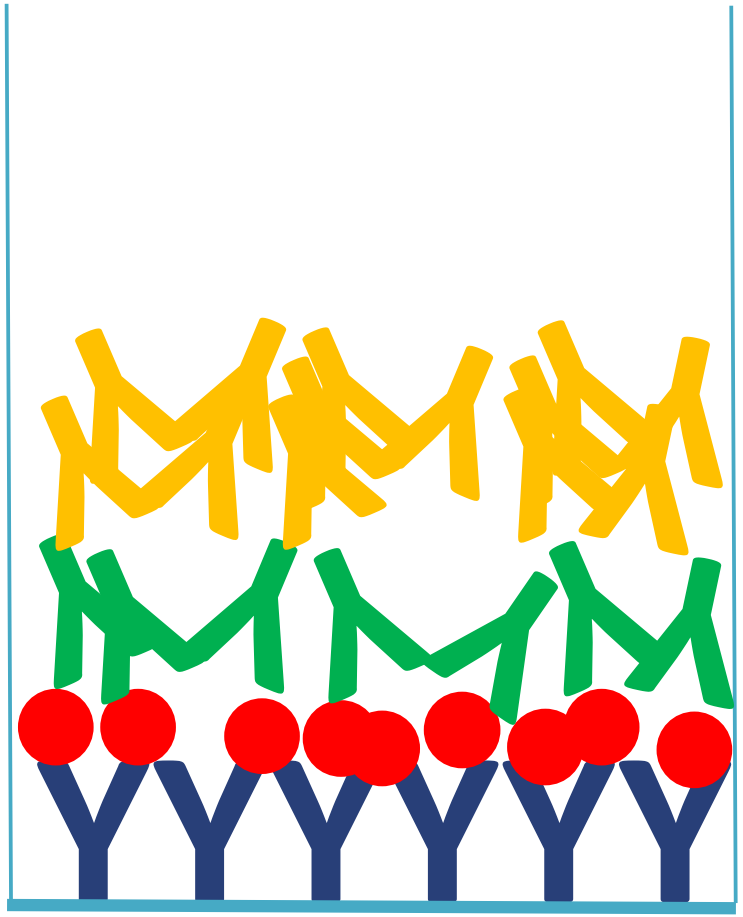
Capture antibody

Primary antibody

secondary antibody

substrate





Add the secondary antibody

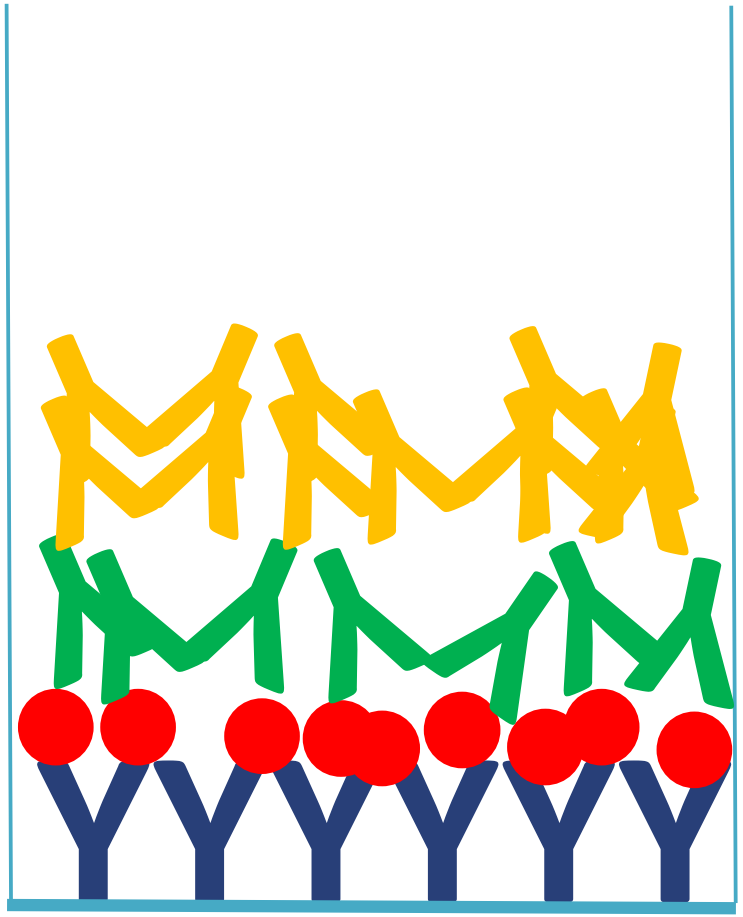
antigen

Capture antibody

Primary antibody

secondary antibody

substrate



Wash the wells 3x to get rid of the excess

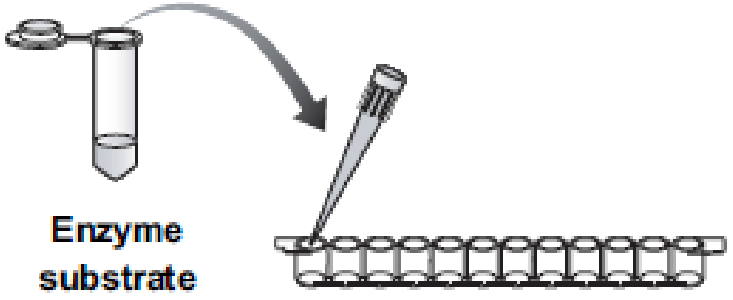
antigen

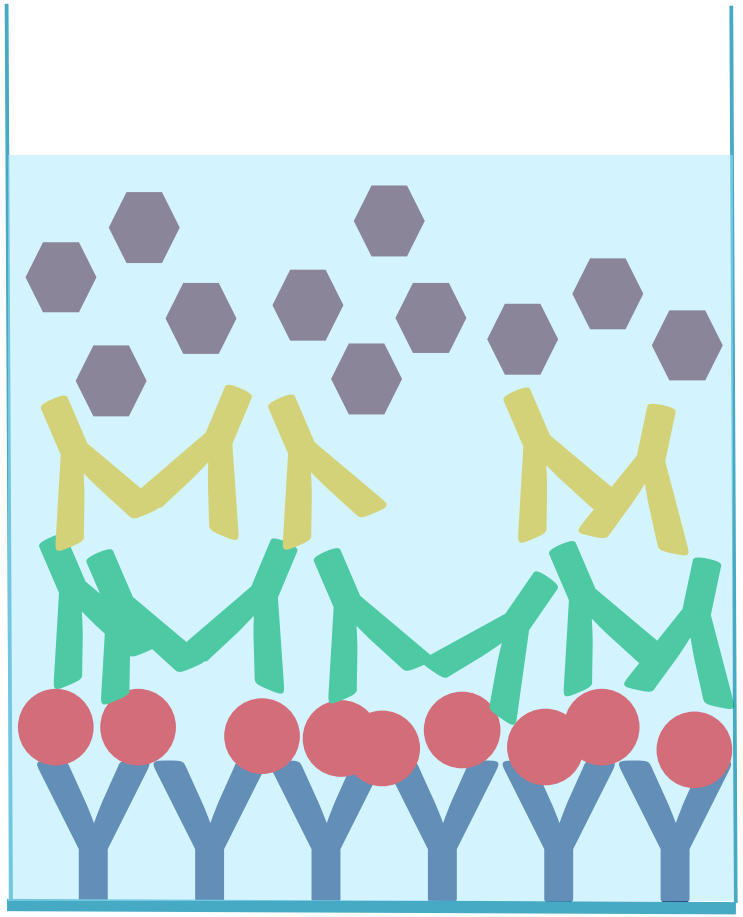
Capture antibody

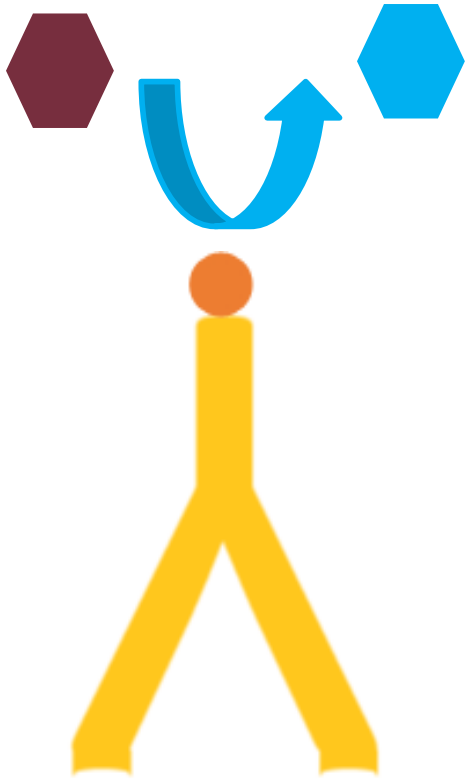
Primary antibody

secondary antibody

substrate







Add the substrate + Add acid -> **Results!**

antigen

Capture antibody

Primary antibody

secondary antibody

substrate

Today's experiment

Create a **standard curve** using known **flu antigen** concentrations

Test serum samples from chickens on farms A, B, C & D for presence of flu virus antigen

Safety first!

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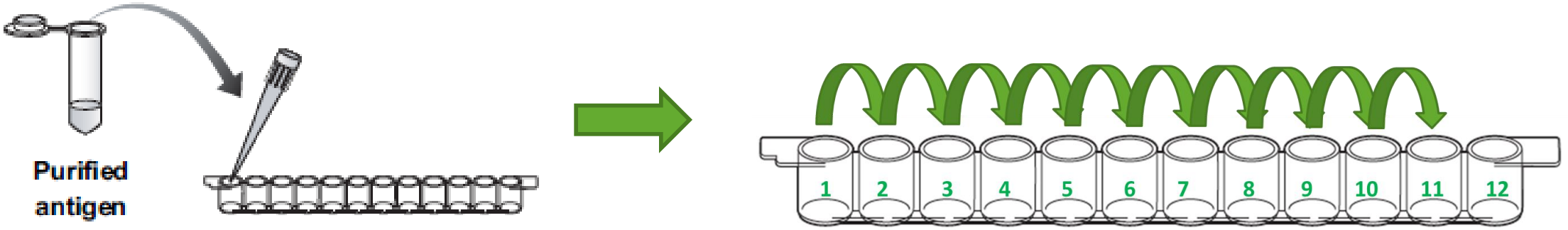
How do you create a standard curve?

1. Create a serial dilution of known concentration of flu virus antigen

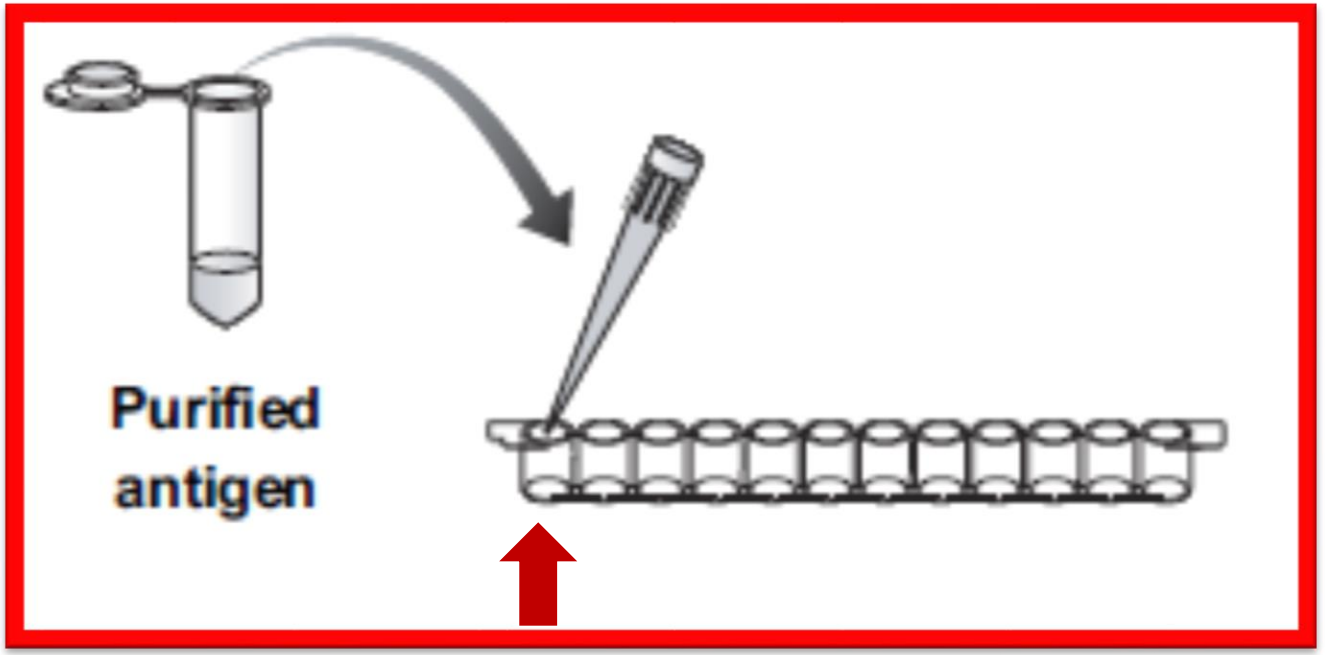


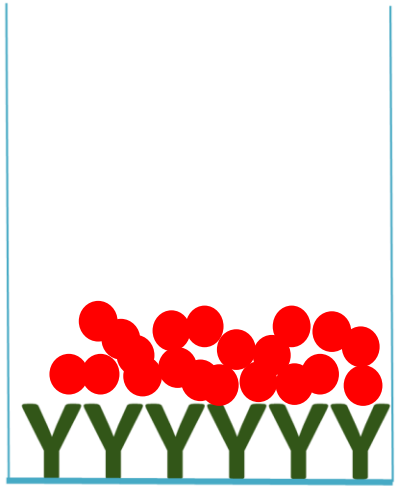
2. Test these samples using the ELISA technique

3. Plot absorbance readings from ELISA against antigen concentration to create standard curve

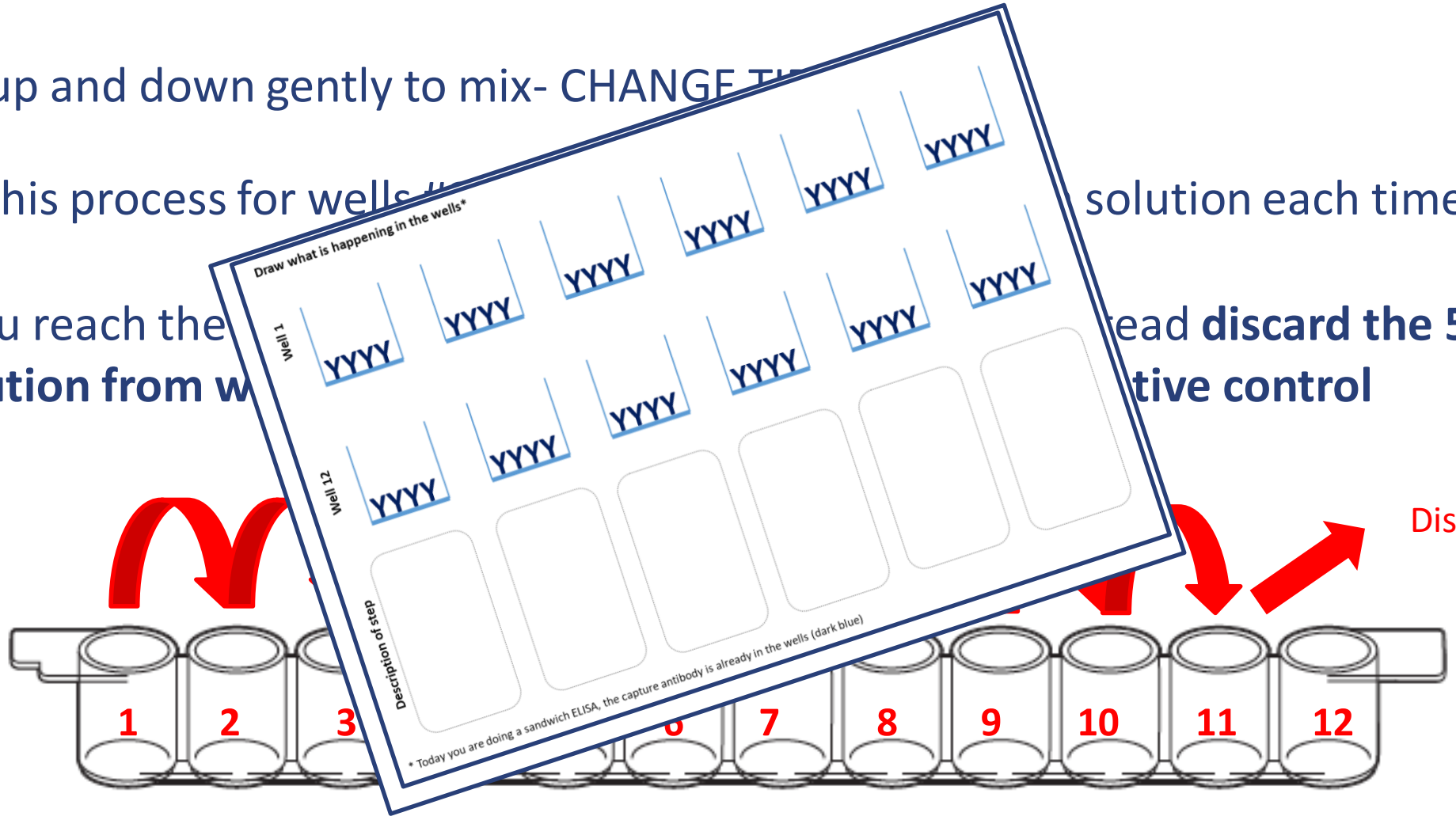
Method – Serial dilution of antigen

1. Label the outside of **one** of your 12-well strips with numbers 1-12
2. Add **50µl PBS** from the tube labelled “PBS” to wells labelled **#2 to #12**
3. Add **100µl antigen** from the red tube to well #1



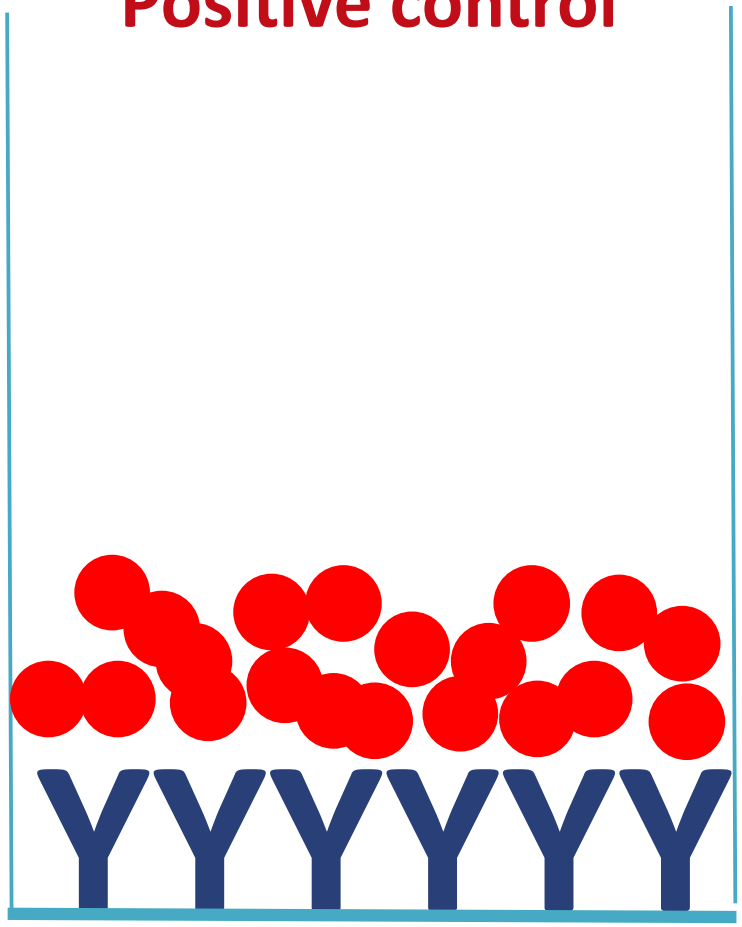


4. Using your pipette, **transfer 50µl antigen** from well #1 and add it to the PBS solution in well #2
5. Pipette up and down gently to mix- CHANGE TI
6. REPEAT this process for wells # solution each time
7. When you reach the ead **discard the 50µl antigen solution from w tive control**



8. Incubate for **5 min** to allow antigen to bind- what is happening in well 1 and 12?

Well 1
Positive control



Well 12
Negative control

antigen

Capture antibody

Primary antibody

secondary antibody

substrate

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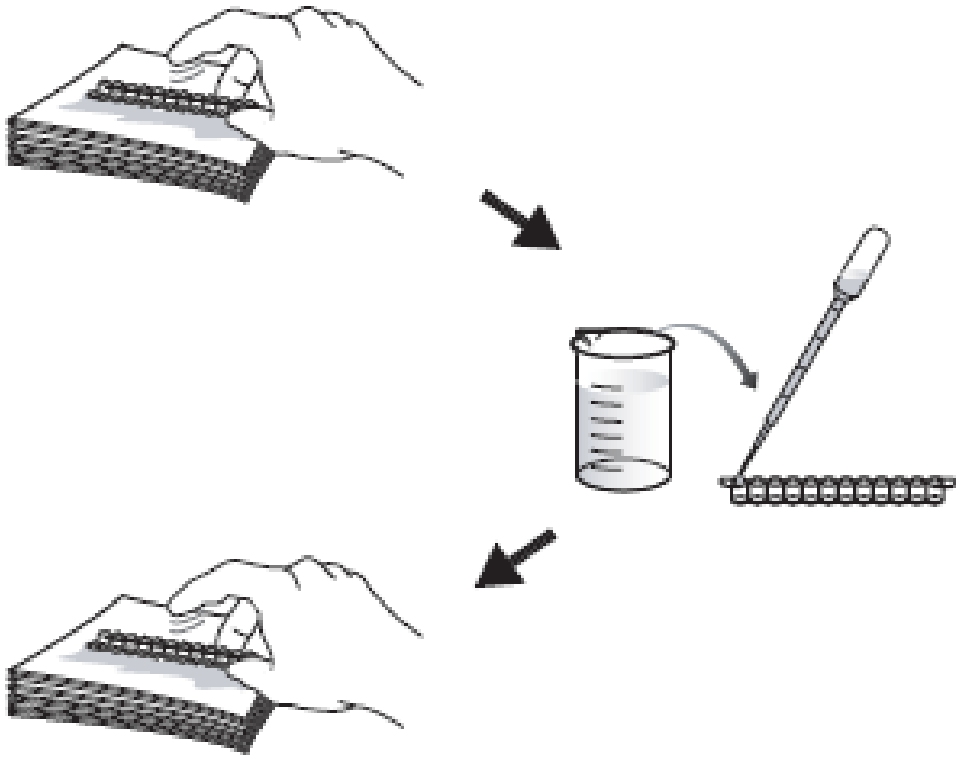
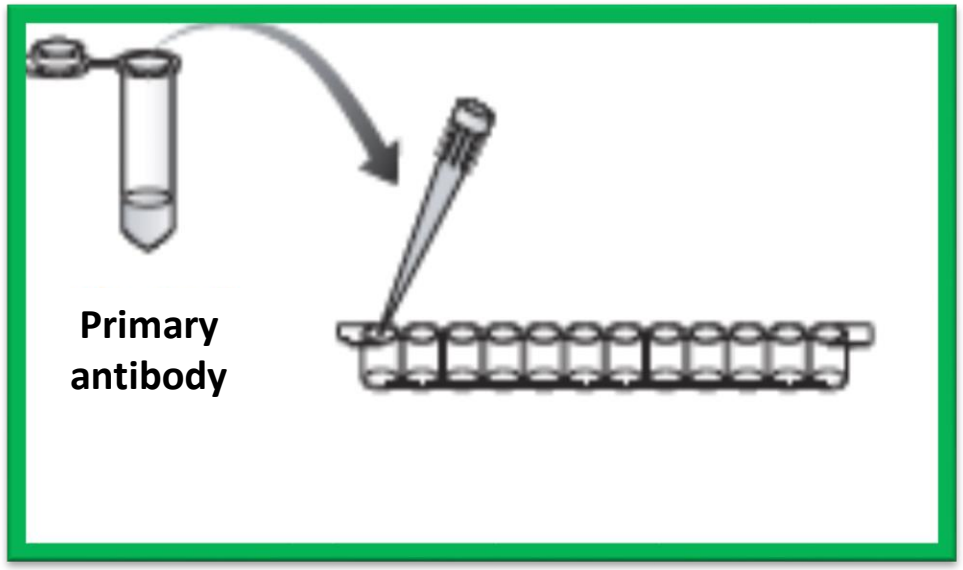
Method – Wash step

1. Tip microplate strip upside down onto paper towel stack and tap to drain wells.
2. Use transfer pipette to **fill each well with wash buffer**
3. Tip microplate strip upside down onto paper towel stack and tap to drain wells.
4. Discard the top 2–3 paper towels.
5. Repeat steps 1 to 4

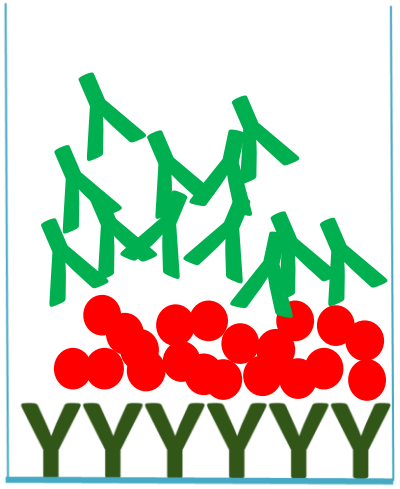


Method – Primary antibody

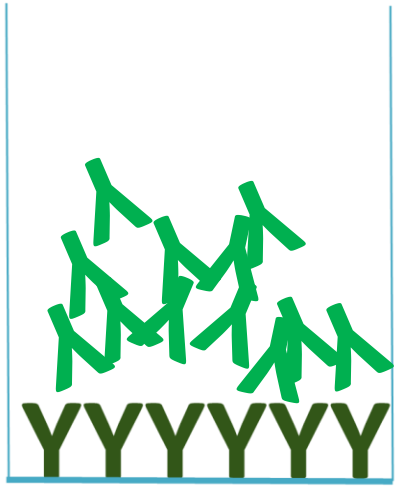
1. Add **50µl primary antibody** from the green tube to all wells
2. Wait for 5 minutes- what is happening in wells 1 and 12?
3. Wash all wells with wash buffer **2 times**



Well 1
Positive control

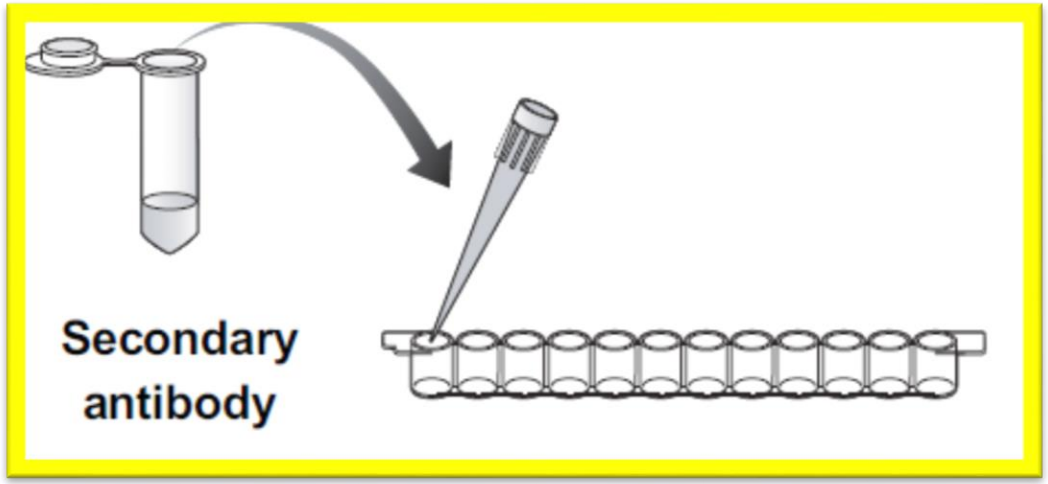


Well 12
Negative control

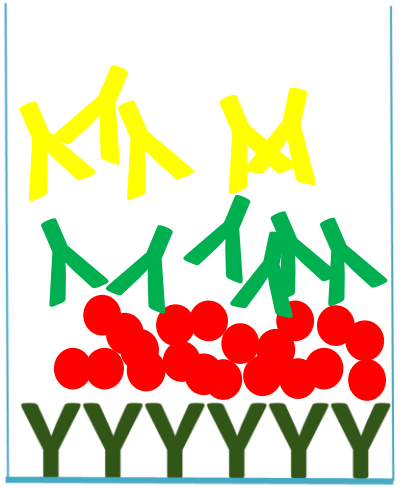


Method – Secondary antibody

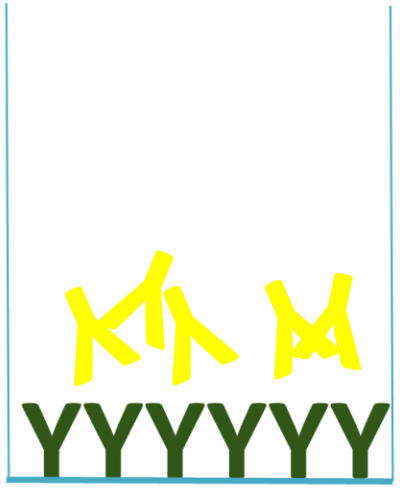
1. Transfer **50µl secondary antibody** from the yellow tube into all 12 wells
2. Wait for 5 minutes- what is happening in wells 1 and 12?
3. Wash all wells with wash buffer **3 times**



Well 1
Positive control

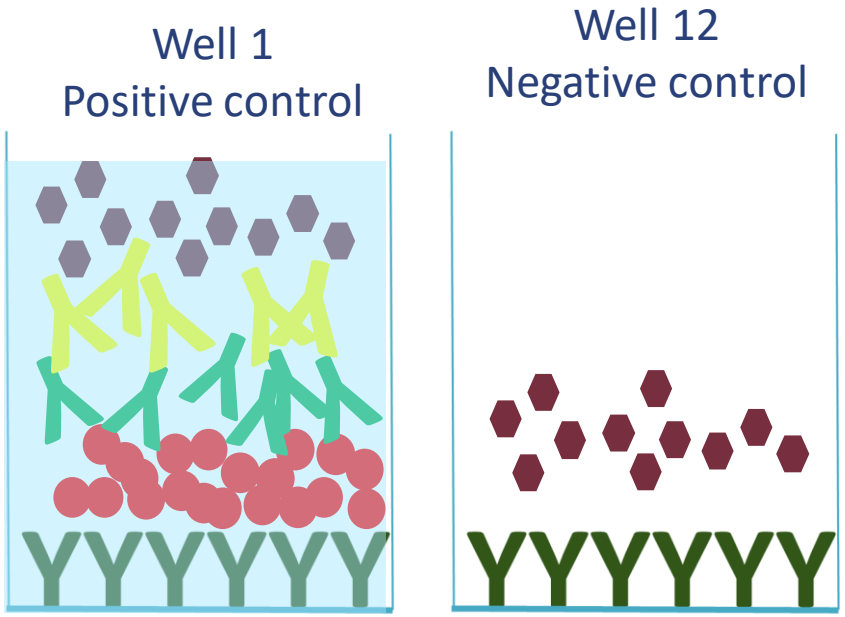


Well 12
Negative control



Method – reporter reaction

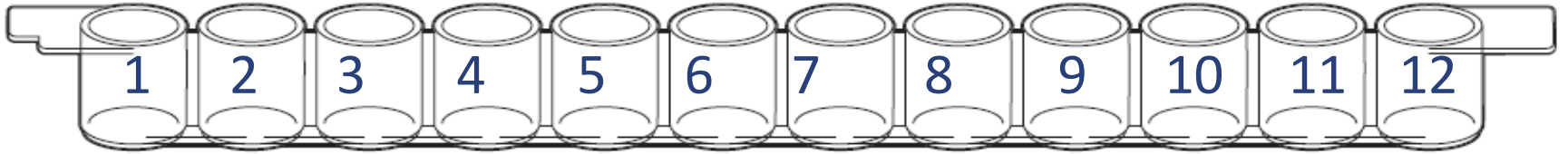
1. Transfer **50µl substrate** from the brown tube into all 12 wells
2. Wait 5 minutes- what is happening in wells 1 and 12?



3. Using a fresh tip add **100µl 0.18M sulphuric acid** to each well **<u>to stop the reaction</u>**

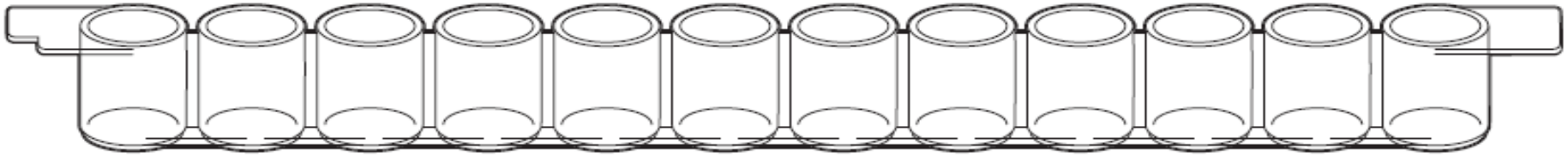
Qualitative Data Analysis Observe the colour change after the acid has been added and rate the colour change on a scale of your choice.

Well numbers:



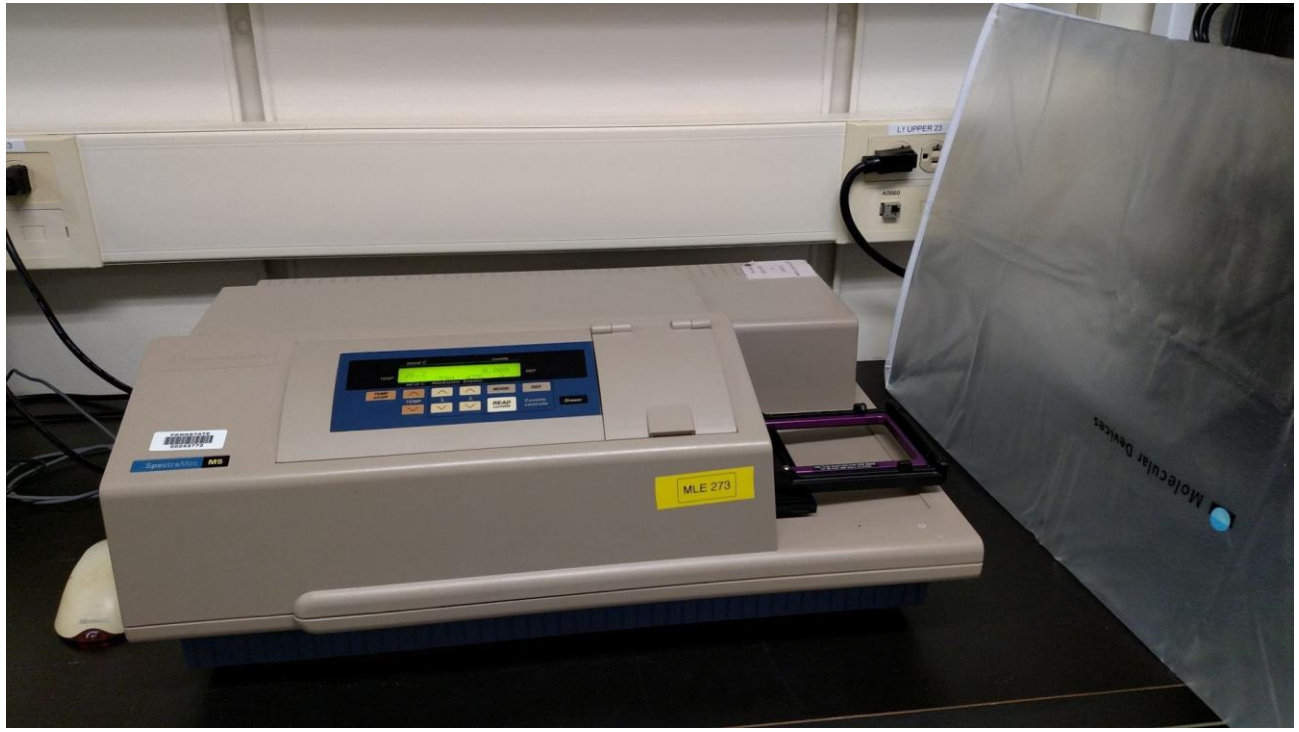
Colour intensity scale:

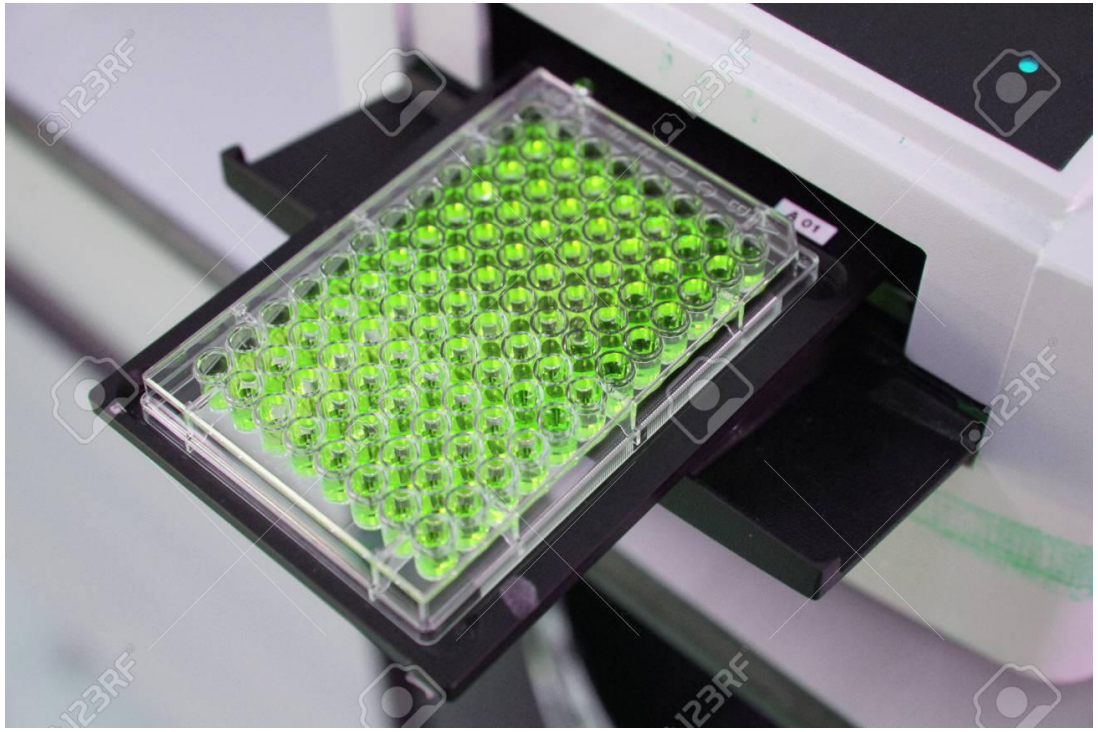
Your scores here:



Notes:

Quantitative Data Analysis Using the data from the plate reader, record the absorbance of your dilution series.





The plate reader will measure the intensity of the yellow colour

-absorbance of each well will be measured at a wavelength of 450nm-

Quantitative Data Analysis

Quantitative data : Using the data from the plate reader, record the absorbance of your dilution series and that of the entire classes. Use the average values to plot a standard curve

Well	1	2	3	4	5	6	7	8	9	10	11	12
Antigen conc. (ng/ml)	1000	500	250	125	62.5	31.3	15.6	7.8	3.9	2	1	0

Data provided on a separate print out

LUNCH

Please be back here for 12:45pm!

So far….

Created a standard curve using known antigen concentrations

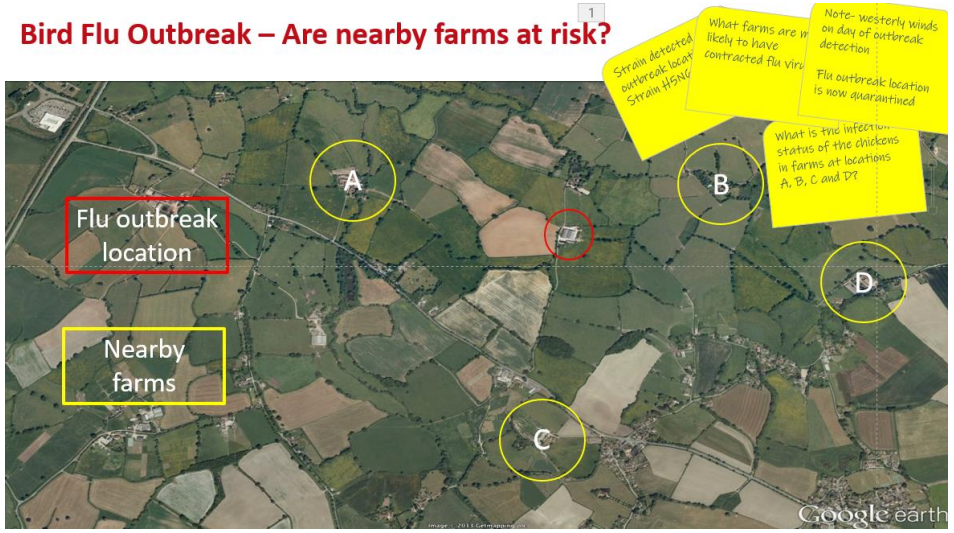
Next…

Test serum samples from chickens on farms A, B, C & D for presence of flu virus antigen

What is your hypothesis?

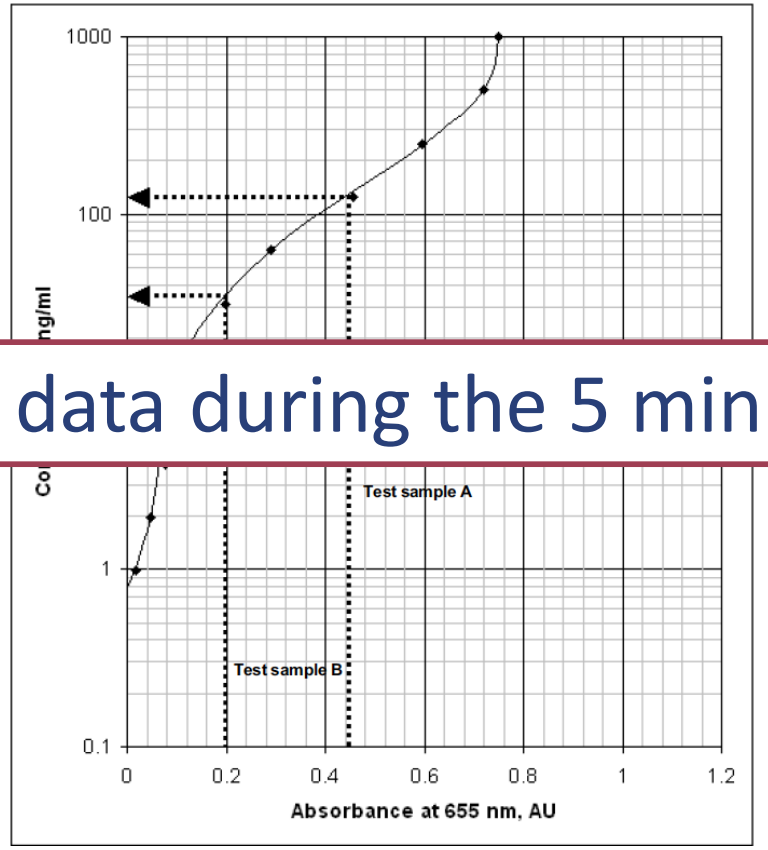
Which of the surrounding farms (A-D) do you think could have infected chickens?

- Analyse the information
- Identify the important evidence
- Write down your hypothesis



Why have we created a standard curve?

- Creating a standard curve using **known** concentrations of flu antigen will allow us to convert the readings from our **unknown** test samples into antigen concentrations



Plot your data during the 5 minute waits

ELISA Masterclass: Flu Fighters

27th August 2019

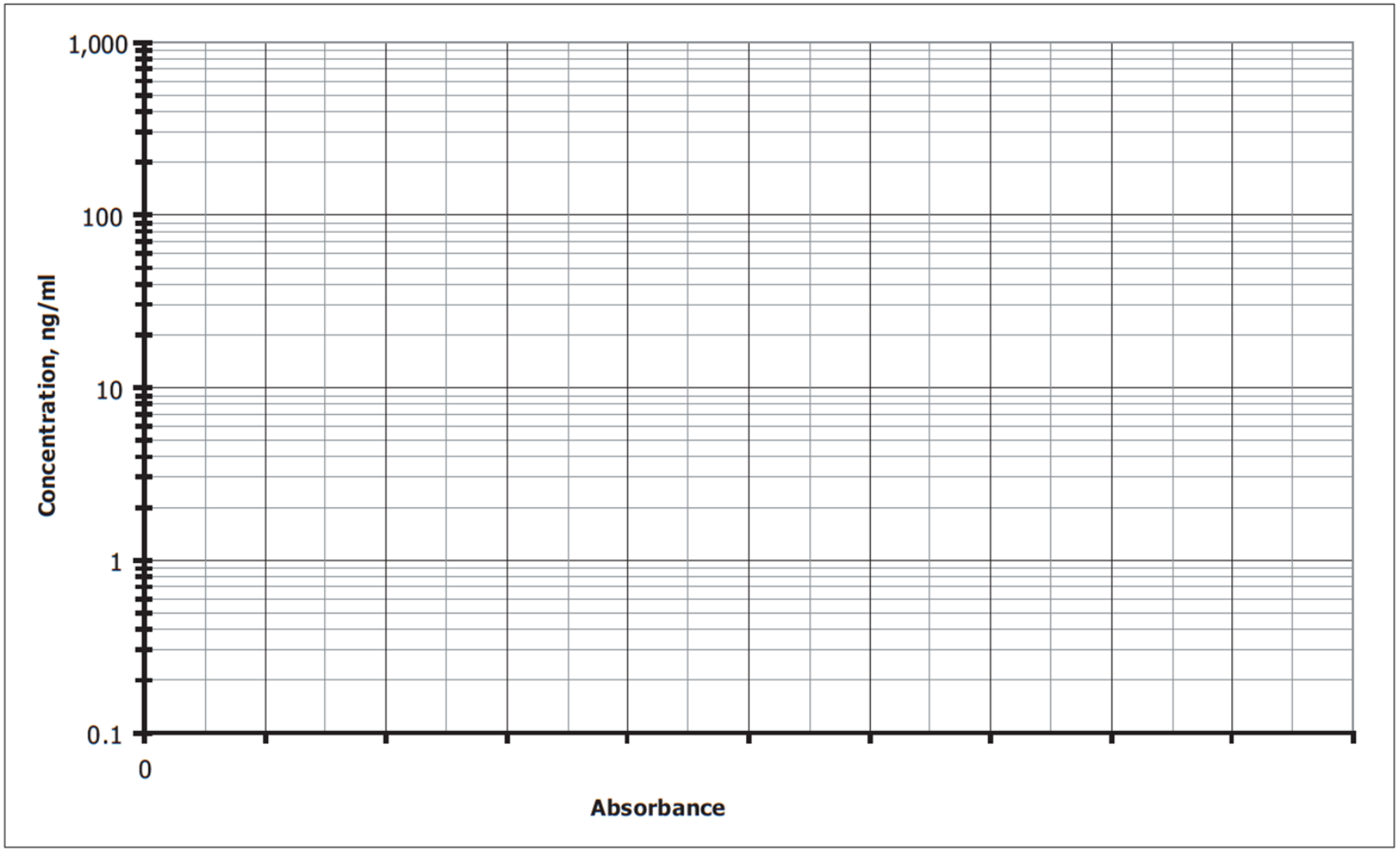
ELISA #1: Flu antigen standard curve

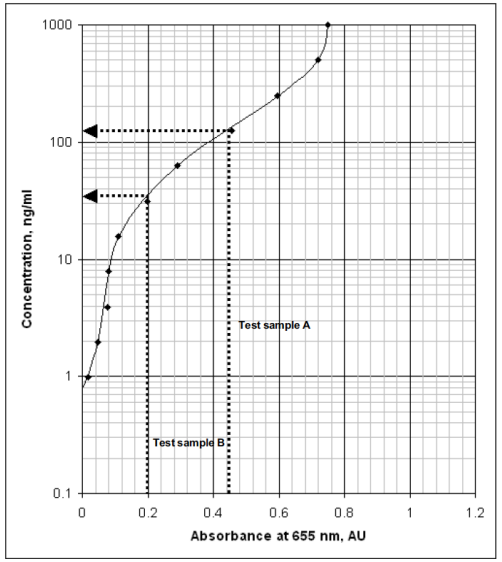
Group	Well number and antigen concentration (ng/ml)											
	1 (1000)	2 (500)	3 (250)	4 (125)	5 (62.5)	6 (31.3)	7 (15.6)	8 (7.8)	9 (3.9)	10 (2.0)	11 (1.0)	12 (0)
A1 & A2	1.952988	1.786888	1.724988	1.598688	1.004988	0.642488	0.209888	0.087588	0.019688	-0.01121	-0.01501	-0.02691
A3 & A4	2.110688	1.977288	1.485188	1.059788	0.610788	0.275888	0.175088	0.073588	0.037788	0.003588	-0.03501	-0.04541
B1	1.488488	1.310388	0.607988	0.021888	-0.01151	-0.02501	-0.03221	-0.01981	-0.02791	-0.03051	-0.03071	-0.02401
B2 & B3	2.142188	1.596088	1.005688	1.192288	0.608688	0.068188	0.076288	0.033988	0.060488	-0.01061	-0.01141	-0.00071
C1	1.294988	1.576988	1.036988	1.016288	0.395388	0.116388	0.141588	0.349288	0.165088	0.050688	0.045988	0.206588
C3	1.768388	2.011188	1.612288	1.207288	0.723788	0.216688	0.352588	0.051188	0.213988	0.053788	-0.00071	-0.00781
D	2.290188	2.216188	1.950088	1.551788	0.975188	0.514088	0.196088	0.134088	0.025288	0.009688	-0.00721	-0.02971
E1 & 2	1.818125	1.813425	1.399525	0.907225	0.488025	0.131625	0.067025	0.004825	-0.02688	-0.03498	-0.03218	-0.01868
E3	2.399625	2.220325	1.502625	1.042725	0.966725	0.087225	-0.01058	-0.04608	-0.04718	-0.04278	-0.04058	-0.04858
F 1 & 2	2.161825	1.773925	1.732725	0.970625	0.340525	0.507125	0.308625	0.193225	0.099025	0.137525	0.245425	0.202925
F 3 & 4	2.179325	2.236325	1.690625	1.302125	0.780825	0.647825	0.313125	0.399825	0.683725	0.115425	0.798125	0.177125
Average	1.964256	1.865365	1.431701	1.079156	0.625765	0.28932	0.16341	0.114701	0.109374	0.021874	0.083337	0.034983

Quantitative Data Analysis

Plot standard curve

Plot the average absorbance for your standard curve on this semi log graph paper. Draw a best-fit line





Get hands-on with real-life science

Safety first!

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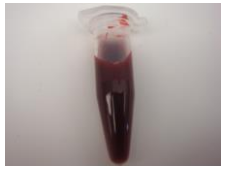
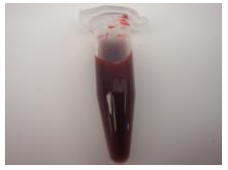
Science Outreach Centre

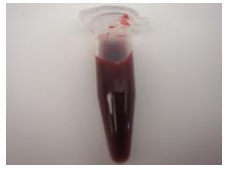
Get hands-on with real-life science

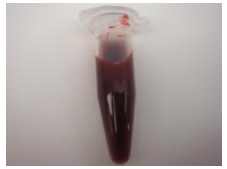
We have a pure serum pure serum for 4 chickens

Chicken blood sample from

Farm A 

Farm B 

Farm C 

Farm D 

Centrifuged chicken blood sample from

Farm A 

Farm B 

Farm C 

Farm D 

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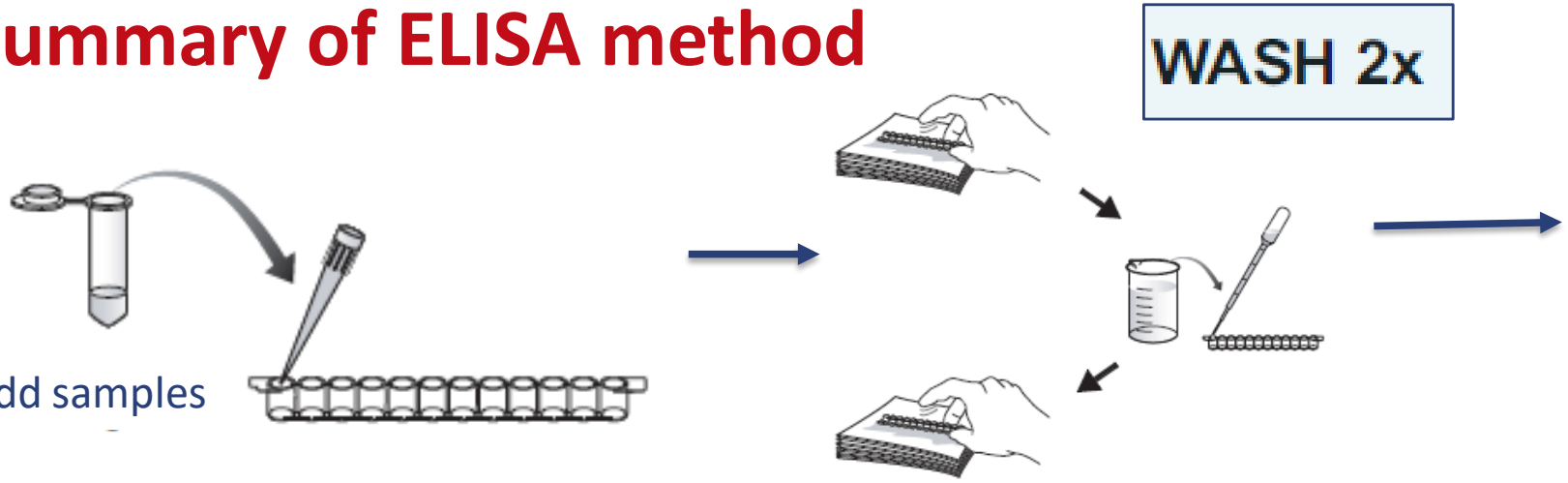
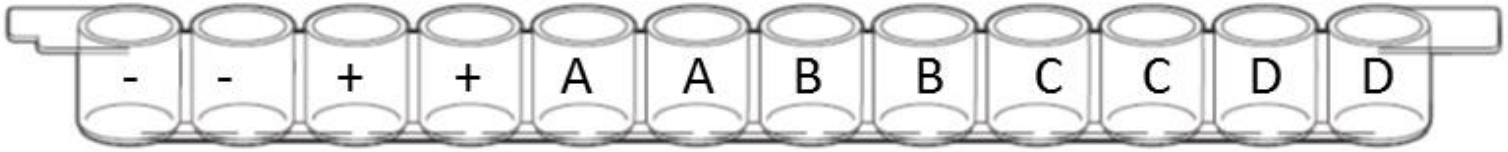
Summary of ELISA method

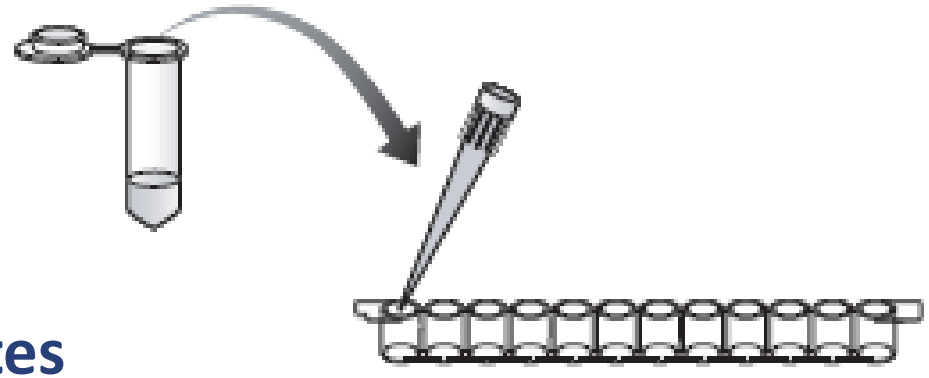


Part 2 – Testing the chicken serum samples

1. Label your second 12-well strip – you will need two wells each for your **negative control**, **positive control** and the **4 farm samples A-D**.



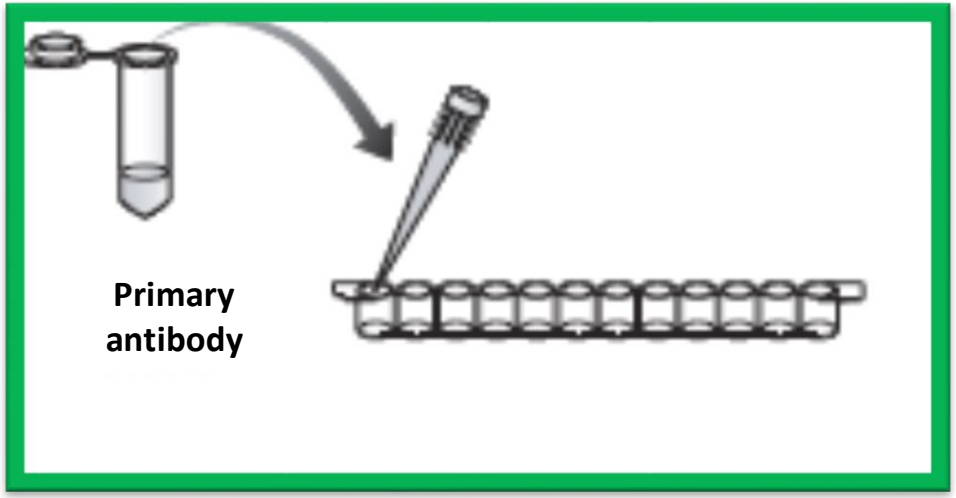
2. Transfer **50µl each sample** into **2 wells** of the microplate strip.



3. Wait for **5 minutes**
4. Wash all wells with wash buffer **2 times**

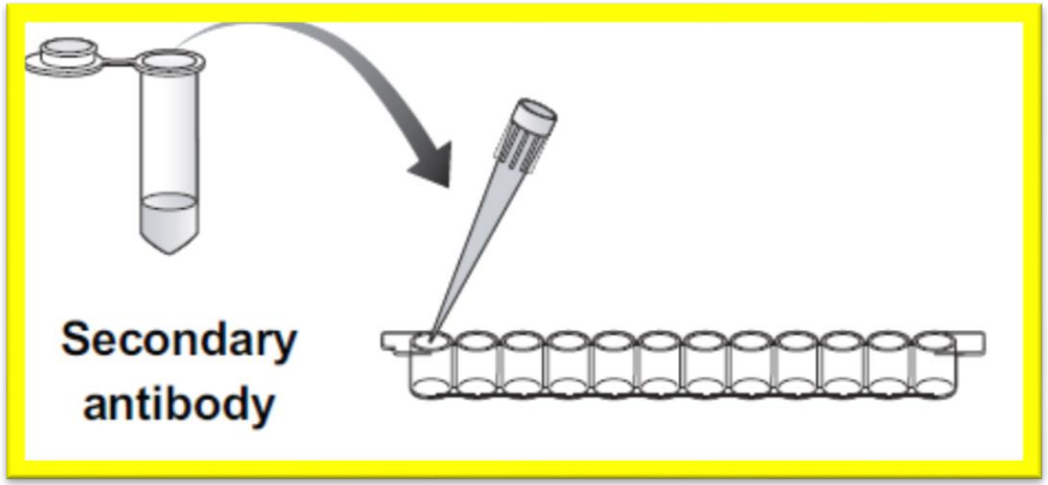
Method – Primary antibody

1. Add **50µl primary antibody** from the green tube to all wells.
2. Incubate for 5 minutes
3. Wash all wells with wash buffer **2 times**



Method – Secondary antibody

1. Transfer **50µl secondary antibody** from the yellow tube into all 12 wells
2. Wait for 5 minutes
3. Wash all wells with wash buffer **3 times**



Method – reporter reaction

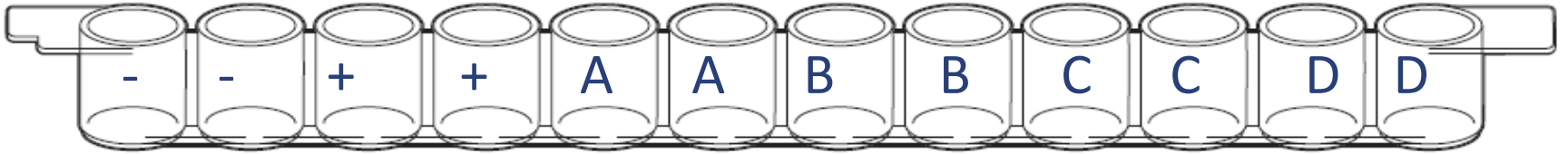
1. Transfer **50µl substrate** from the brown tube into all 12 wells

1. Wait 5 minutes

3. Using a fresh tip add **100µl 0.18M sulphuric acid** to each well **to stop the reaction**

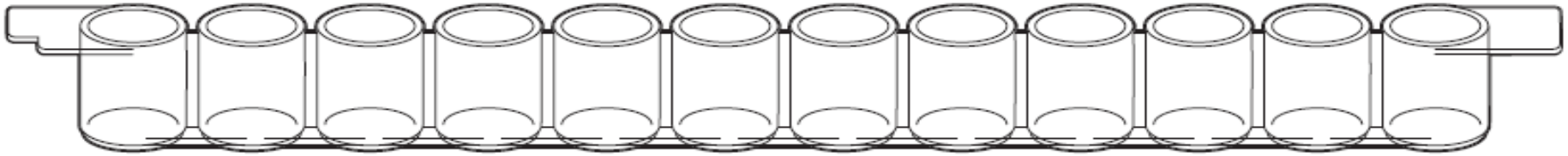
Qualitative Data Analysis Observe the colour change after the acid has been added and rate the colour change on a scale of your choice.

Well numbers:



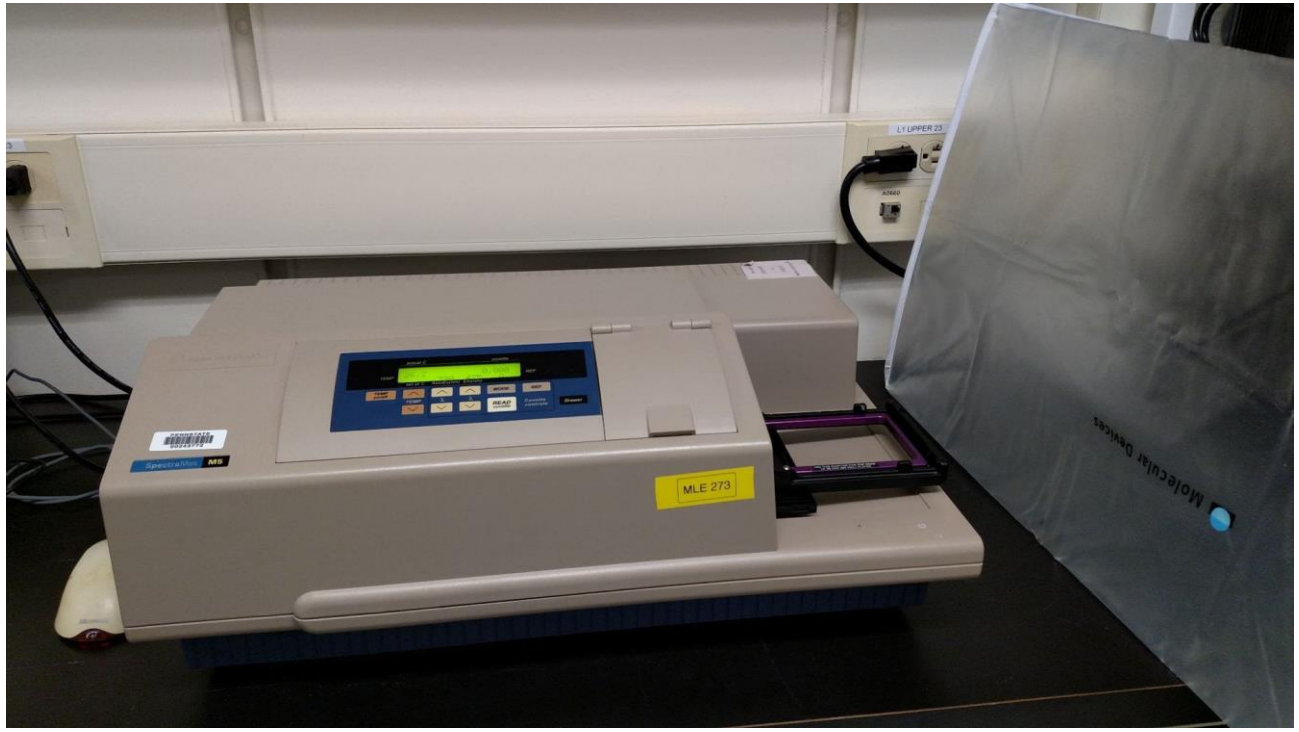
Colour intensity scale:

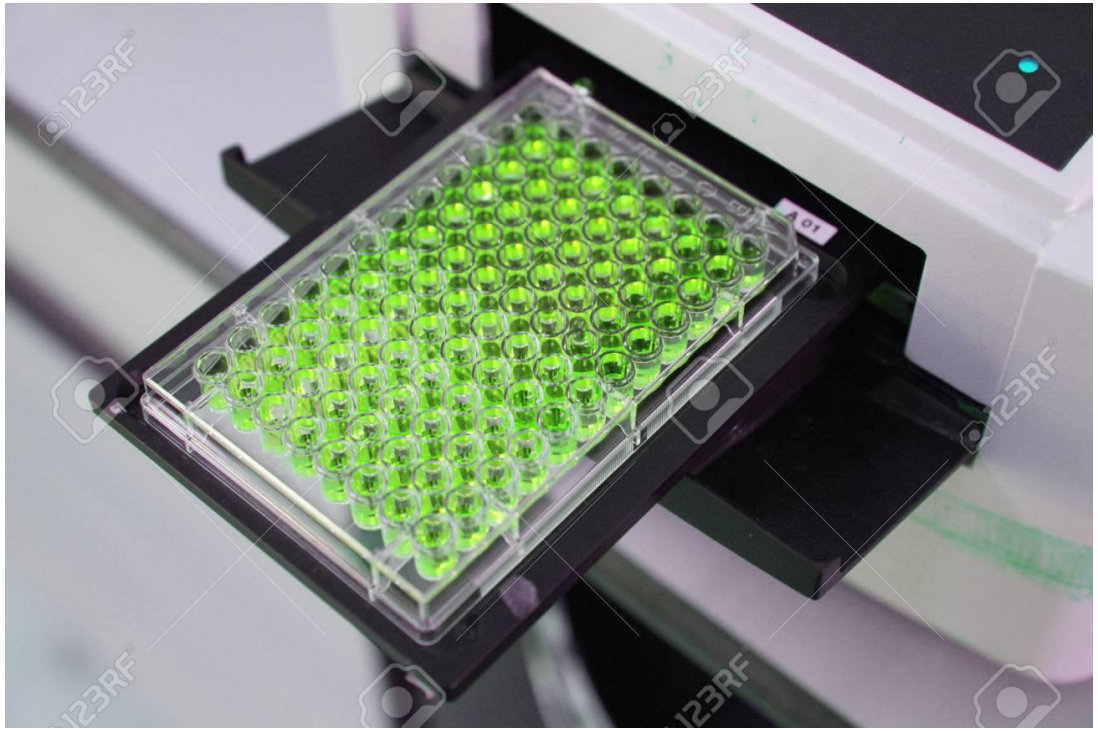
Your scores here:



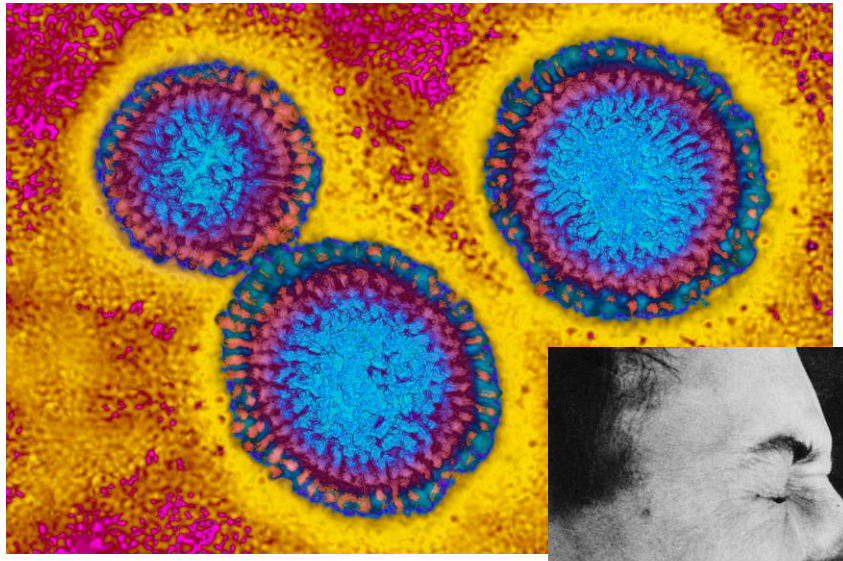
Notes:

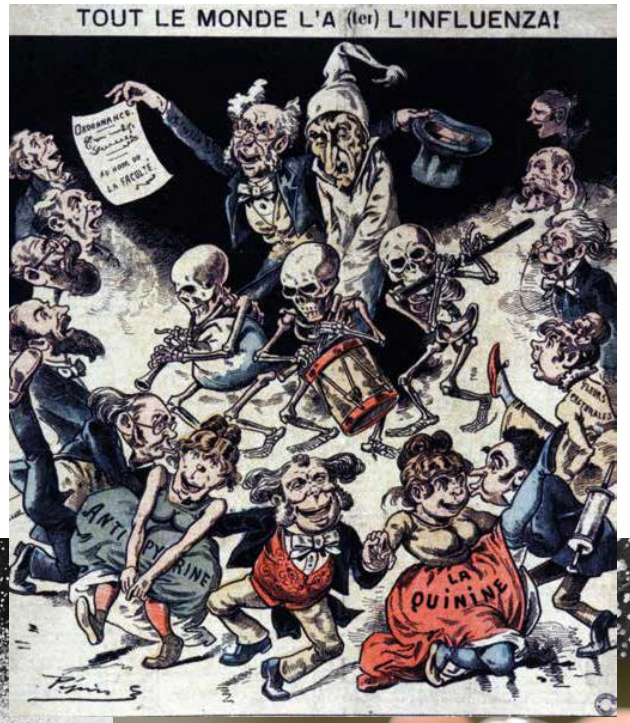
Quantitative Data Analysis

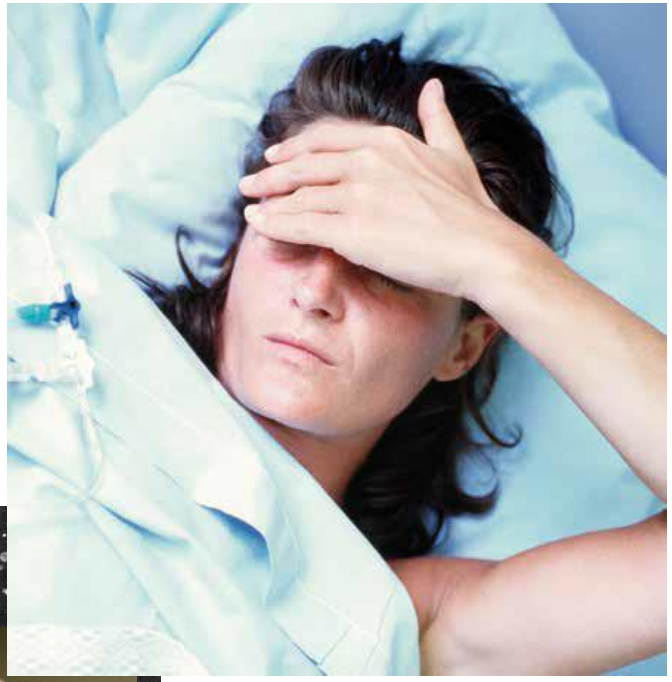




Let’s focus on flu







Qualitative Data Analysis

Quantitative data: Record the absorbance reading for each sample and use your standard curve to calculate the level of antibodies in the chicken samples from each farm

Sample	Absorbance reading 1st well	Absorbance reading 2nd well	Average absorbance	Concentration of flu antibodies in serum	Diagnosis
- control					
+ control					
A					
B					
C					
D					

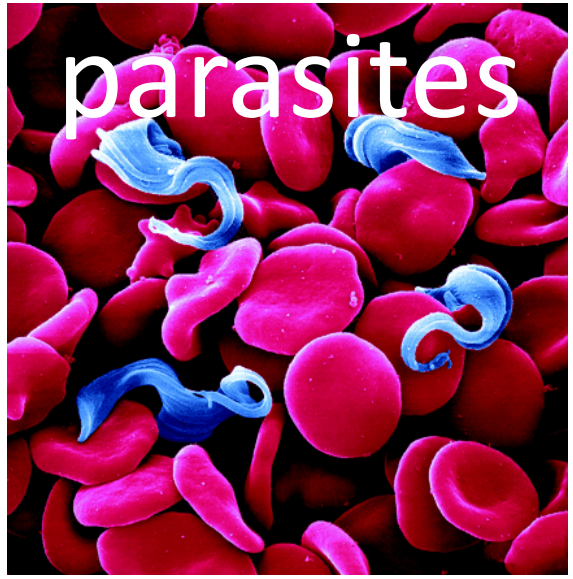
Results and Conclusions

Meet the Scientists

ROSLIN

Control of Infectious Diseases

- Our scientists study:



That infect animals, especially farm animal species

- Many of these diseases can also be transmitted to humans

= ZOONOSES

The Roslin Institute- Improving animal health and welfare

BBSRC
bioscience for the future

Summary of the day

Created a standard curve using known antigen concentrations

Test serum samples from chickens on farms A, B, C & D for presence of flu virus antigen

Worked with Roslin Institute scientists and learned what it is like to work in a research lab

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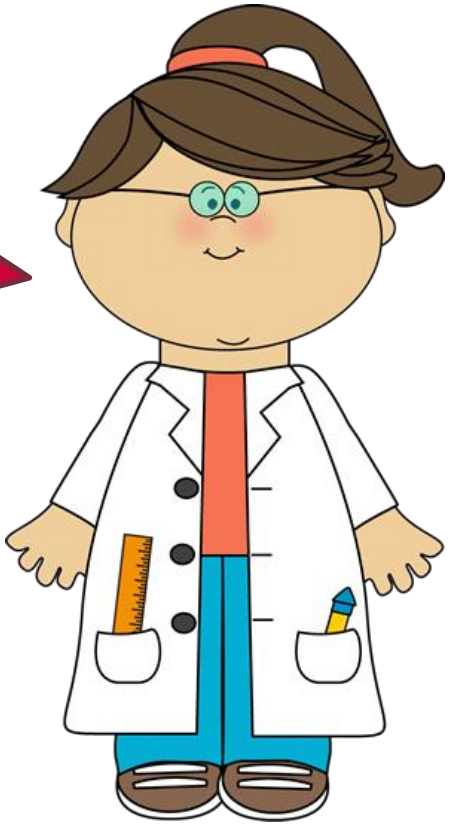
Final instructions

- Fill out online feedback form
- Tidy your tables & trays
- Collect your pencils and worksheets
- Check your lab coat pockets
- Wash your hands



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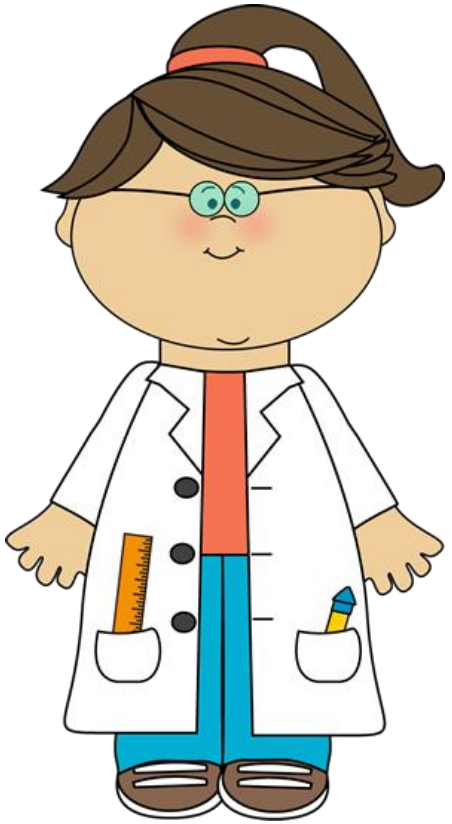
Please write down three words on our wall that describe your experience today!



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Fun

Boring

Informative

Inspiring

Rewarding

Uninteresting

Interesting

Confusing

Enjoyable

Challenging

Thought-provoking

Frustrating

Dull

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