5. Using a clean pipet, add 3 drops of Patient B sample to wells A10, A11, and A12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
6. Using a clean pipet, add 3 drops of Patient C sample to wells B1, B2, and B3 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
7. Using a clean pipet, add 3 drops of Patient D sample to wells B4, B5, and B6 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
8. Using a clean pipet, add 3 drops of Patient E sample to wells B7, B8, and B9 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
9. Using a clean pipet, add 3 drops of Patient F sample to wells B10, B11, and B12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA assay, at this point, the wells would be washed with a buffer to remove any molecules that have not bound to the adhered antigen. In this simulated lab activity, the washing step has been eliminated.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+C	+C	+C	–C	–C	–C	A	A	A	B	B	B
B	C	C	C	D	D	D	E	E	E	F	F	F

+C = positive control, –C = negative control, A–F = Patients A–F

Figure 3. Microtiter plate sample wells

10. Using a clean pipet, add 3 drops of simulated secondary antibody to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA, the wells would again be washed to remove unbound molecules. This step has been eliminated from this simulation.
11. Using the remaining clean pipet, add 3 drops of simulated chromogen to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
12. All the reaction wells will turn light green when the chromogen is added. A change from light green to purple indicates a positive result. Incubate the microtiter plate at room temperature for a minimum of 5 minutes and a maximum of 10 minutes to allow for color development.
13. After 5–10 minutes of incubation, record your results in the data table on page S-5. Compare the color of each patient sample to that of the positive and negative control. Colored results that are in between the positive and negative control should be scored as weak positives.

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