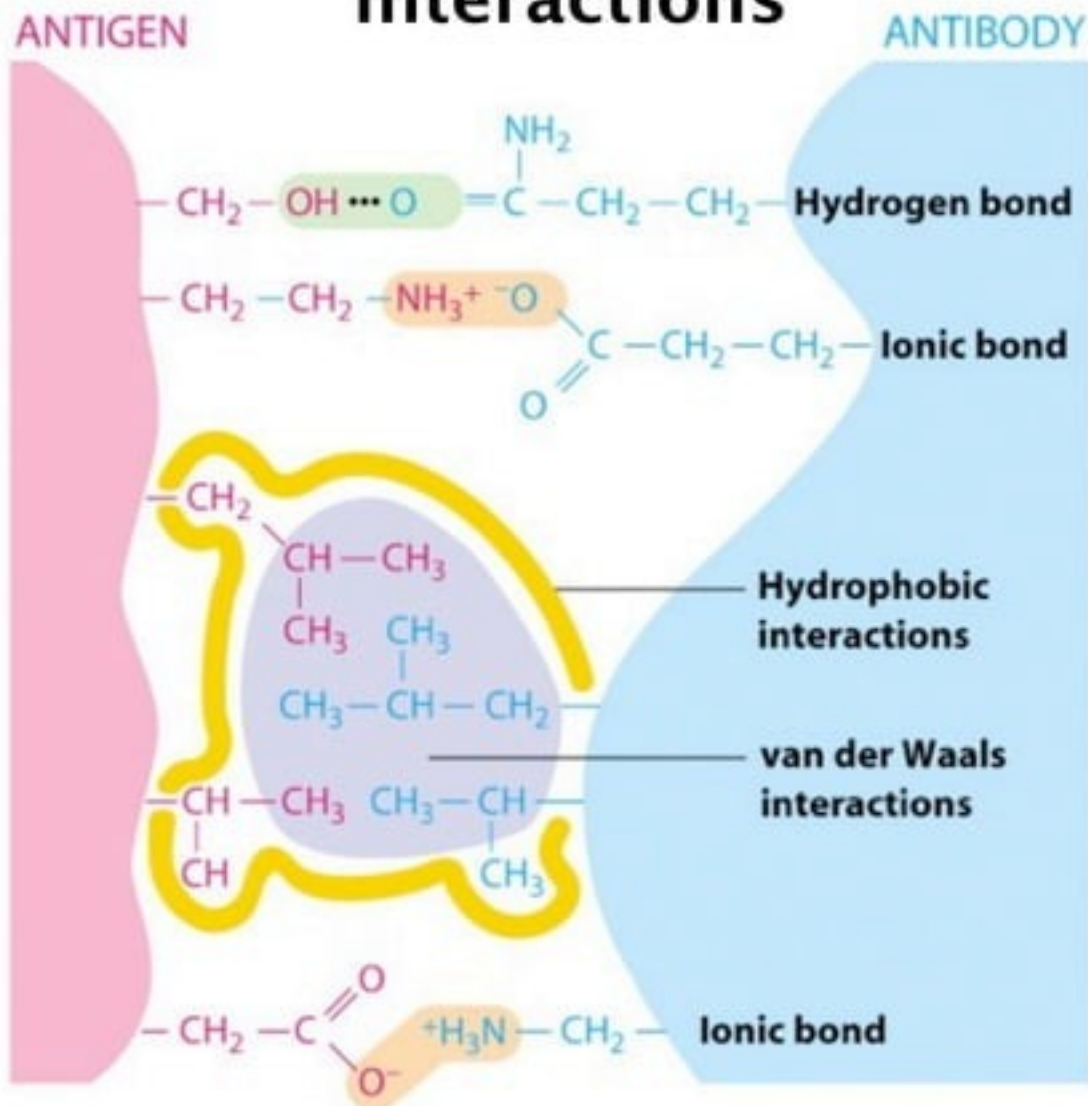


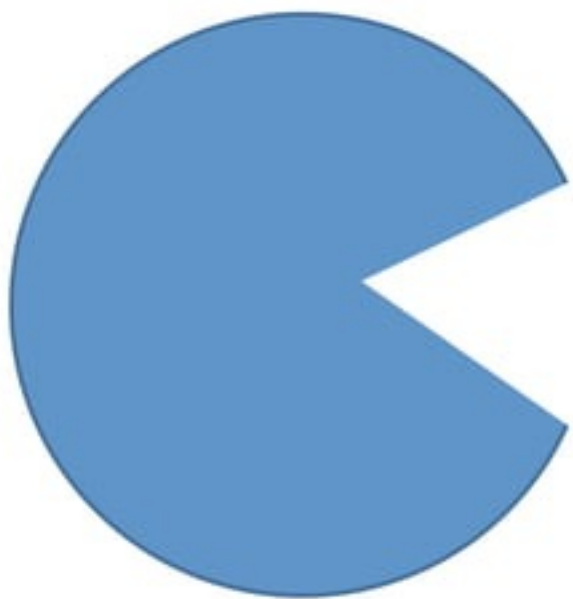
Precipitin Reaction

Purpose: To understand the biological basis of the precipitin reaction, and how to perform, and analyze the reaction.

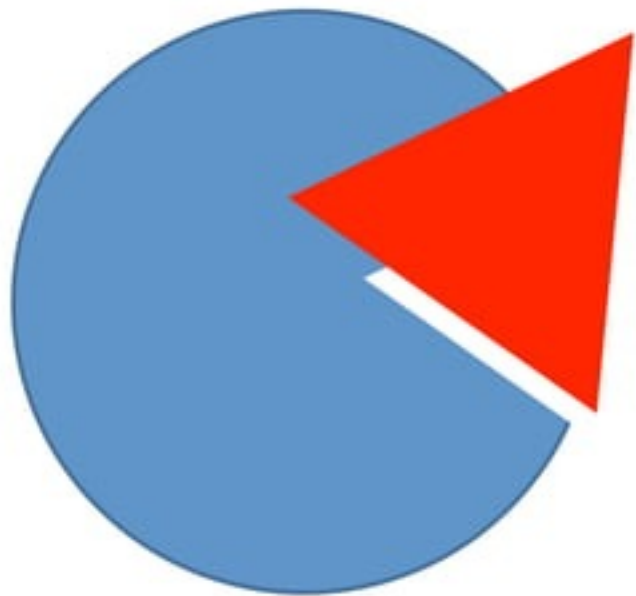
Forces that stabilize Ab-Ag interactions



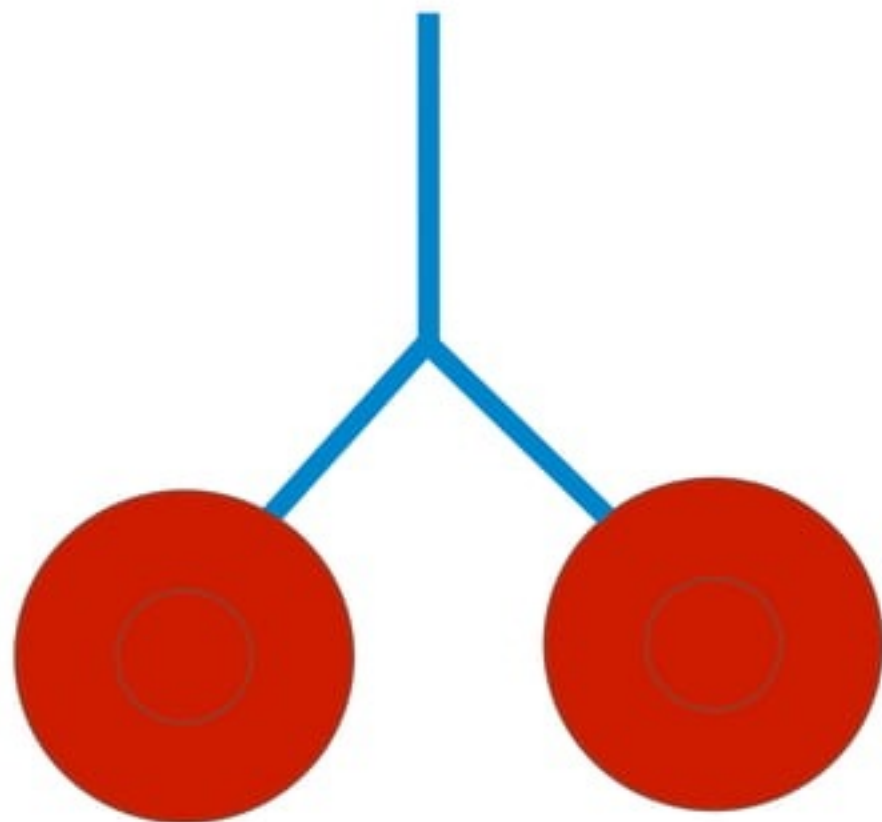
**Complementary shape: maximizes
affinity and specificity**

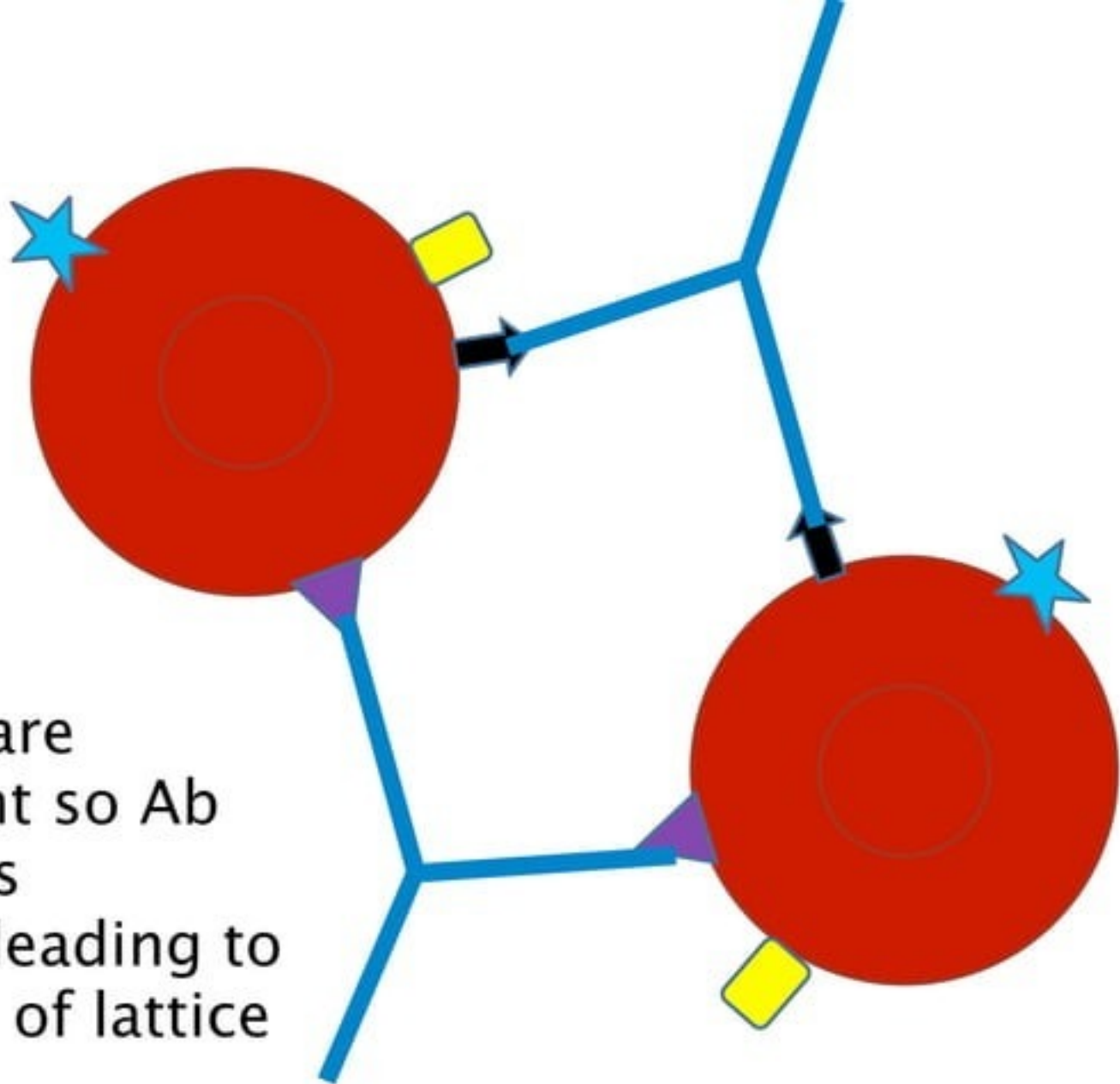


**Complementary shape: maximizes
affinity and specificity**



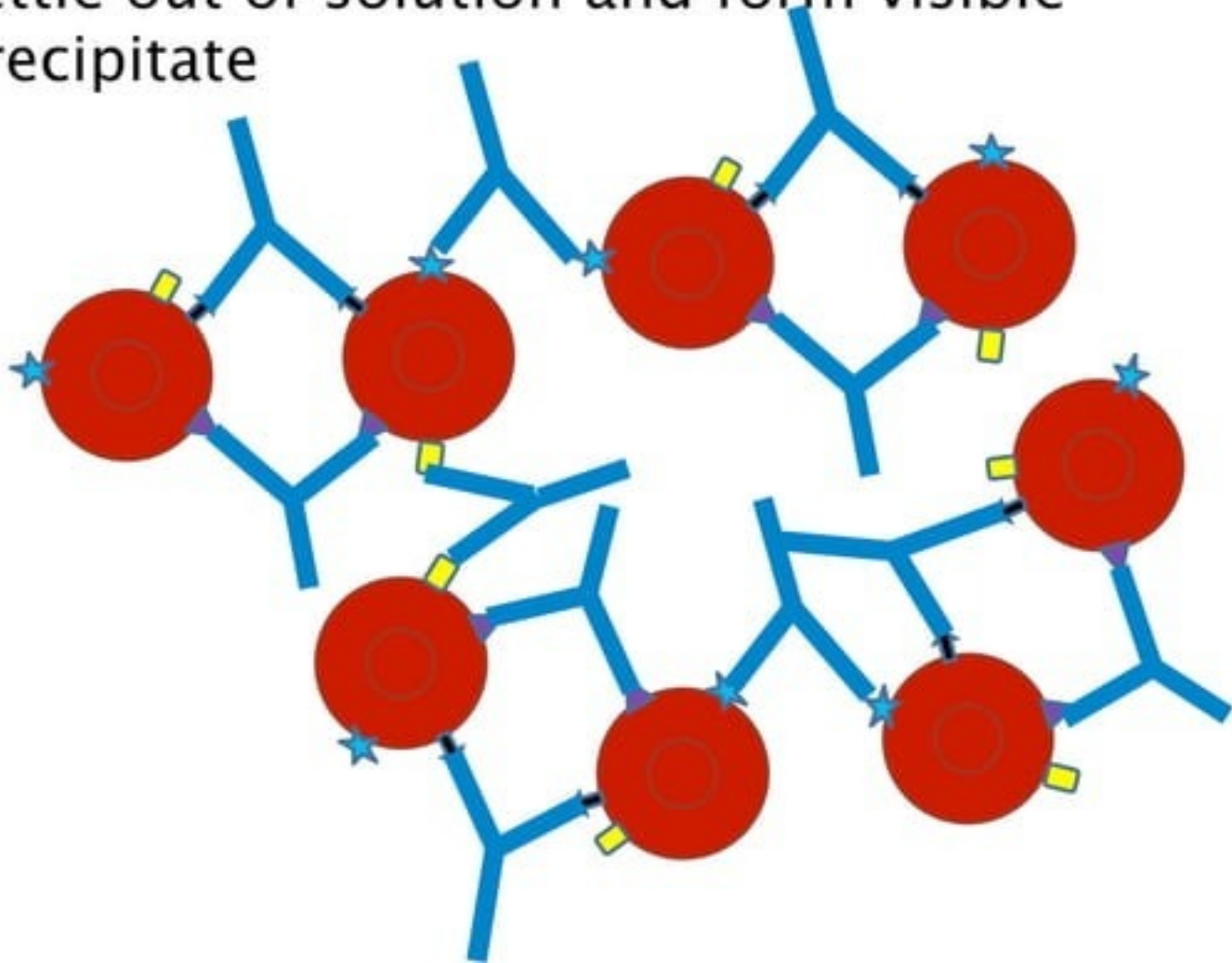
Antibodies are divalent so they can link Ag together



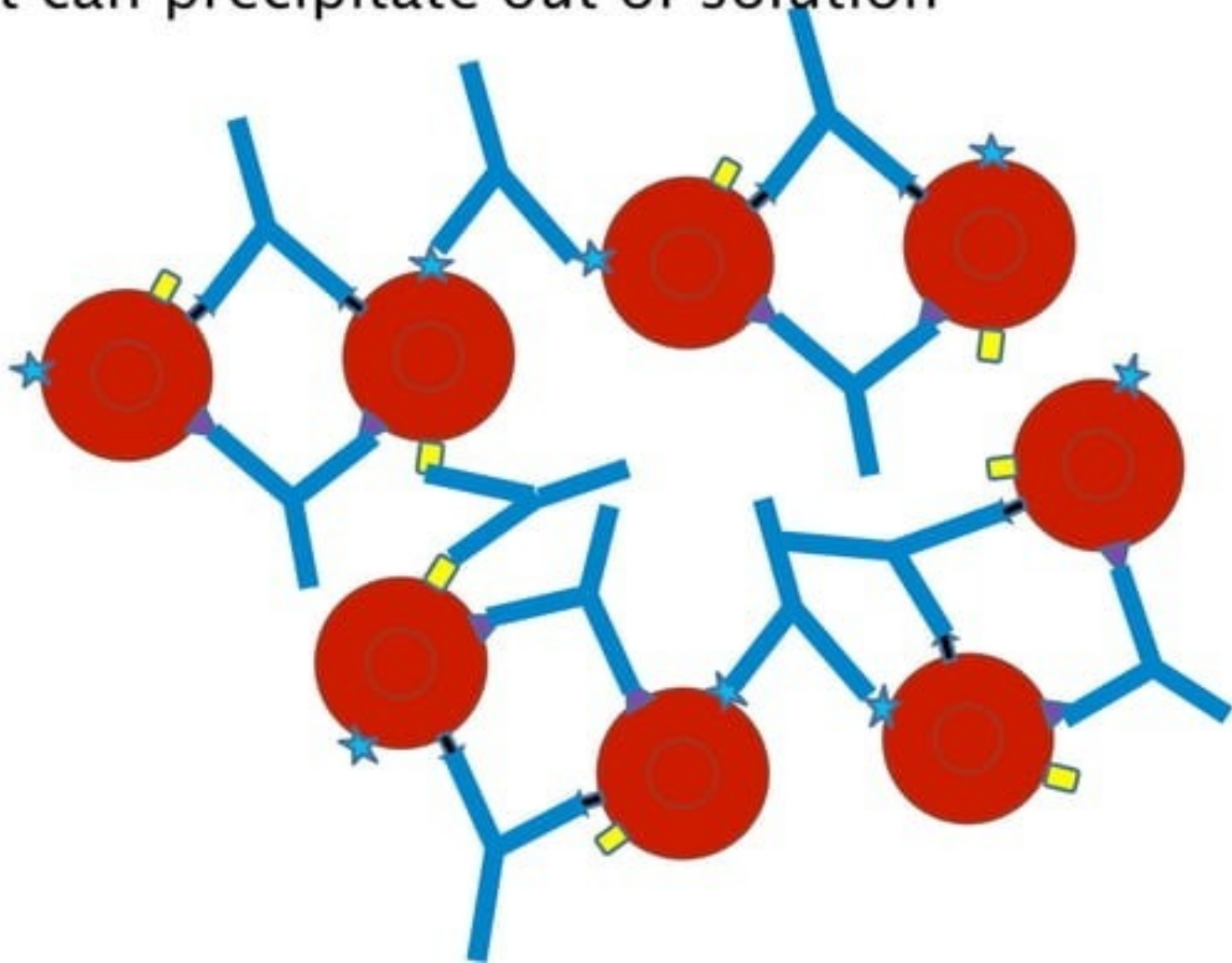


Antigens are multivalent so Ab bridging is possible, leading to formation of lattice

Precipitin reaction: continuation of lattice formation results in large aggregates which settle out of solution and form visible precipitate

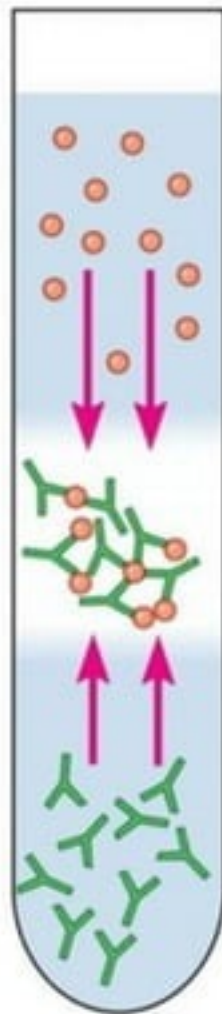


Precipitin reaction: Ab binds soluble Ag to form aggregation of Ab-Ag complex (immune complex) that can precipitate out of solution



qualitative
test (ring
test)

tests for
presence
of Ab
specific
for the Ag



Antigens
(soluble)

Zone of
equivalence:
visible precipitate

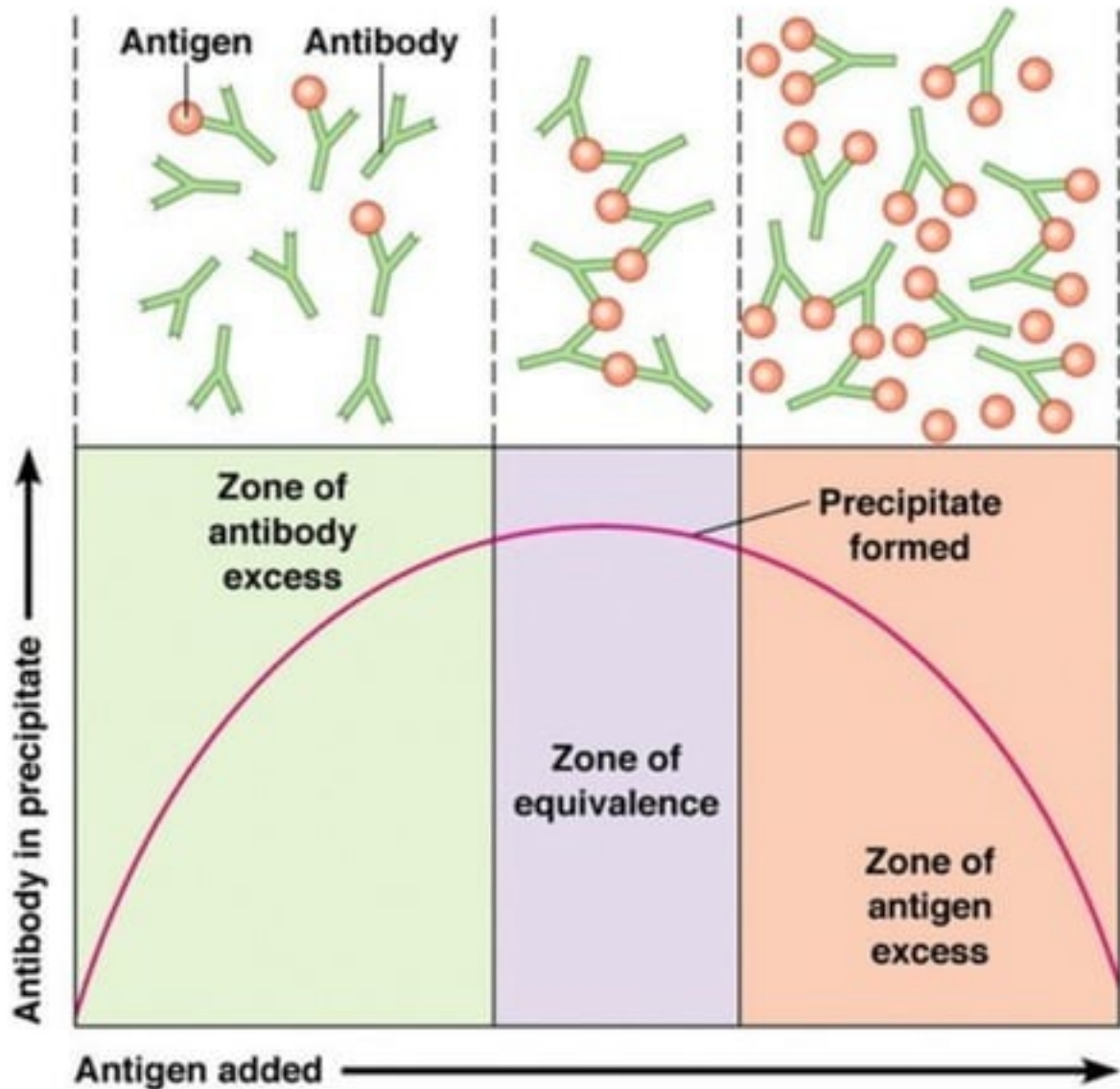
Antibodies

(a)

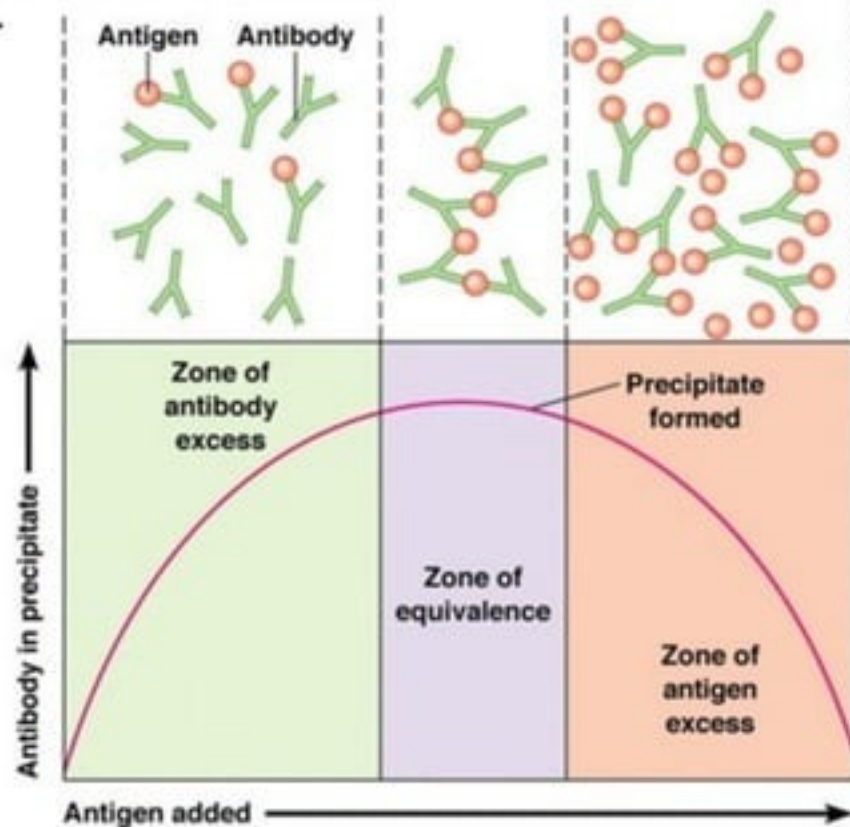


(b)

Consideration of concentration of both Ag and Ab is necessary to produce insoluble aggregates



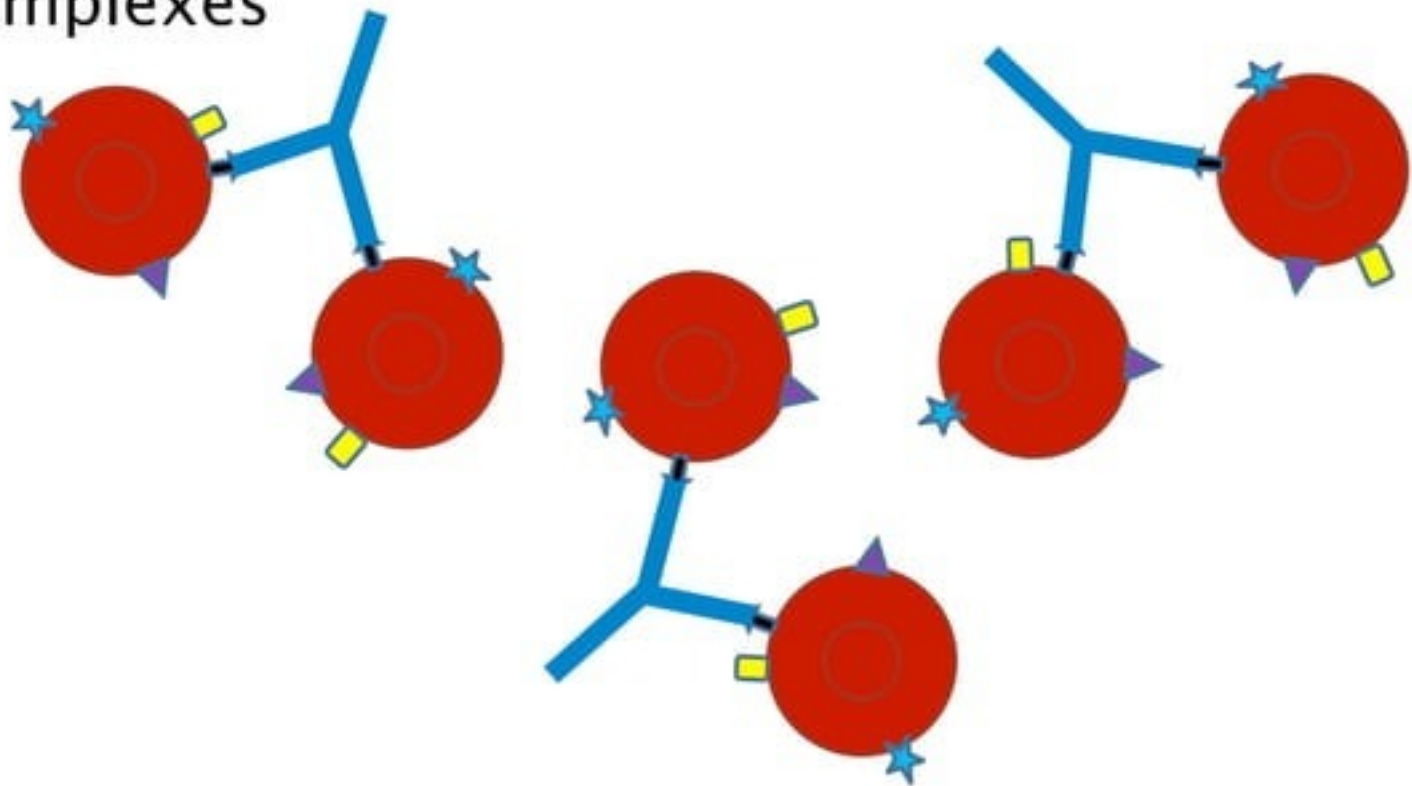
Optimal mutual proportions (OMP) aka equivalent proportions: usage of all available Ag and Ab in formation of precipitate



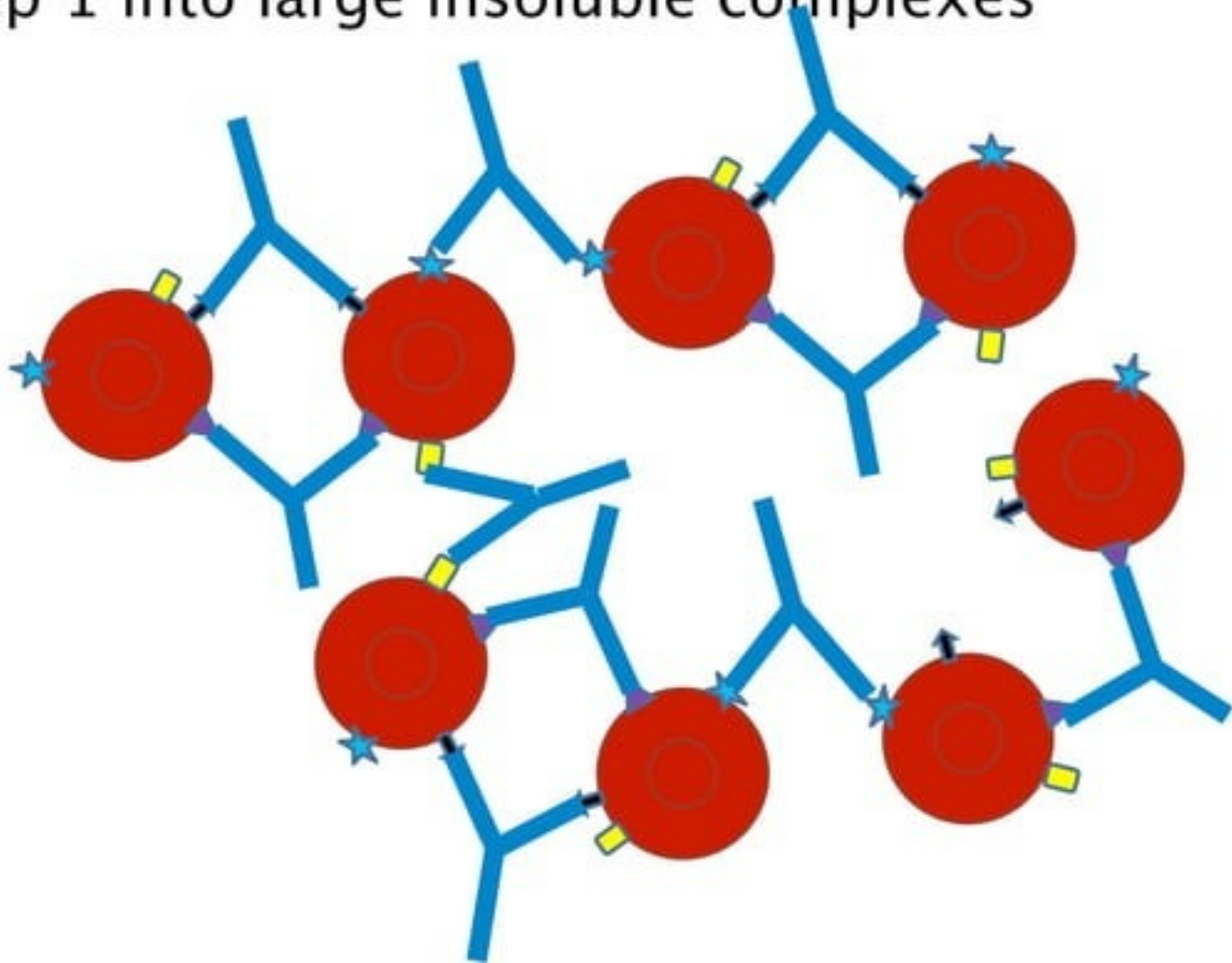
At OMP, addition of more Ag or Ab shows no additional precipitation. All reactants participated in initial reaction

Methodology:

Ab reacts with Ag for 1–2 h at 37°C to allow rapid binding of Ab–Ag; forms small, soluble complexes



Incubate for 24–28 h at 4°C to allow slow development of small, soluble complexes from step 1 into large insoluble complexes



Hence want to determine OMP= optimal concentration of Ab and Ag to allow precipitation reaction to occur

SO dilute Ag, keep Ab constant, measure amount of precipitation, and determine dilution of Ag that gives greatest amount of precipitation

Dilution of Ag that gives greatest precipitation = OMP aka equivalence

- Change Ag amounts (mg), add to constant amount of Ab, measure amount of precipitation
- Plot amounts of precipitate VS amount of Ag added
- To determine if a particular SN has excess Ag or Ab, Add more Ag or Ab and see if additional precipitation occurs

Our Antigen

Ovalbumin:

- Most prevalent protein in chicken eggs
- Used as an allergen in test patients
- Used as molecular weight marker for calibrating

Agglutination	Precipitin Reaction
Qualitative (slide test)	Qualitative (ring test)
Ag - SRBC/Ab - α -SRBC	Ag - OA /Ab - α -OA
Quantitative (microtiter plate)	Quantitative (microtiter assay)
Result: Titer	Results: OMP
Serially diluted Ab	Serially diluted Ag
More sensitive (need . 8-16mg)	Less sensitive (need 80-320mg)
Ag is not soluble	Ag is soluble

Serum: The clear liquid that can be separated from clotted blood. Serum differs from plasma, the liquid portion of normal unclotted blood containing the red and white cells and platelets. It is the clot that makes the difference between serum and plasma.

Antiserum: antibody containing serum

Protocol

1) Aliquot 120 μ l of OA into a clean, dry eppendorf tube and label accordingly. Set aside for now.

120 μ l
OA



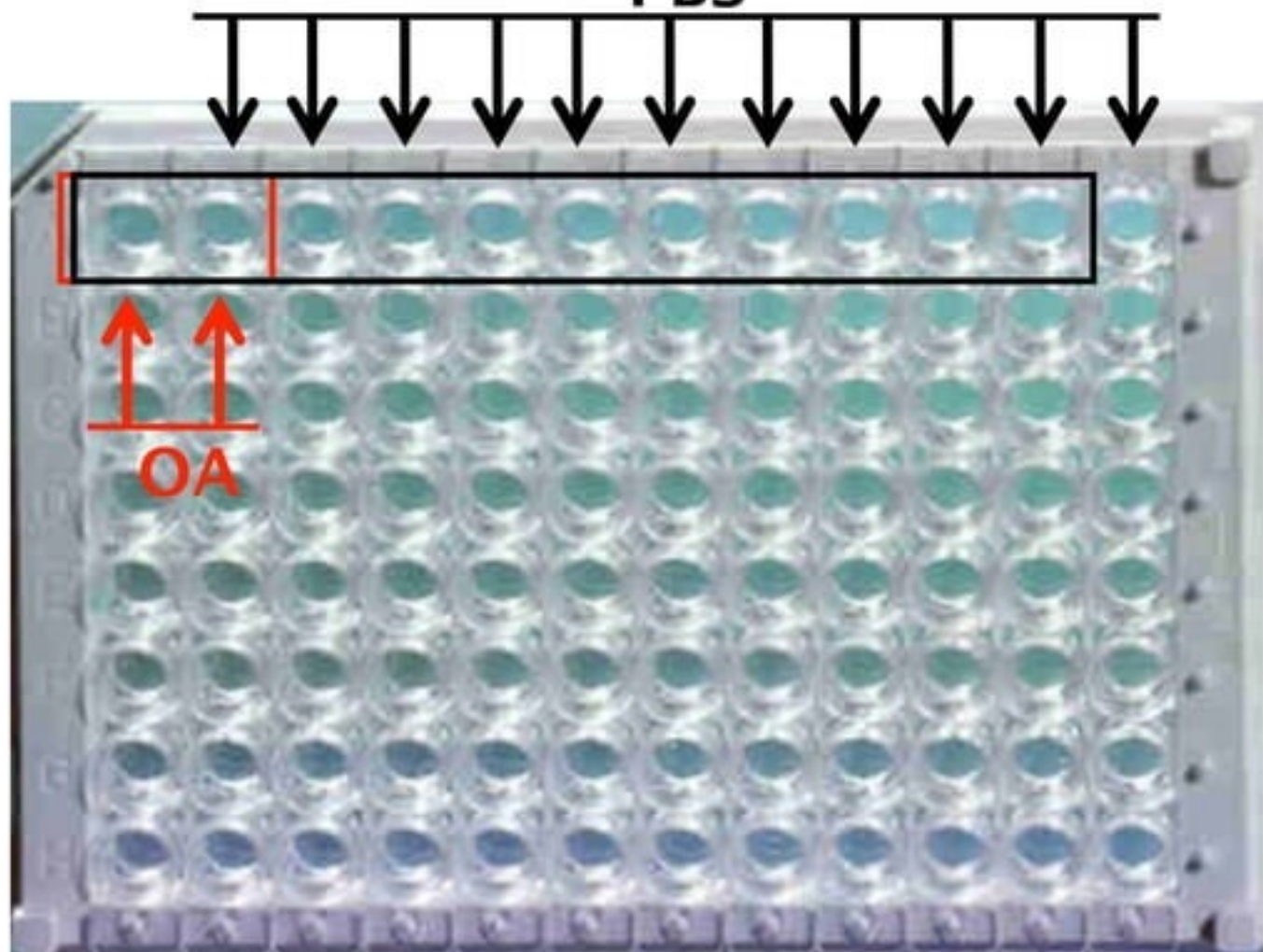
2) Set up a 1:4 dilution (180 μ l of anti-OA serum + 540 μ l of PBS) in a clean, dry eppendorf tube and label accordingly. Set aside for now.



50 μ l PBS

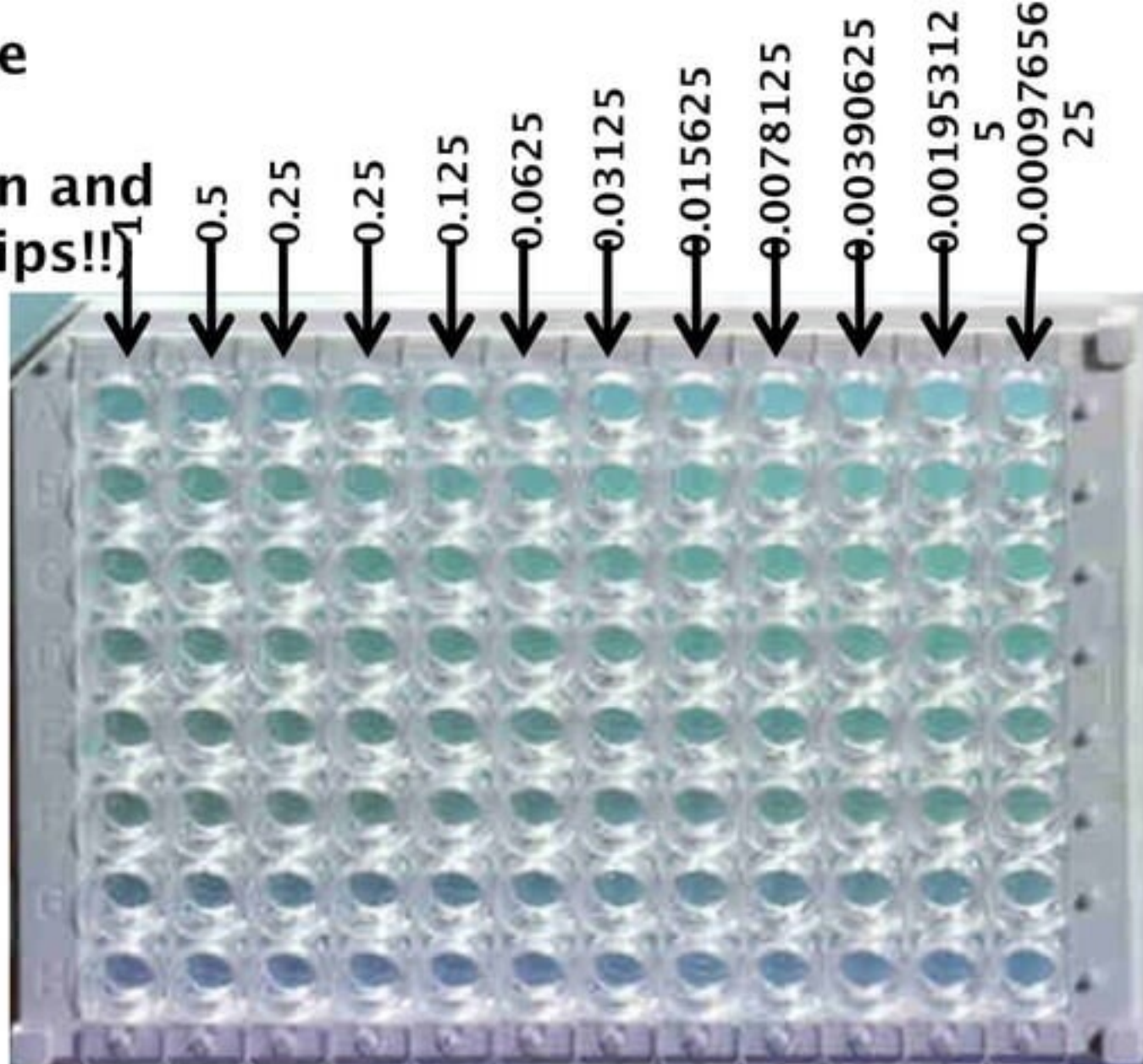
50 μ l OA

PBS

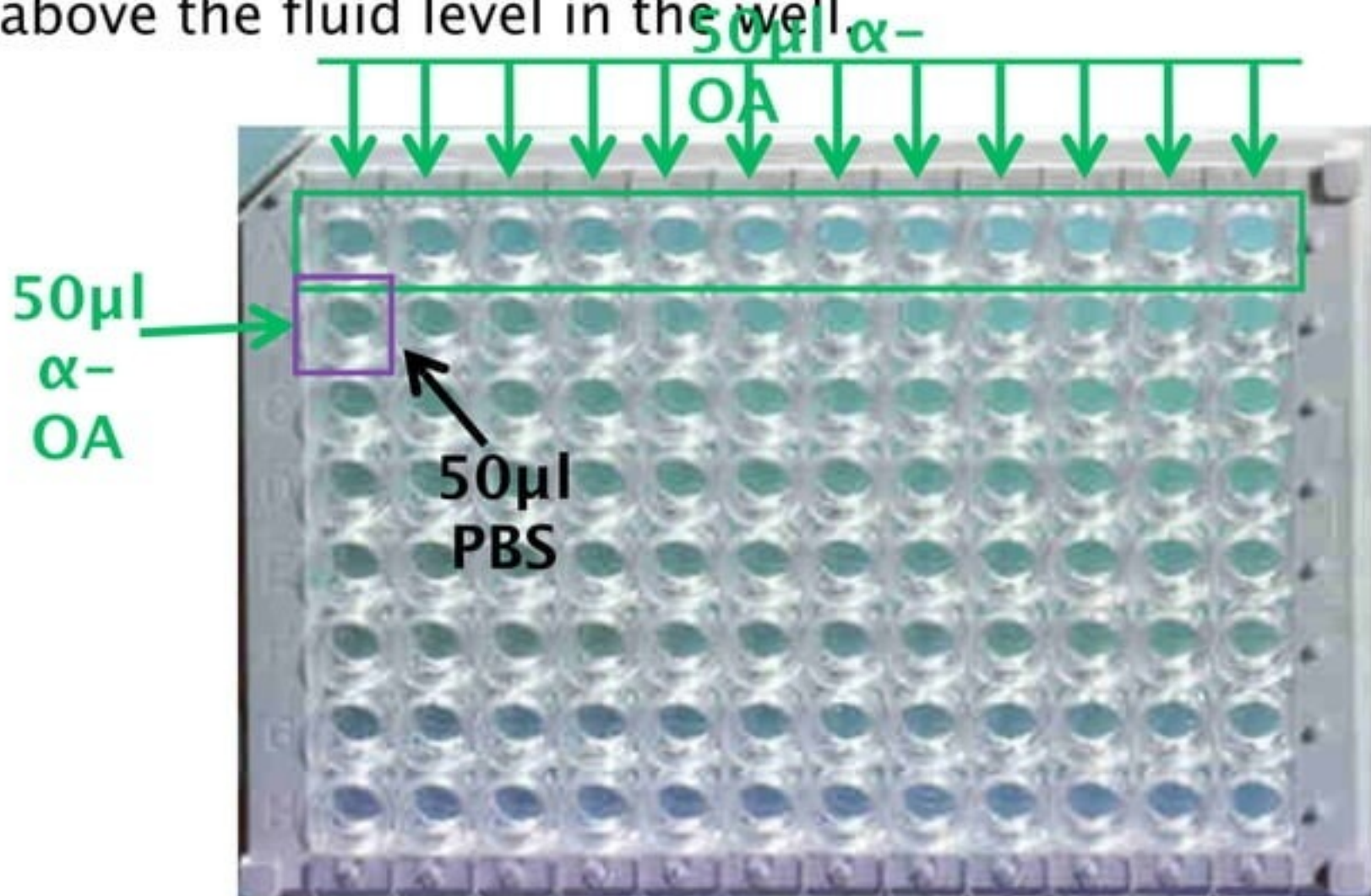


(Minimize
bubble
formation and
change tips!!!)

1:2
serial
dilution
of OA



When adding the serum solution, touch the dispensing tip to the upper surface of the well above the fluid level in the well.



Immunodiffusion plate =
Immunodiffusion gel

Purpose: To test if the well showing
the largest amount of precipitate is
your OMP

Excess Ab

Your sample (centered on predicted
OMP)

Day 2

- Centrifuge plate (microtiter) 10 mins at 1200 rpm
- Prepare 2 plates (petri dishes): 10mL of agar to each dish
- Label plates
- Incubate plates in fridge for 45–60 mins.
- Remove plates

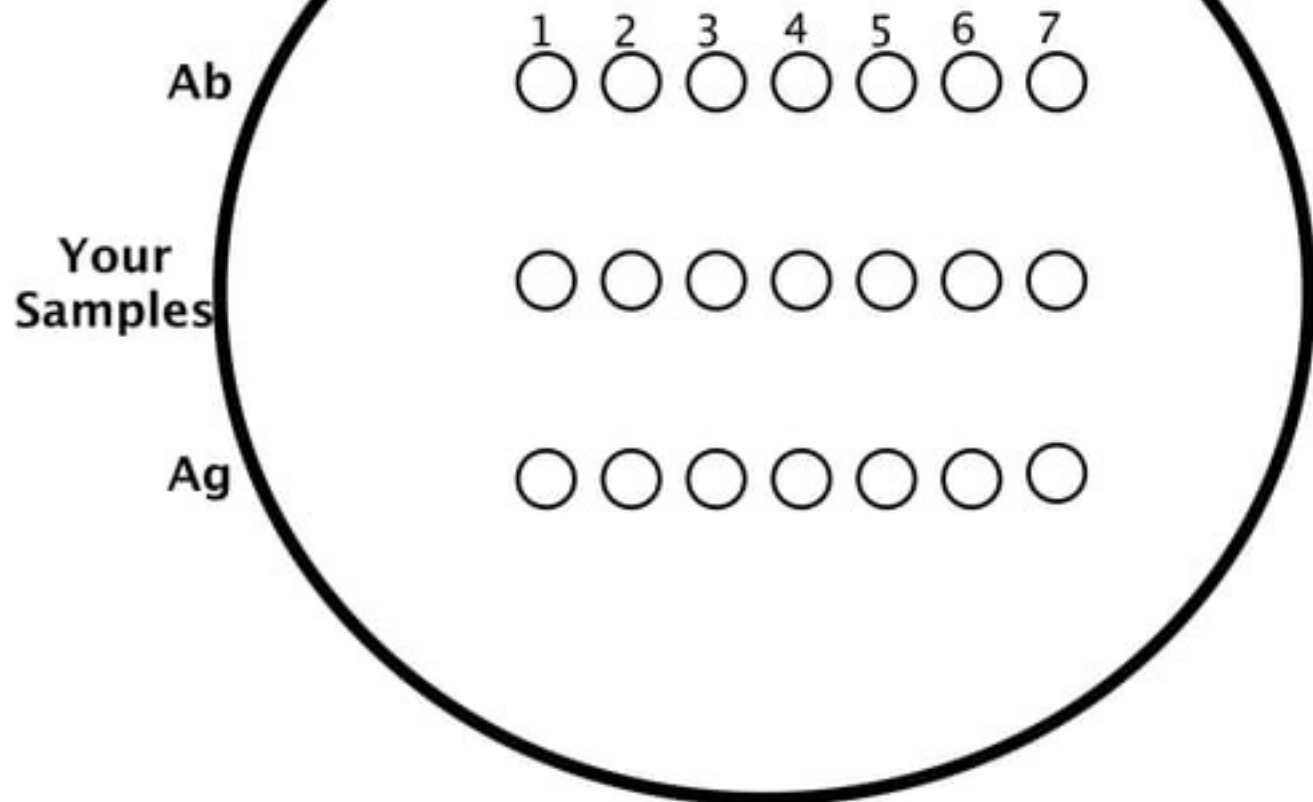
Day 2

- Examine Day 1 plates
- Determine well(s) that show largest amount of precipitate...OMP?

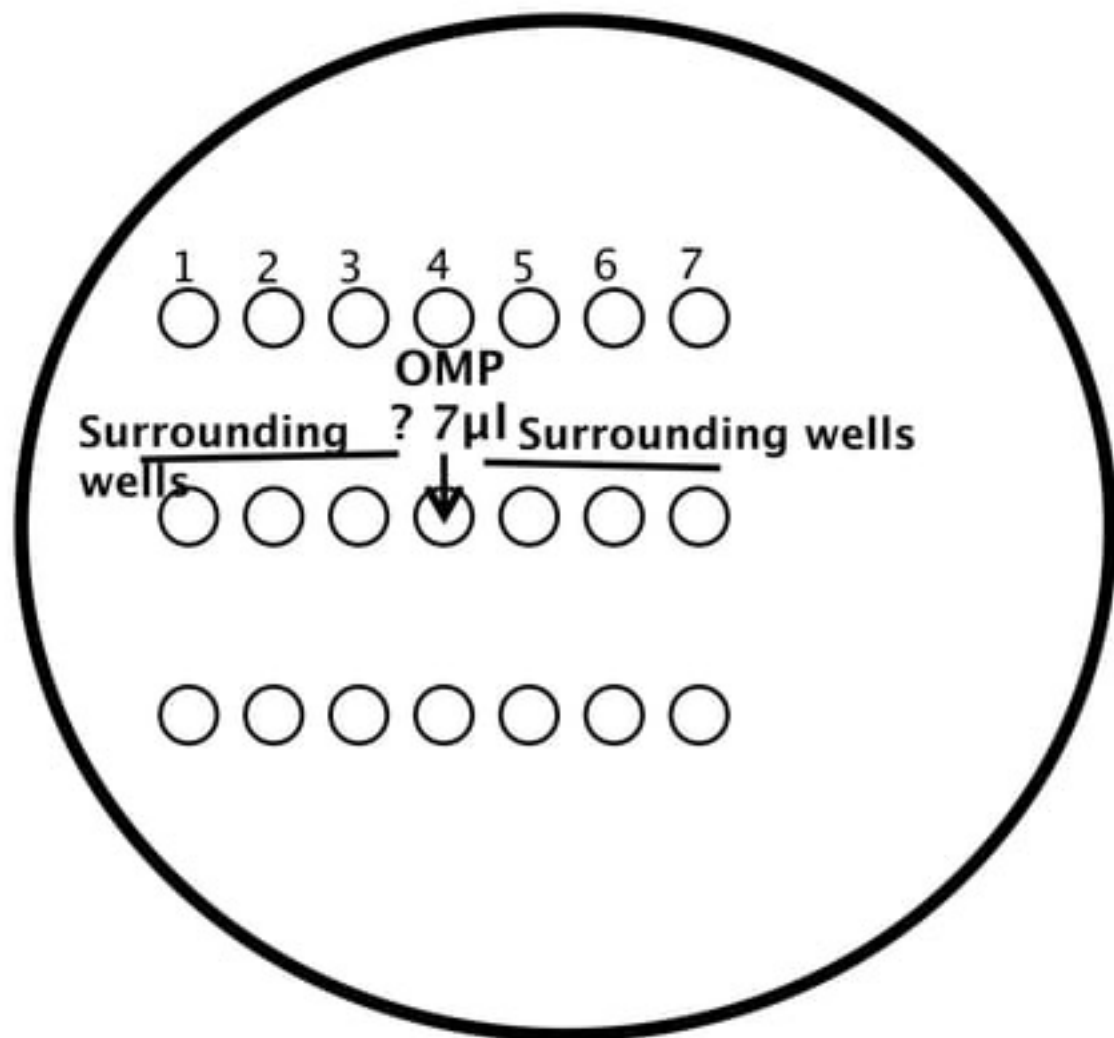
*Be very careful to not to disrupt the precipitate
(do not shake)!

Immunodiffusion plate = Immunodiffusion gel

7 μ
I



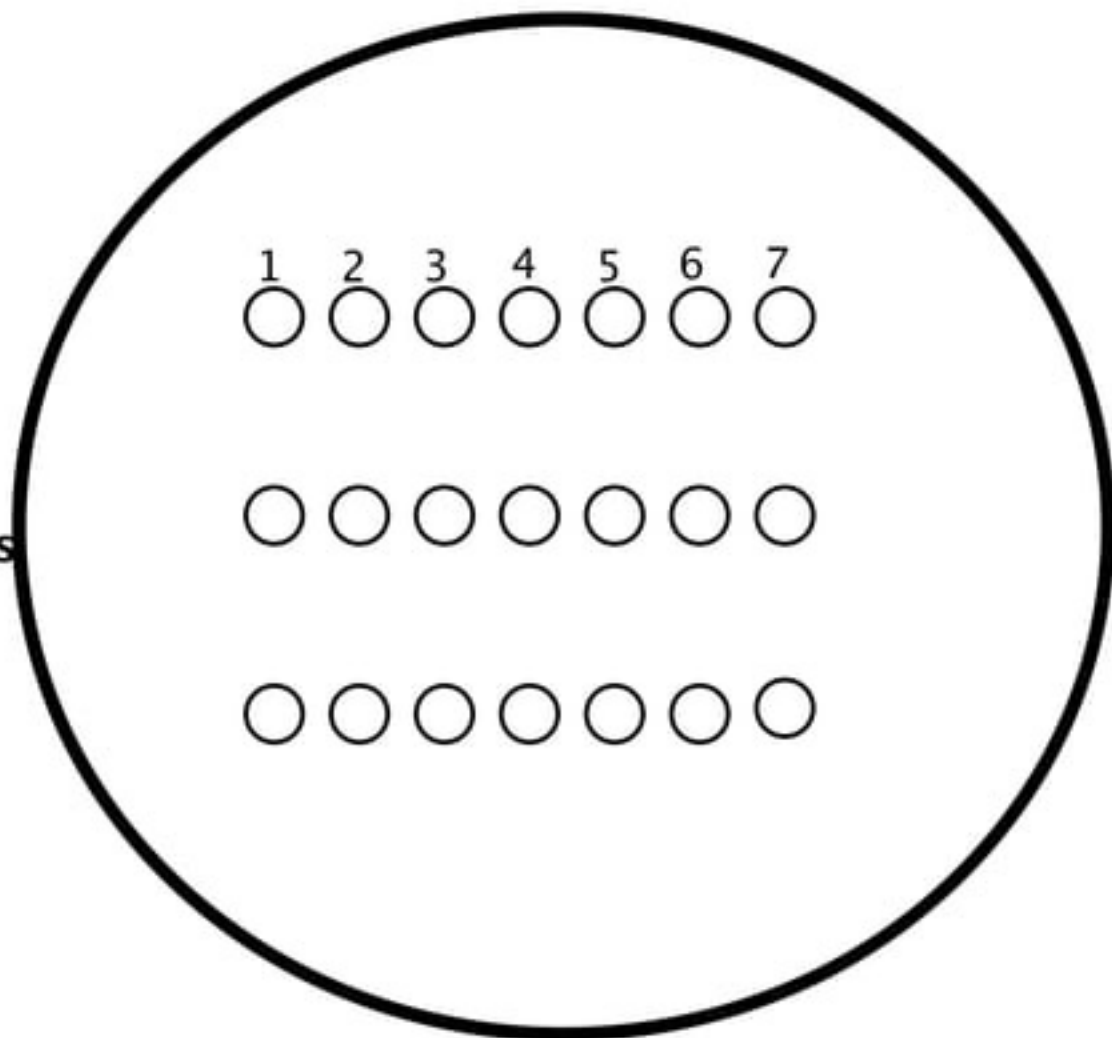
To plate your samples:



To plate your samples:

7 μ
l

Your
Samples



Example

Most precipitate OMP?

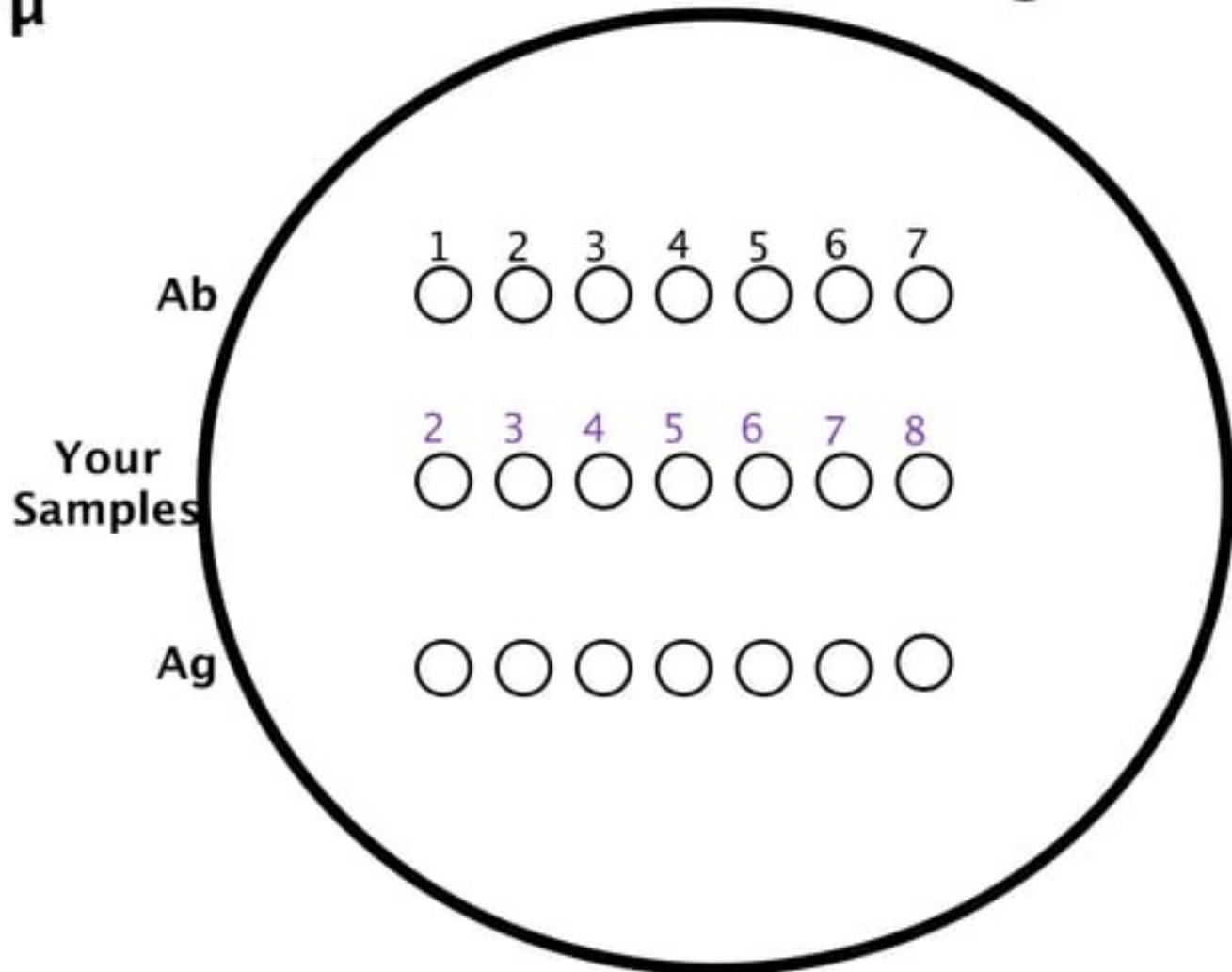


1 2 3 4 5 6 7 8 9 10 11 12



Immunodiffusion plate = Immunodiffusion gel

7 μ
I



Schedule a time to view your plate tomorrow

If you know when you can make it, let me know

If not, find time tomorrow to come find me in
MH-317

I will go with you to view your
immunodiffusion
plate

Dilutions of Ag

Final Volume = 750 μ l

[OMP]

1



2



3



4



5



Add 750 μ l of antiserum

1



2



3



4



5



Week 2 Day
1



1



2



3



4



5



Excess

Ag

1

2



OMP

3



Excess

Ab

4

5



Week 2 Day 2

Centrifuge at 1500 rpm, 4°C for 10 min.
Aspirate the SN_Resuspend pellet_Biorad

1



2



3



4



5



Calculations

- Construct a standard curve (total protein concentration vs antigen concentration)
- Calculate the amount of antibody protein at equivalence
 - $\text{OMP, [ppt]} = [\text{antigen}] + [\text{antibody}]$
- Using the dilution of the original antiserum at equivalence, calculate the amount of antibody protein per ml of original undiluted antiserum