Indirect ELISA

The purpose of an ELISA (Enzyme-Linked ImmunoSorbent Assay) is to detect one specific protein out of a complex mixture of proteins using the immunological principles of antigen-antibody specificity. The purpose of this indirect ELISA is to detect antibodies to HIV (one specific protein) in patient’s blood (complex mixture of proteins). This is the actual test done to determine if someone has been exposed to HIV. There are no safety issues regarding this ELISA, as the samples are simulated.

Materials Needed
ELISA plate (12 wells)
plate-coating buffer + antigen (HIV)
plate-washing buffer
assay-blocking buffer + detecting antibody (anti-human IgG-HRP)
substrate solution
10 patient samples (serum)
1 positive control sample
1 negative control sample
p200 pipetman
p200 tips
pasteur pipets

Day 1: Procedure
1. pipet 100µl plate-coating buffer/antigen (HIV) solution into each of the 12 wells of the ELISA plate (you do not need to change pipet tips in between)
2. incubate the plate in the 37°C incubator for 30 minutes
3. remove the liquid from the ELISA plate by gently tapping the liquid into the sink; tap plate "dry" onto a paper towel
4. add 50µl of patient sample #1 to well #1 (keep careful records of each well # and patient #)
5. using a new pipet tip each time (you don’t want to cross-contaminate patient samples), add the remaining 9 patient samples to the ELISA plate (each patient sample gets its own well)
6. add 50µl of the positive control to one well (keep track of which well!)
7. add 50 ml of the negative control to one well (keep track of which well!!)
8. give your plate to your wonderful instructor, and she will incubate it at 4°C for you until the next lab period

Day 2: Procedure

9. remove patient samples from the ELISA plate by gently tapping into sink
10. gently wash the plate 3x with plate-washing buffer (use a pasteur pipet to fill the wells) (i.e. fill wells, tap liquid out, fill wells, tap liquid out...)
11. tap the liquid into sink; tap plate "dry" onto paper towel
12. add 100µl of the detecting antibody solution to each of the 12 wells (you do not need to change pipet tips in between)
13. incubate at room temperature for 30 minutes
14. remove detecting antibody solution by tapping it into the sink
15. wash plate 3x with plate-washing buffer (use a pasteur pipet to fill the wells)
16. tap liquid into sink; tap plate "dry" onto paper towel (this wash step is the most crucial)
17. add 100µl substrate solution to each of the 12 wells (you do not need to change pipet tips in between)
18. watch for a color change (yellow = HIV positive)

Results
Fill out the table:

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Diagram, using symbols, this ELISA. Make one diagram for a positive result, and one diagram for a negative result. Try to do this WITHOUT looking at a PICTURE (i.e. try to just reference the steps above)