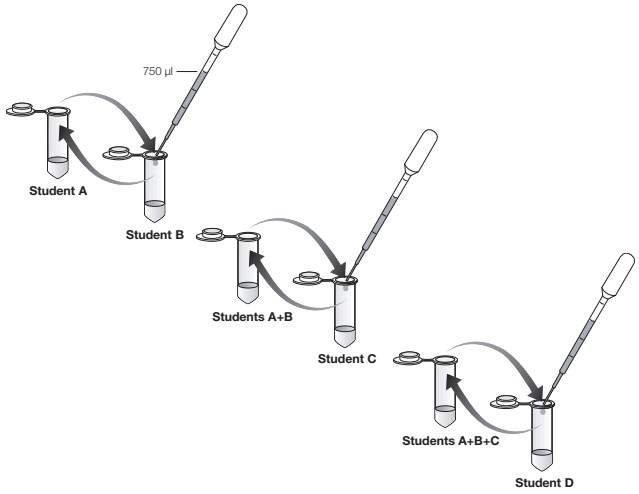
Laboratory Quick Guide

ELISA for Tracking Disease Outbreaks
Student Workstation Checklist

One workstation serves 4 students.

Item (Label)	Contents	Number	(✔)
Yellow tubes	Student test samples (0.75 ml)	4 (1 per student)	❑
Violet tube (+)	Positive control (0.5 ml)	1	❑
Blue tube (–)	Negative control (0.5 ml)	1	❑
Green tube (PA)	Primary antibody (1.5 ml)	1	❑
Orange tube (SA)	Secondary antibody (1.5 ml)	1	❑
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	❑
12-well microplate strips		2	❑
50 µl fixed-volume micropipet or 20–200 µl adjustable micropipet		1	❑
Yellow tips		10–20	❑
Disposable plastic transfer pipets		5	❑
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	1	❑
Large stack of paper towels		2	❑
Black marking pen		1	❑



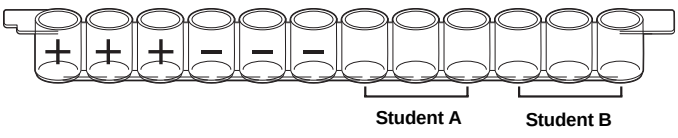
1. Label a yellow tube and a plastic transfer pipet with your initials.
2. Use the pipet to transfer all your "bodily fluid" sample into the tube of another student. Gently mix the samples, then take back half of the shared sample (750 µl) to your own tube. Write down the name of the student next to "Sharing Partner #1".
3. When instructed to do so, repeat the sharing protocol two more times. Discard this transfer pipet after this step.

   Optional stopping point: Samples may be stored at 4°C overnight.
4. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your and your lab partner's initials (3 wells each).

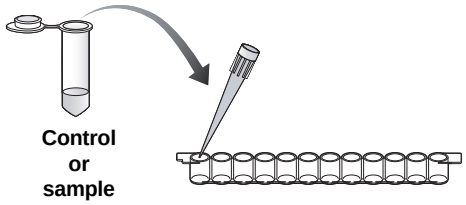
Sharing Partner #1 _____

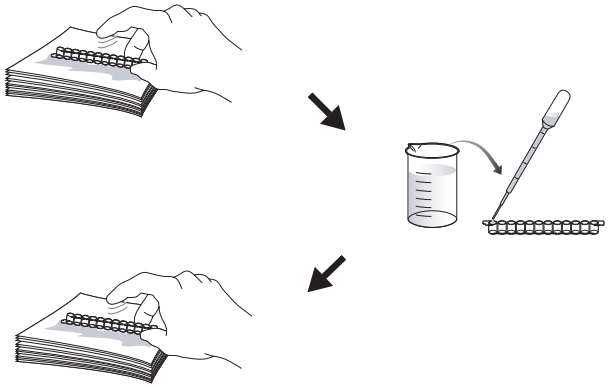
Sharing Partner #2 _____

Sharing Partner #3 _____



5. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.
6. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.
7. Transfer 50 µl of each of your team's samples from step 3 into the appropriately initialed three wells, using a fresh pipet tip for each sample.
8. Wait 5 minutes while all the proteins in the samples bind to the plastic wells.
9. WASH:
   a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid samples splashing back into wells.
   b. Discard the top paper towel.
   c. Use a fresh transfer pipet to fill each well with wash buffer, taking care not to spill over into wells. Note: the same transfer pipet is used for all washing steps.
   d. Tip the microplate strip upside down onto the paper towels and tap.
   e. Discard the top 2–3 paper towels.

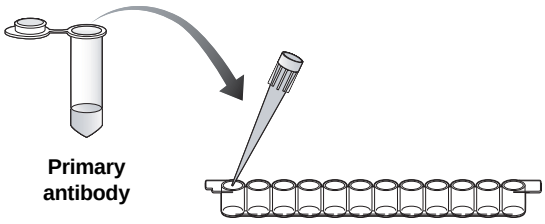




WASH

10. Repeat wash step 9.

11. Use a fresh pipet tip to transfer 50 µl of primary antibody (PA) into all 12 wells of the microplate strip.

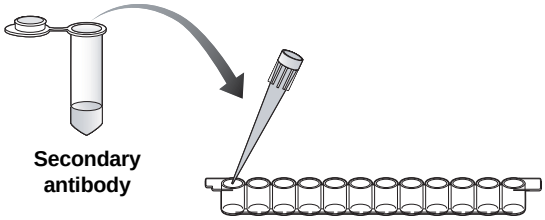


12. Wait 5 minutes for the antibodies to bind to their targets.

13. Wash the unbound primary antibody out of the wells by repeating all of wash step 9 **two** times.

WASH 2x

14. Use a <u>fresh</u> pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip.

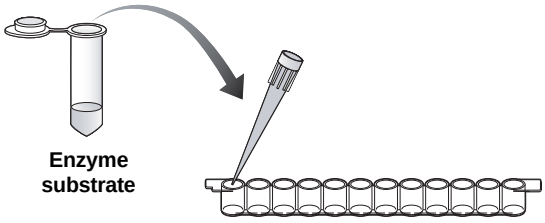


15. Wait 5 minutes for the antibodies to bind to their targets.

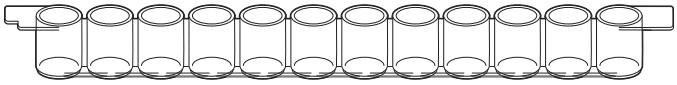
16. Wash the unbound secondary antibody out of the wells by repeating wash step 9 **three** times.

WASH 3x

17. Use a <u>fresh</u> pipet tip to transfer 50 µl of enzyme substrate (SUB) into all 12 wells of the microplate strip.



18. Wait 5 minutes. Observe and record the results.



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