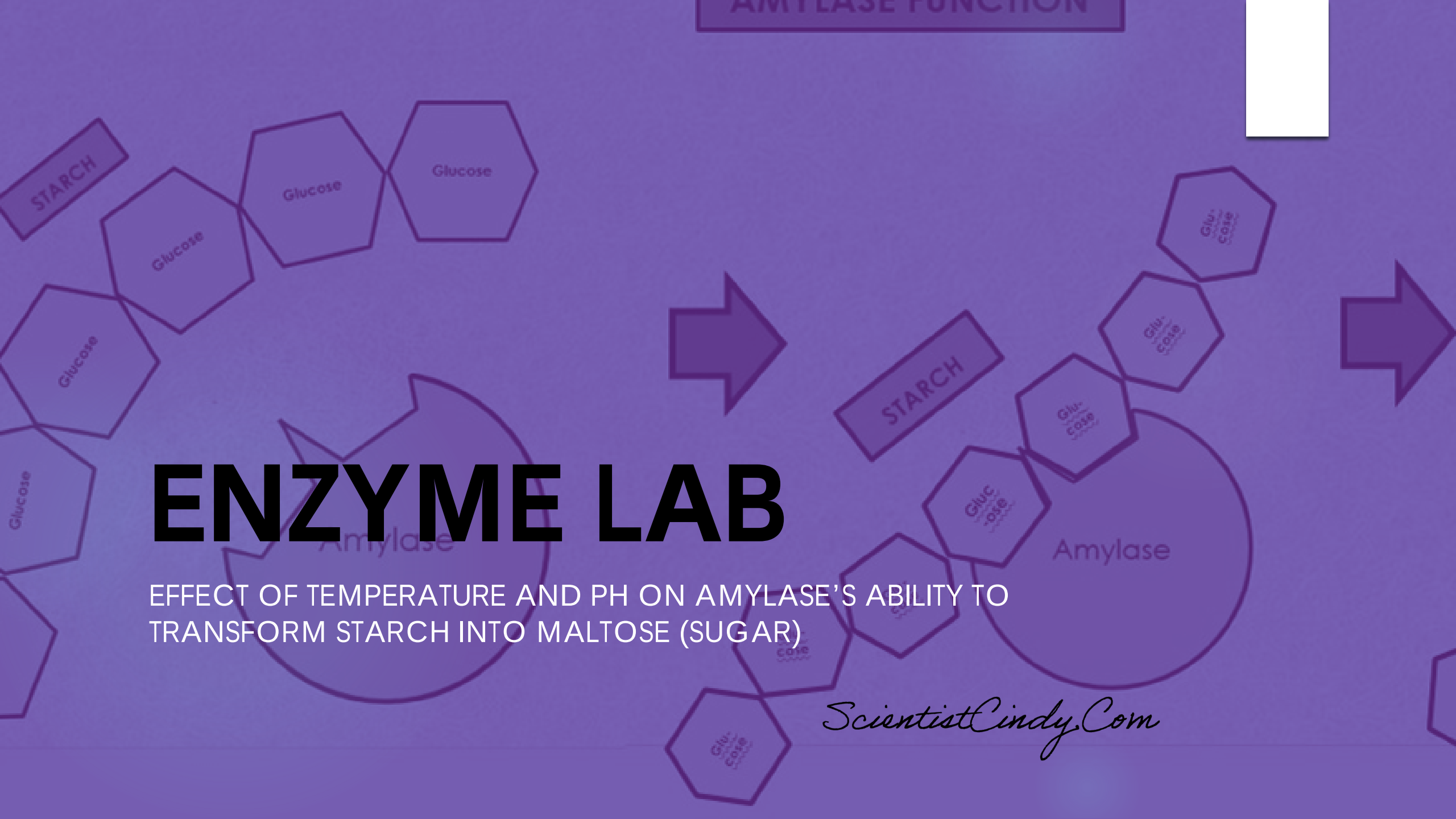


ENZYME LAB

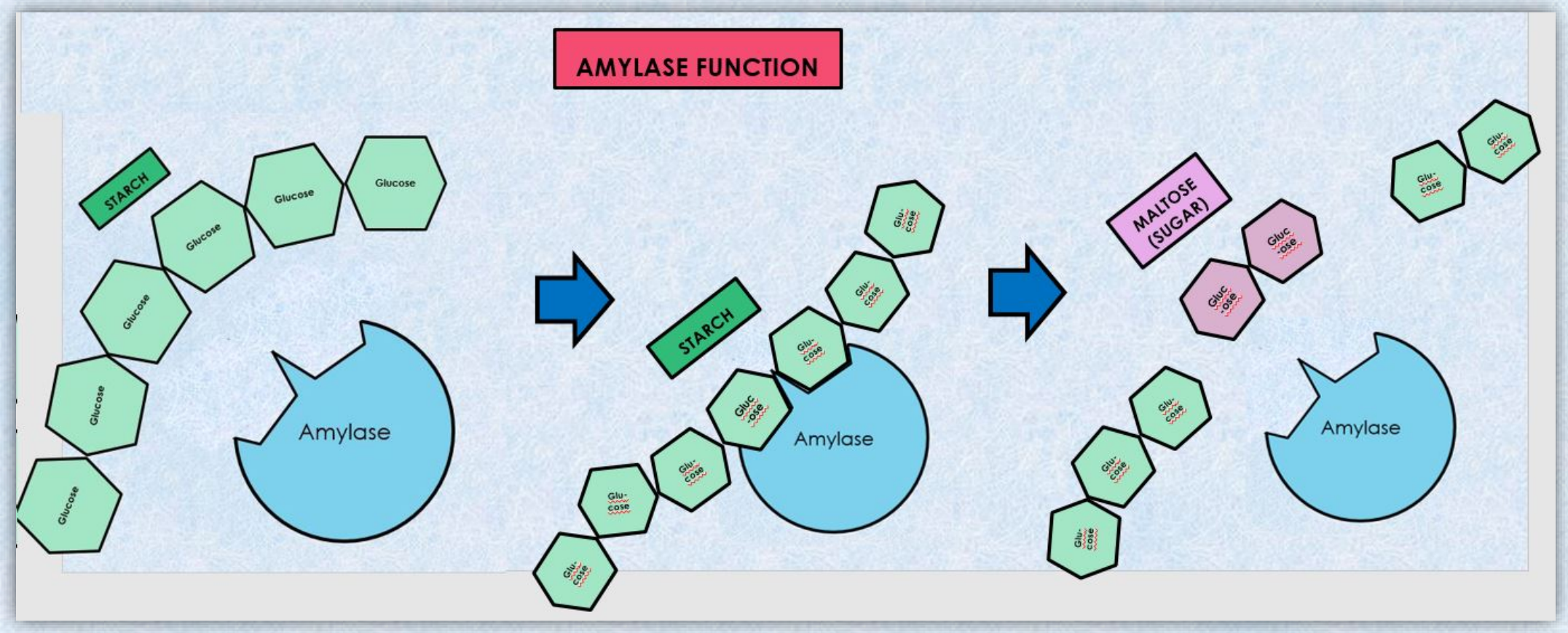
EFFECT OF TEMPERATURE AND PH ON AMYLASE'S ABILITY TO TRANSFORM STARCH INTO MALTOSE (SUGAR)

ScientistCindy.Com

Amylase

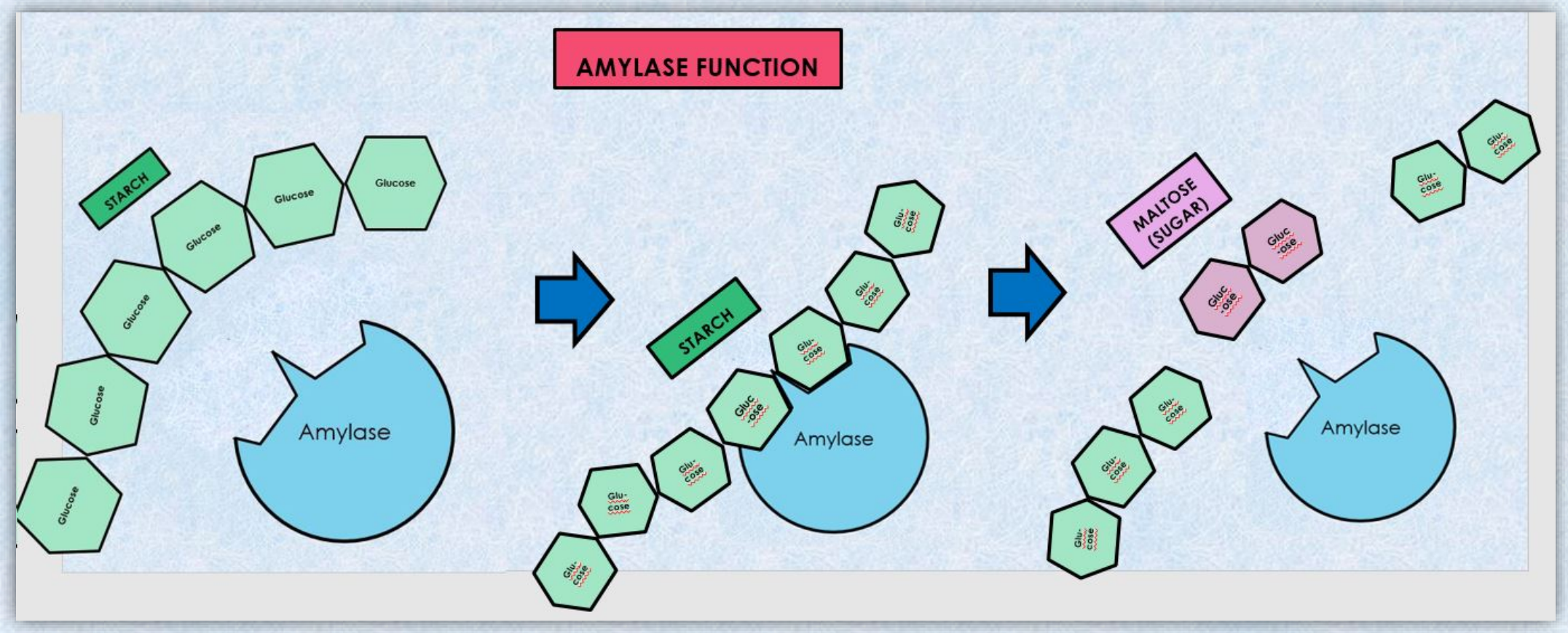
AMYLASE is an enzyme that is found in our bodies that functions to help the body in the digestion food. Amylase is found in saliva and in the pancreas.

Amylase catalyzes the hydrolysis (breaking down) of starch, glycogen and related polysaccharides into more simple and readily usable forms of sugar.



AMYLASE has an OPTIMAL RANGE of pH and Temperature which is pH = 7 (neutral) and 37 degrees C. These are the same conditions that exist in our bodies. When an enzyme is within its Optimal Range or conditions, it will be able to catalyze reactions at its fastest rate.

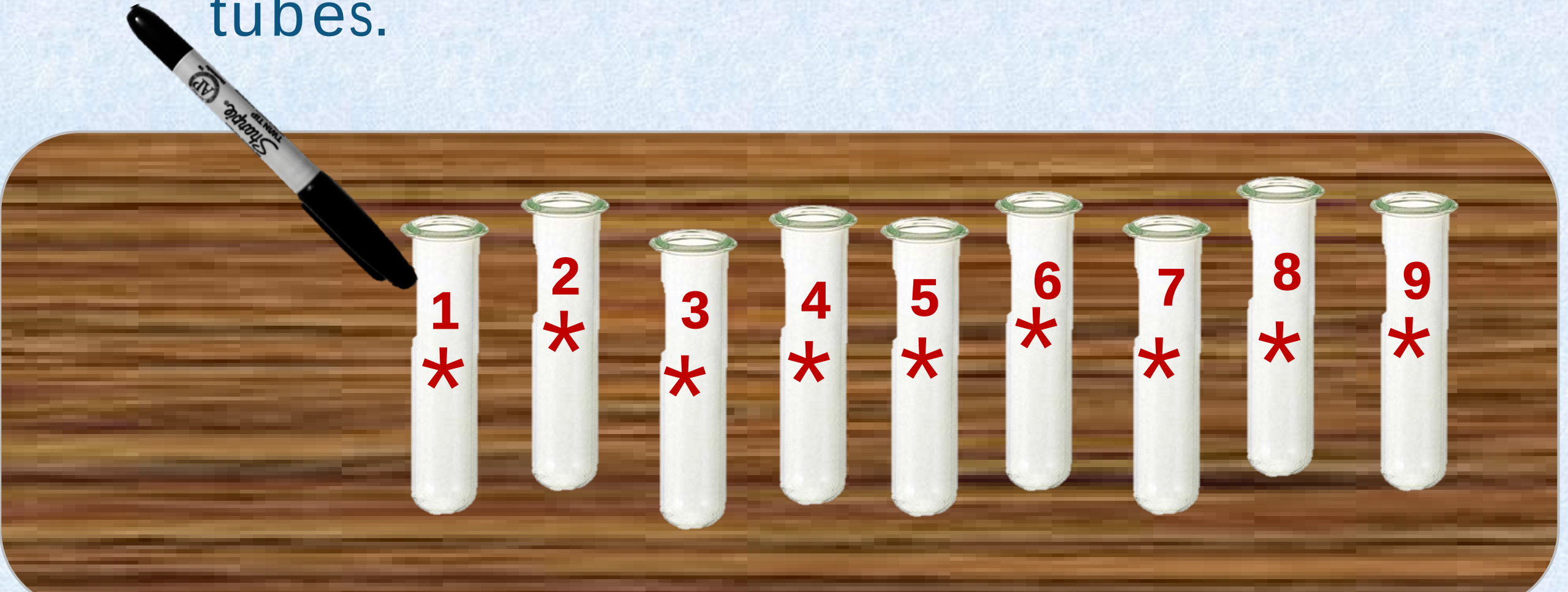
Enzymes are not products or reactants in the chemical reaction, they just assist (catalyze) the reaction by making it proceed much more quickly than it would in the absence of the enzyme. For this reason, amylase can be used again and again!



Gather your materials:

1 TEST TUBE RACK AND 15 TEST TUBES

Label 9 of the tubes 1 through 9 then add an identifying mark so you will be able to identify your team's tubes.

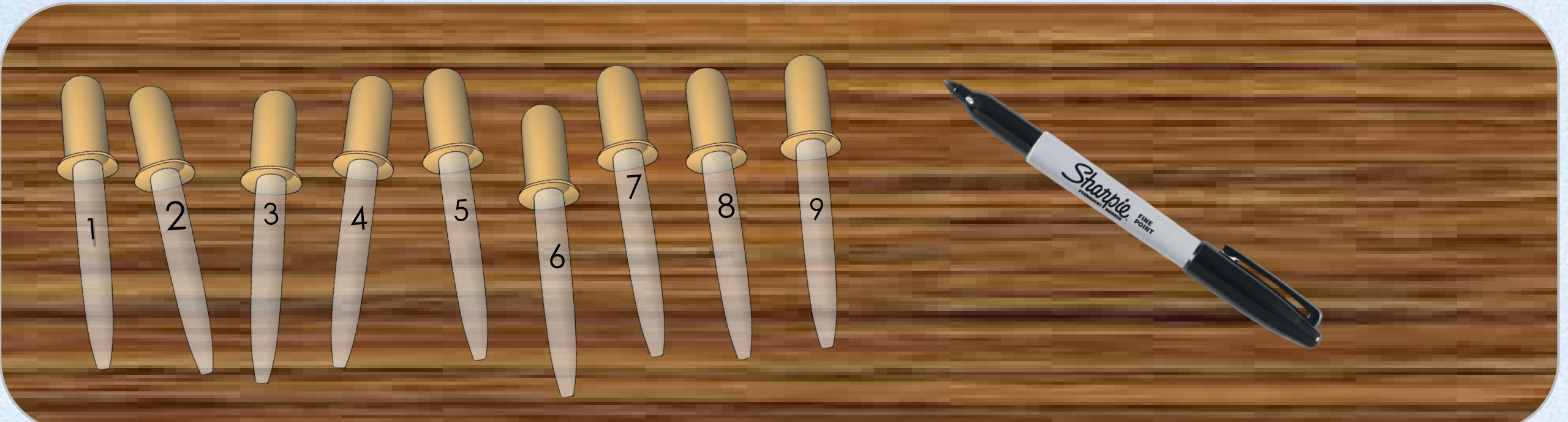


Gather the following

1. A bottle of Distilled Water (DI Water)
2. 1 ice bucket
3. AMYLOSE (STARCH)
4. AMYLASE (ENZYME)
5. NaOH (Sodium Hydroxide)
6. HCl (Hydrochloric Acid)

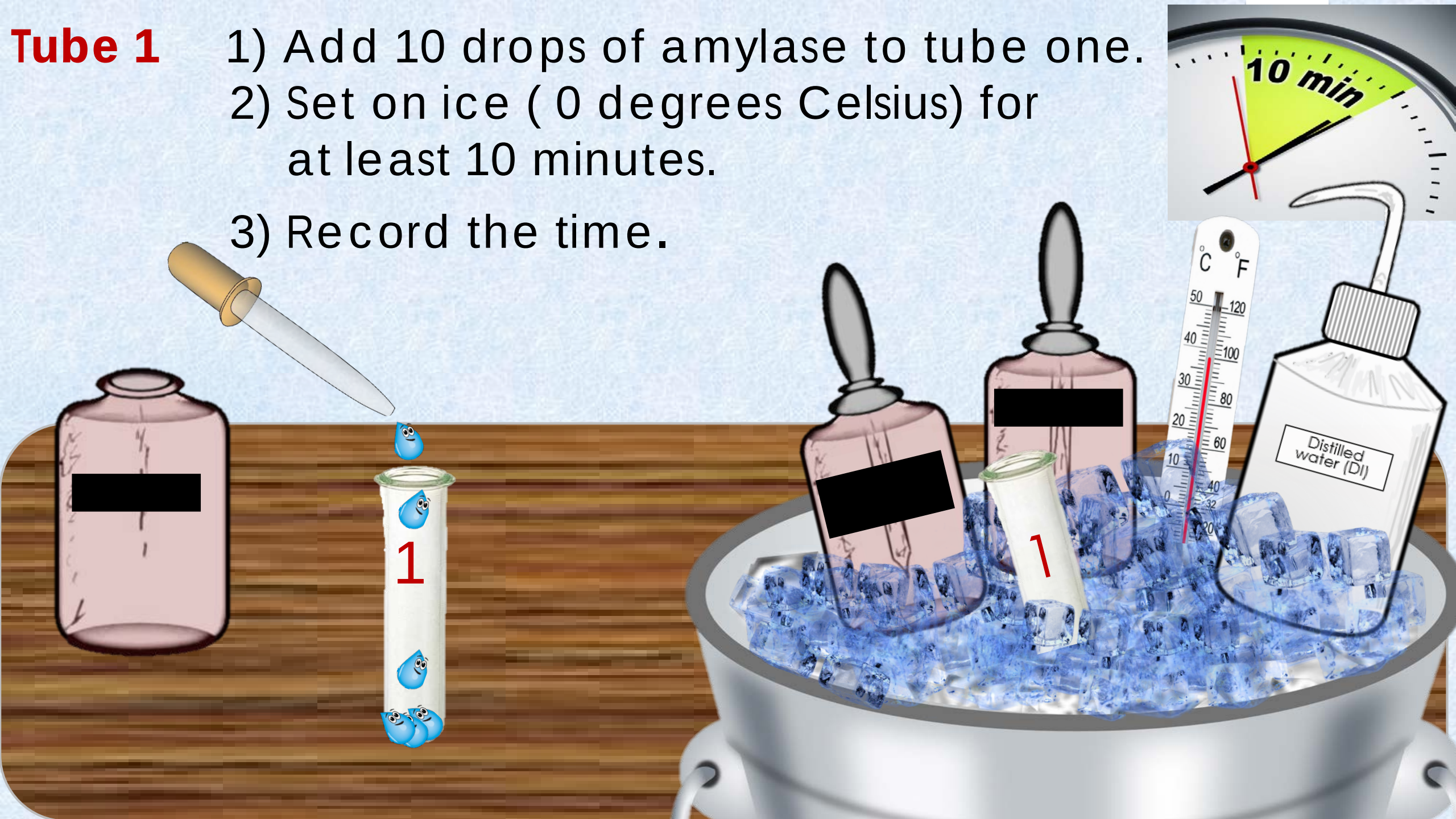
Place the starch, amylase and water in ice. Keep these chilled throughout the experiment.

Get 9 eye droppers or pipettes and label them 1 though 9



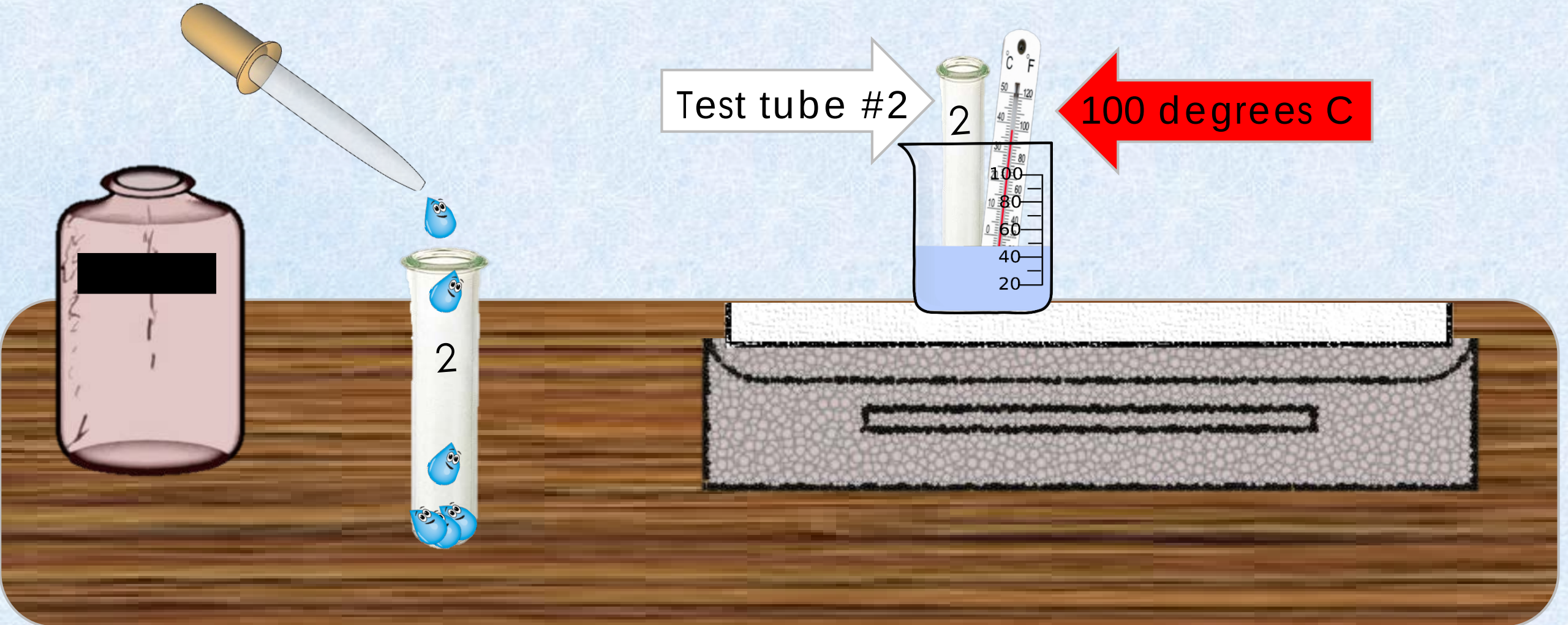
Tube 1

1) Add 10 drops of amylase to tube one.
2) Set on ice (0 degrees Celsius) for at least 10 minutes.
3) Record the time.



TUBE 2

1) Add 10 drops of amylase to tube 2.
2) Set in beaker of hot water on the hot plate (100 degrees Celsius) for at least10 minutes.
3) Record the time.



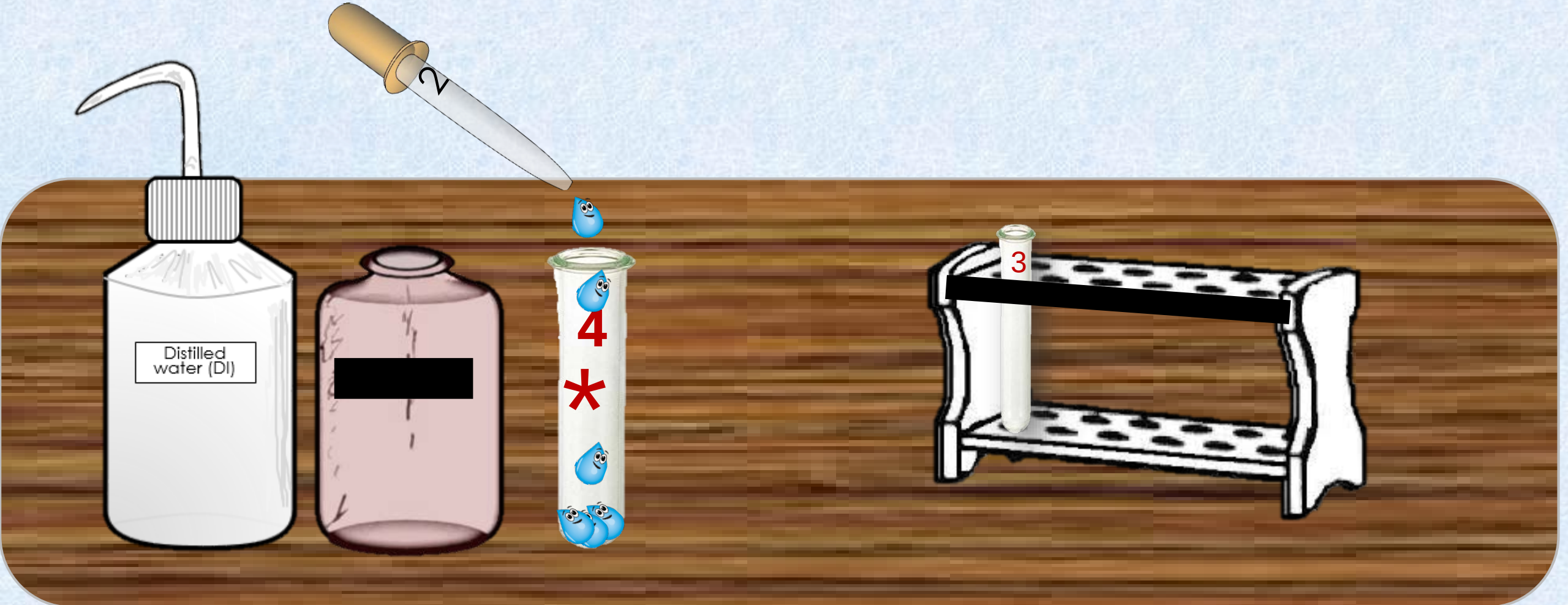
Tube 3

1) Add 20 drops of distilled water to tube 3.

2) Set this aside in rack.

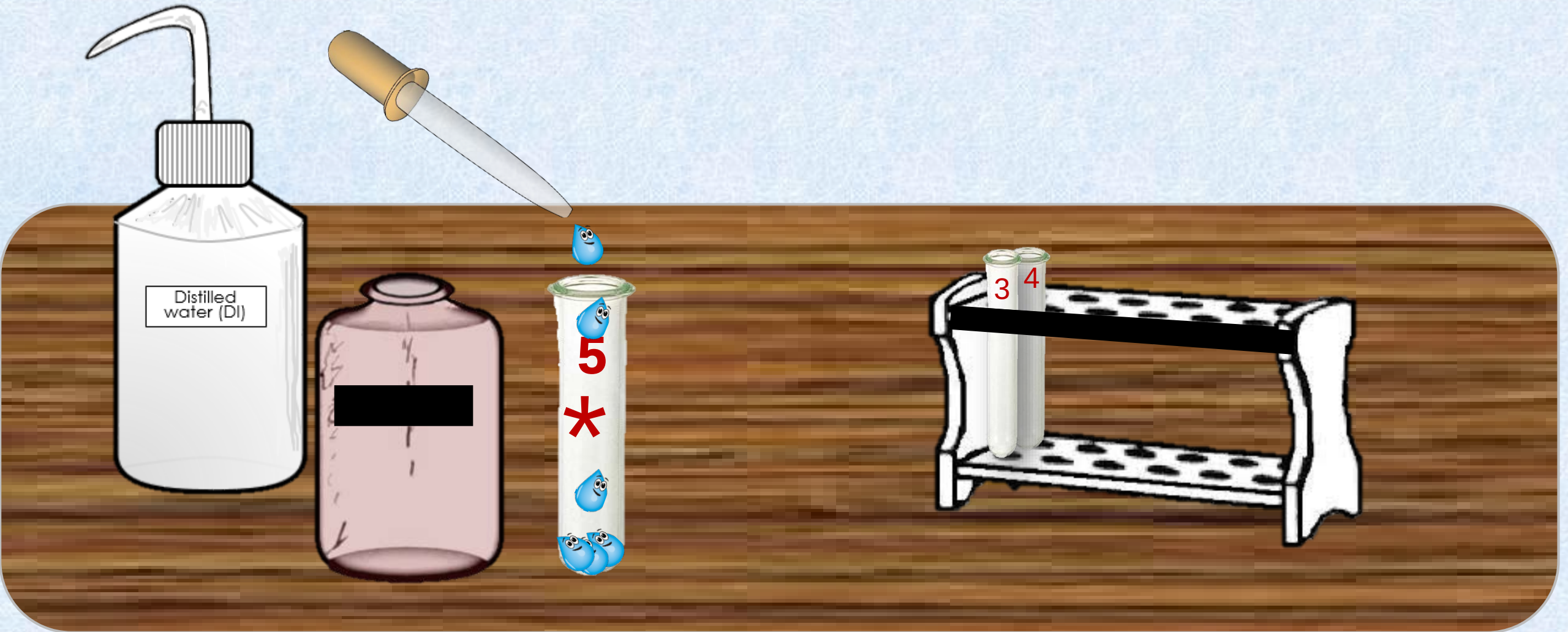
Tube 4

1) Add 1 drop amylase
and 10 drops of distilled water to tube 4.
2) Set this aside in rack.



Tube 5

1) Add 20 drops of amylase
and 1 drop of distilled water to tube 5.
2) Set this aside.

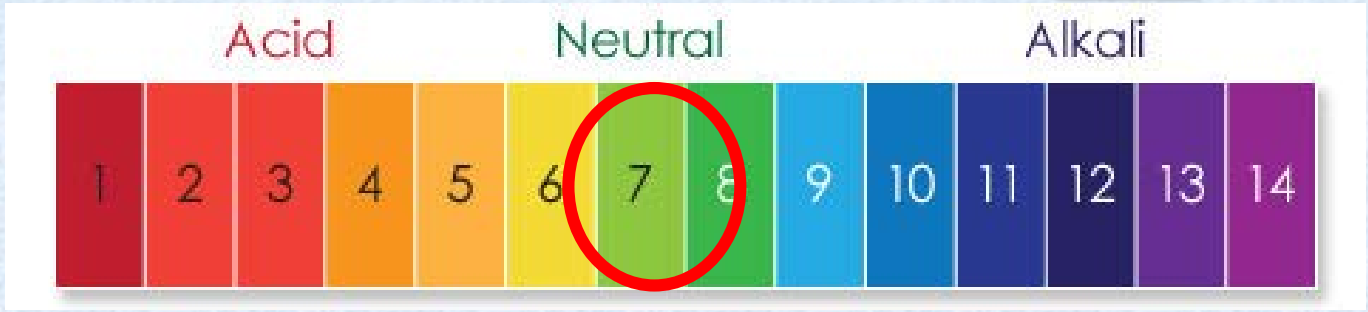


Tube 6

1) Add 10 drops of amylase only to tube 6.

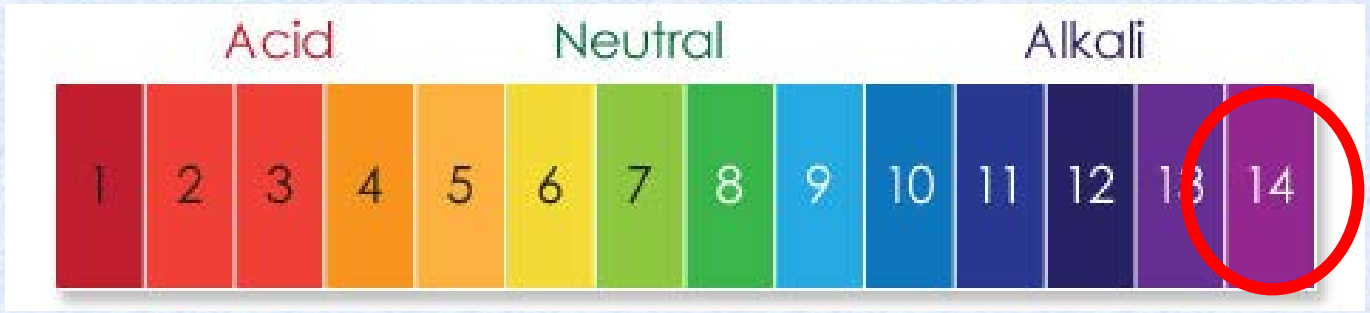
2) Note that this is pH=7.

3) Set this aside.



Tube 7

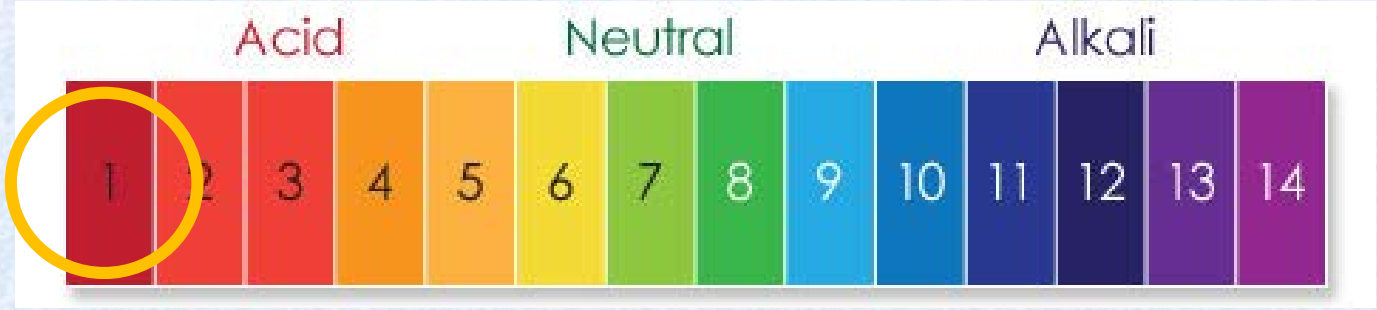
1) ADD 10 drops of amylase and 1 drop of sodium hydroxide (NaOH) to tube 7.
2) Note that this is **pH=14.**
3) Set this aside.



Tube 8 1) ADD 10 drops amylase and 1 drop hydrochloric acid (HCl) to test tube 8.

2) Note that this is **pH=1**

3) Set this aside.



Tube 9

1) Add 10 drops of amylase alone to test tube #9.

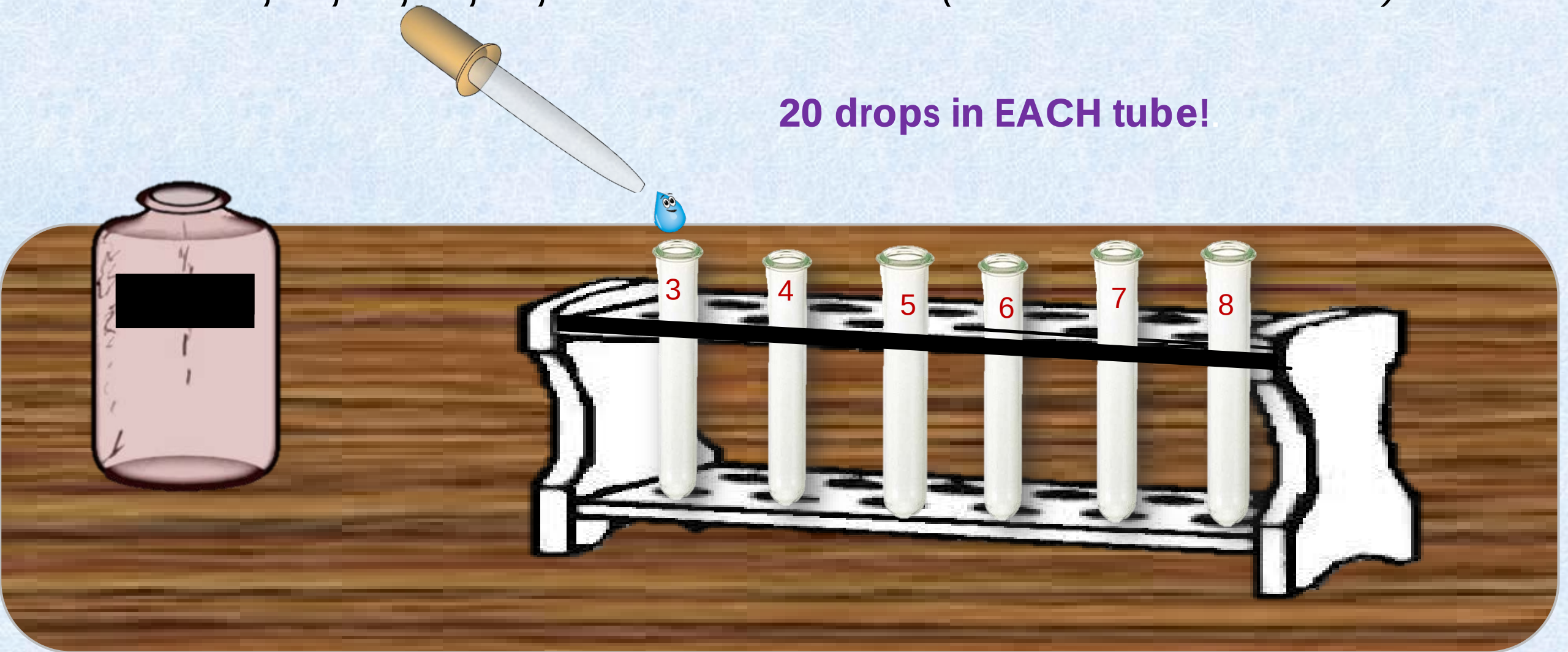
3) Set this aside in test tube rack

-this tube will remain at room temperature which is about 22 degrees C.

TUBES 3, 4, 5, 6, 7, and 8 ONLY

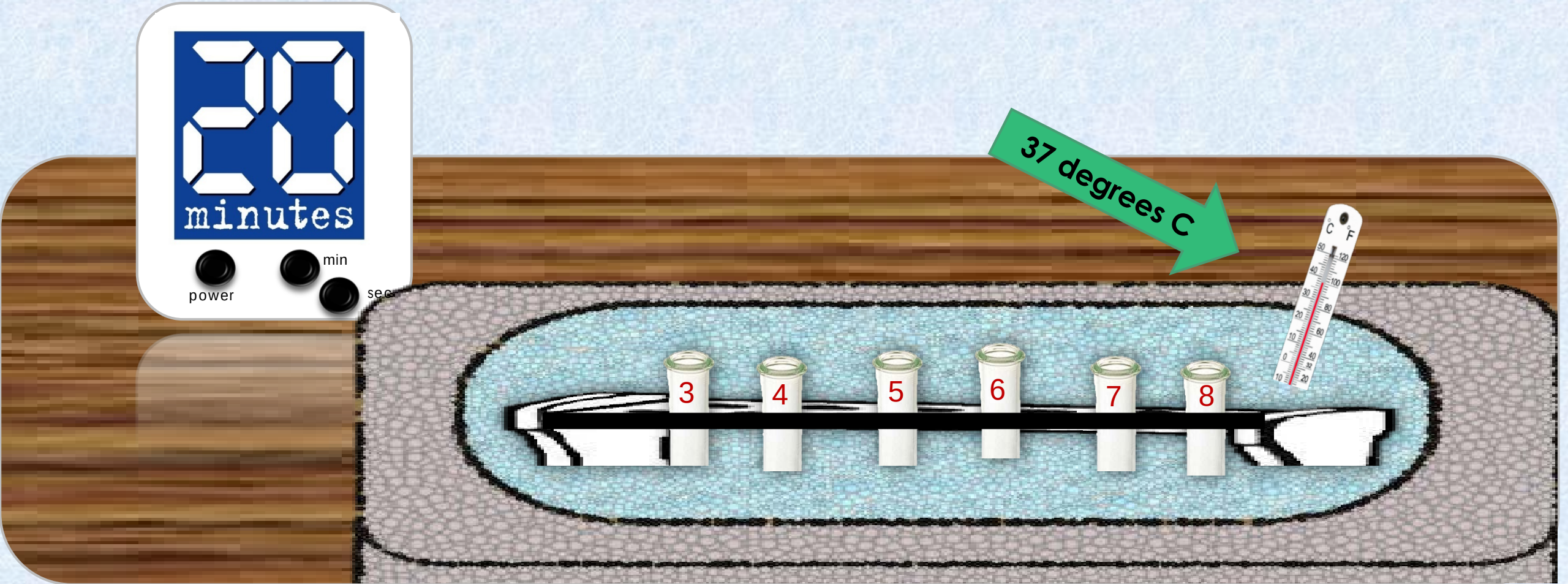
1) Add 20 drops of amylose (starch) to tubes 3, 4, 5, 6, 7, and 8 ONLY *(do not add to tube #9)*

20 drops in EACH tube!



1) Place test tubes 3, 4, 5, 6, 7, and 8 into a test tube rack and immerse in the **37 degree** C water bath.
2) Incubate for 20 minutes.
3) Note the time.

Our body's temperature is 37degrees C!

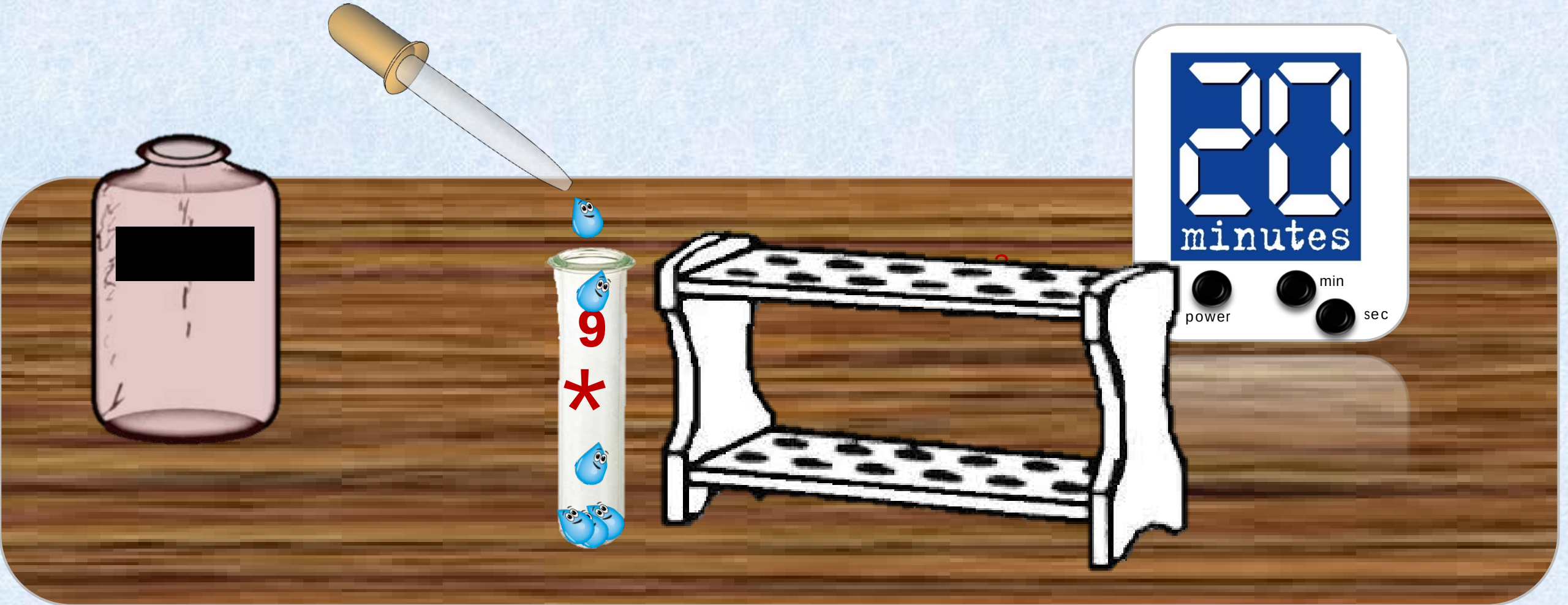


Grab tube 9 again.

1) Add 20 drops of amylose (starch) to test tube 9

3) Allow to incubate for 20 minutes at **room temperature (22 degrees C).**

3) Note the time.



Tube 1

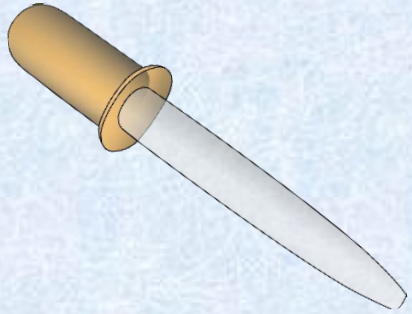
1) Add 10 drops of cold amylose (starch) from the ice bath to test tube 1 *(test tube 1 should have been incubated on ice for at least 10 minutes prior to this step!).*
2) Keep incubating on ice for 20 minutes.
3) Record the time.

Make sure the test tube 1 has been on ice for at least 10 minutes before adding cold starch!

TUBE 2

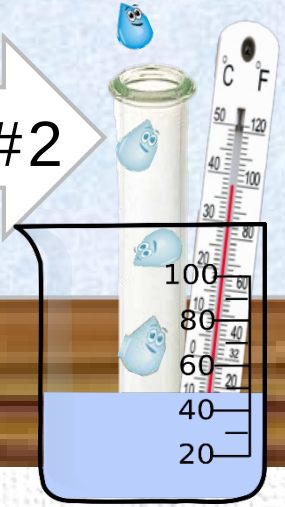
1) Add 20 drops of amylose (starch) to test tube 1 incubating on the hot plate at 100 degrees Celsius *(test tube 1 should have been incubated on ice for at least 10 minutes prior to this step!).*
2) Keep incubating on hot plate for another 20 minutes.
3) Record the time.

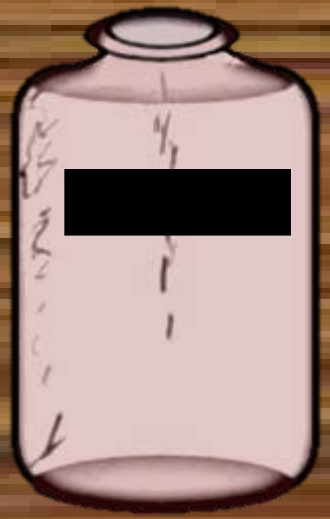
Make sure the test tube 1 has been incubating for at least 10 minutes before adding starch!!



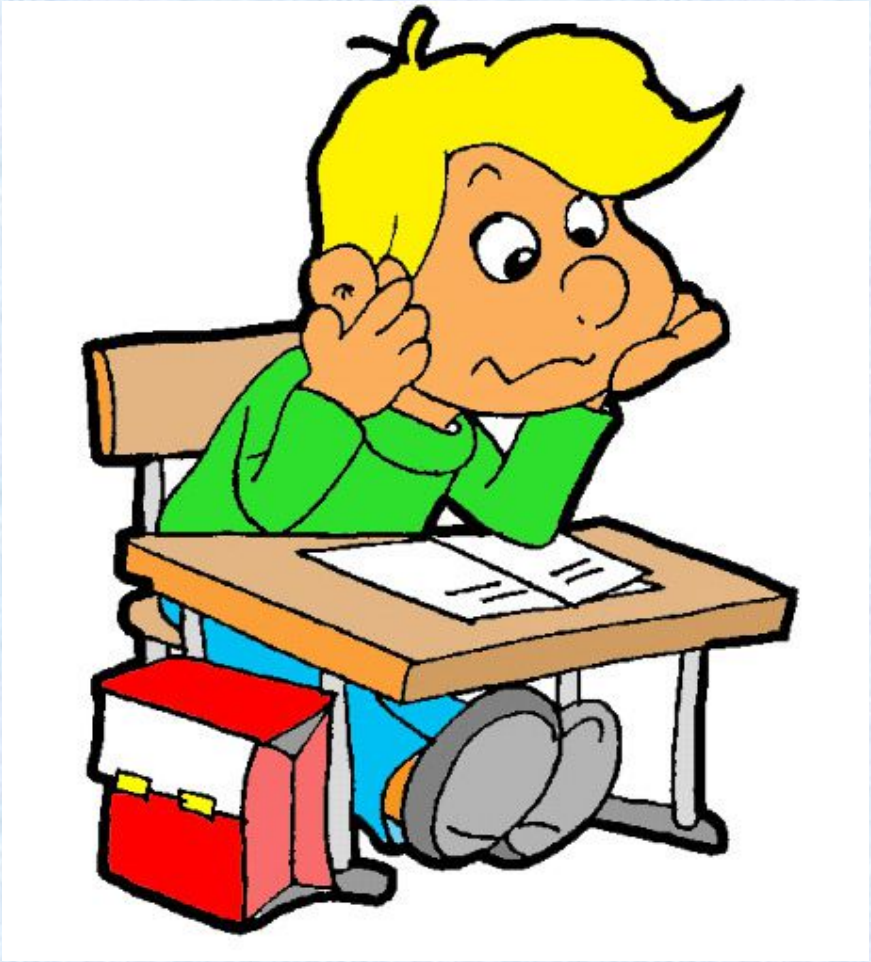
Test tube #2

100 degrees C





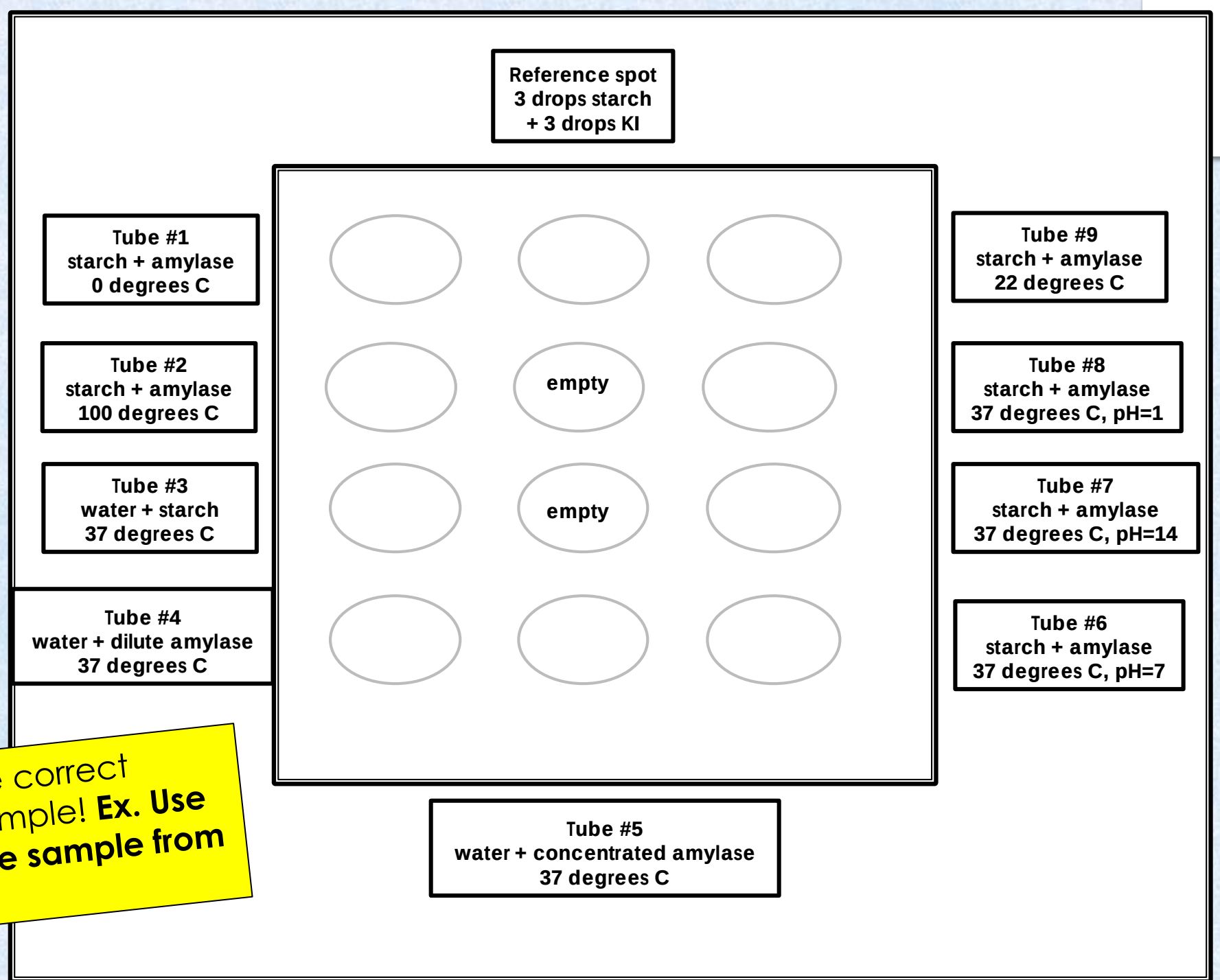
Let tubes 1 through 9 incubate for 20 minutes from the time starch was added



While you are waiting,
set up your SPOT PLATE!
See next slide.

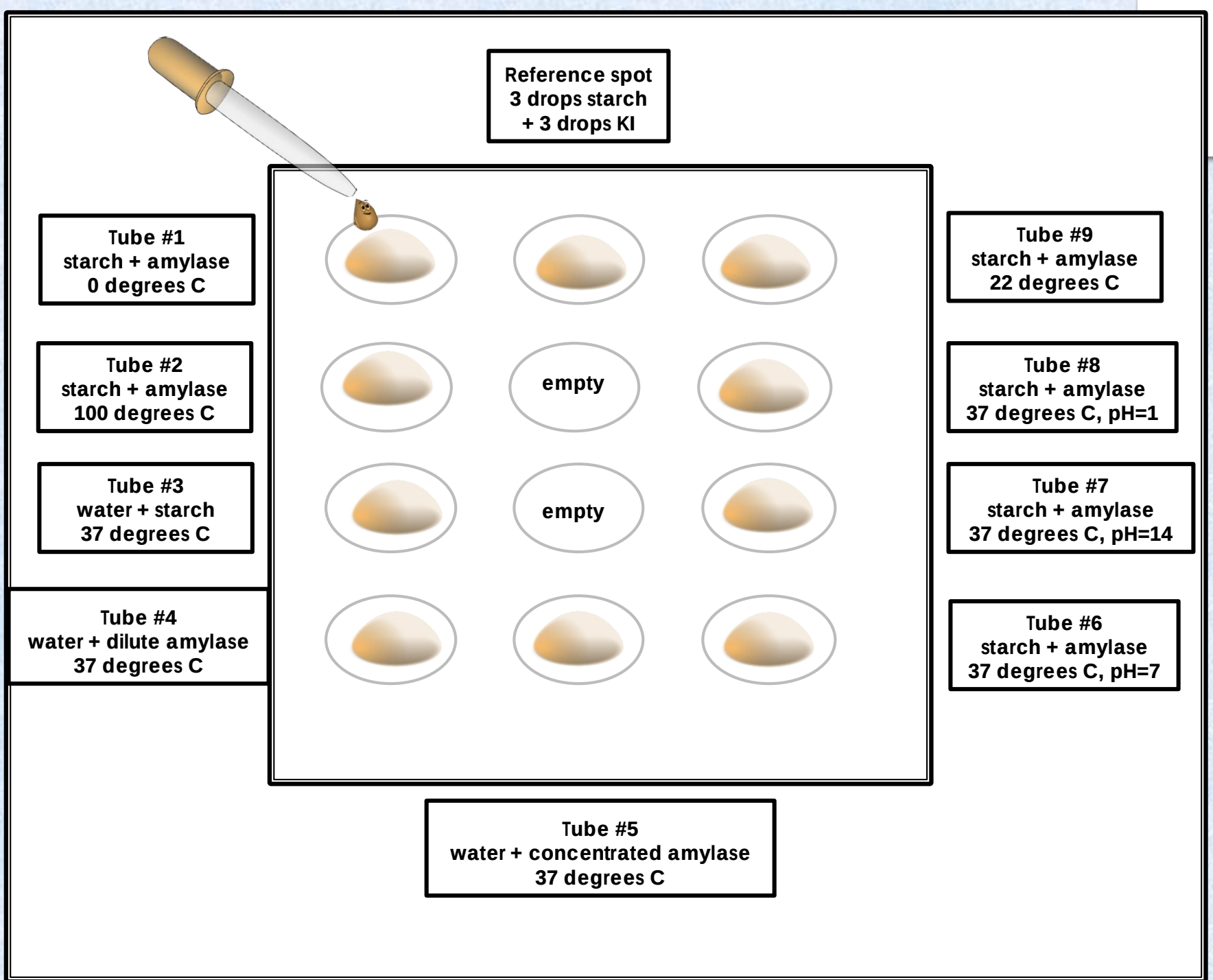
1) While you wait, set up your SPOT PLATE!

Place your welled plate on top of a paper towel. Label the paper towel to correspond to the wells as shown here →

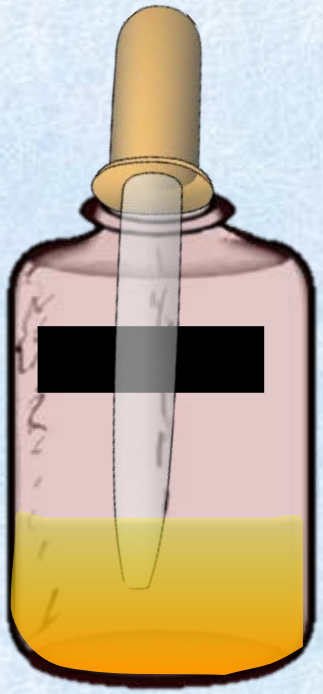


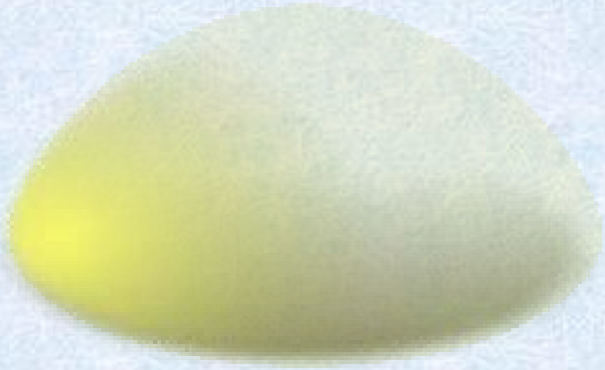
Make sure to use the correct dropper for each sample! **Ex. Use dropper #1 to get the sample from test tube #1!**

1) Add 3 drops of iodine potassium iodide, aka Lugol's solution (IKI) to each well.



Make note of the color of Lugol's Soln (IKI) before anything else is added to it.



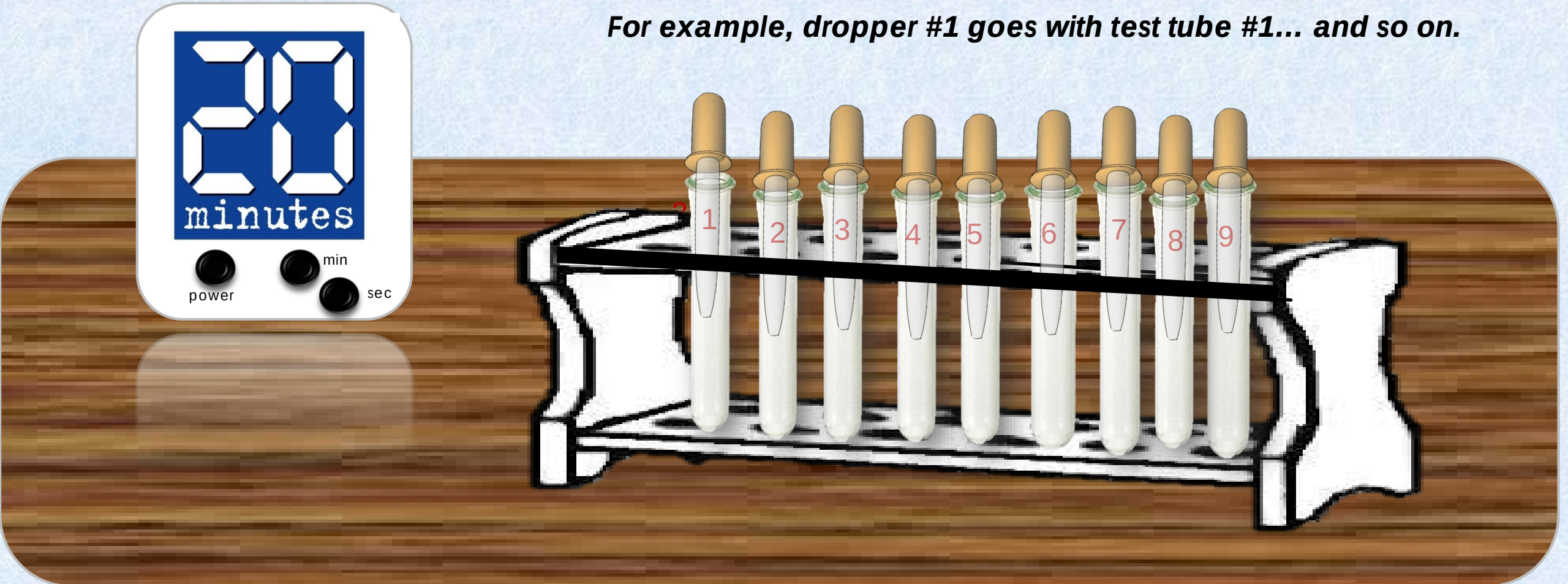


Lugol's soln by itself should be a yellowish – brownish color.

- Lugol's Solution is elemental Iodine dissolved in a potassium iodide solution. In the presence of starch, Lugol's solution turns blue-black. This is due to the formation of polyiodide chains from the reaction of starch and iodine.
- If starch is broken down into smaller units, there will be no color change in the Lugol's Solution.
- Amylase functions to speed up the reaction of amylase breaking down starch into its smaller components such as maltose which is a disaccharide.

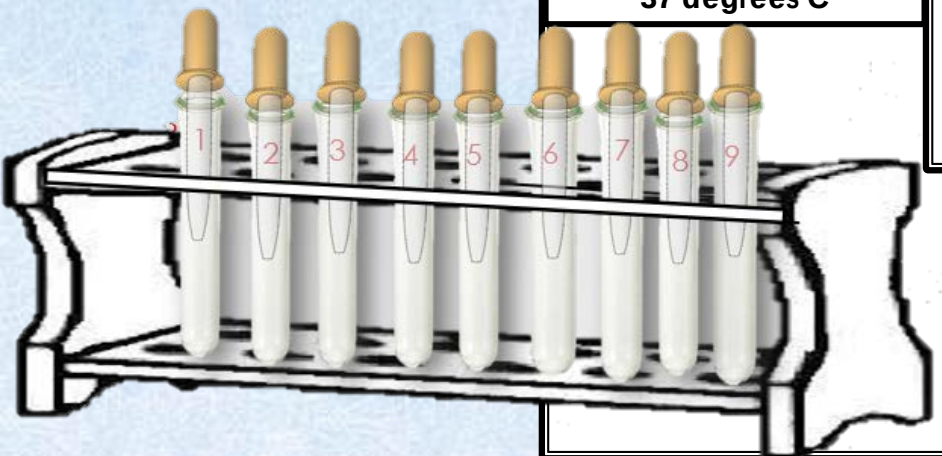
Once the 20 minutes has ended, gather test tubes 1 through 9 and pair each of them with the appropriate dropper as shown here!

For example, dropper #1 goes with test tube #1… and so on.



- Add **3 drops** of sample 1 from test tube 1 using dropper #1 into the well labelled Tube #1.
- Continue likewise for each of the remaining samples.

Be sure to use the correct sample and the appropriate dropper to the correct spot as indicated here →



Reference spot
3 drops starch
+ 3 drops KI

Be sure to put any remaining sample in your dropper back into the correct tube. You will be using it to test for the presence of sugar in the next step.

Tube #1
starch + amylase
0 degrees C

Tube #2
starch + amylase
100 degrees C

Tube #3
water + starch
37 degrees C

Tube #4
water + dilute amylase
37 degrees C

Tube #1 goes here		Tube #9 goes here
Tube #2 goes here	empty	Tube #8 goes here
Tube #3 goes here	empty	Tube #7 goes here
Tube #4 goes here	Tube #5 goes here	Tube #6 goes here

Tube #9
starch + amylase
22 degrees C

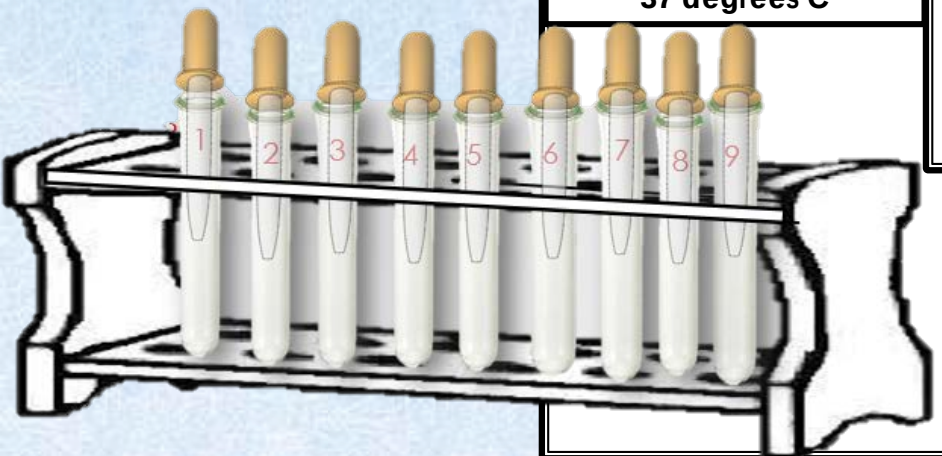
Tube #8
starch + amylase
37 degrees C, pH=1

Tube #7
starch + amylase
37 degrees C, pH=14

Tube #6
starch + amylase
37 degrees C, pH=7

Tube #5
water + concentrated amylase
37 degrees C

Write down observations (*color changes*) **immediately** after adding your sample to the IKI on the welled plate.



Reference spot
3 drops starch
+ 3 drops KI

Results may vary, but you should get similar results. Some colors may change over time. Use the colors that immediately appeared after adding the sample as your data.

Tube #1
starch + amylase
0 degrees C

Tube #2
starch + amylase
100 degrees C

Tube #3
water + starch
37 degrees C

Tube #4
water + dilute amylase
37 degrees C

empty

empty

Tube #9
starch + amylase
22 degrees C

Tube #8
starch + amylase
37 degrees C, pH=1

Tube #7
starch + amylase
37 degrees C, pH=14

Tube #6
starch + amylase
37 degrees C, pH=7

Tube #5
water + concentrated amylase
37 degrees C

COLOR KEY

NO DIGESTION = Blue-Black

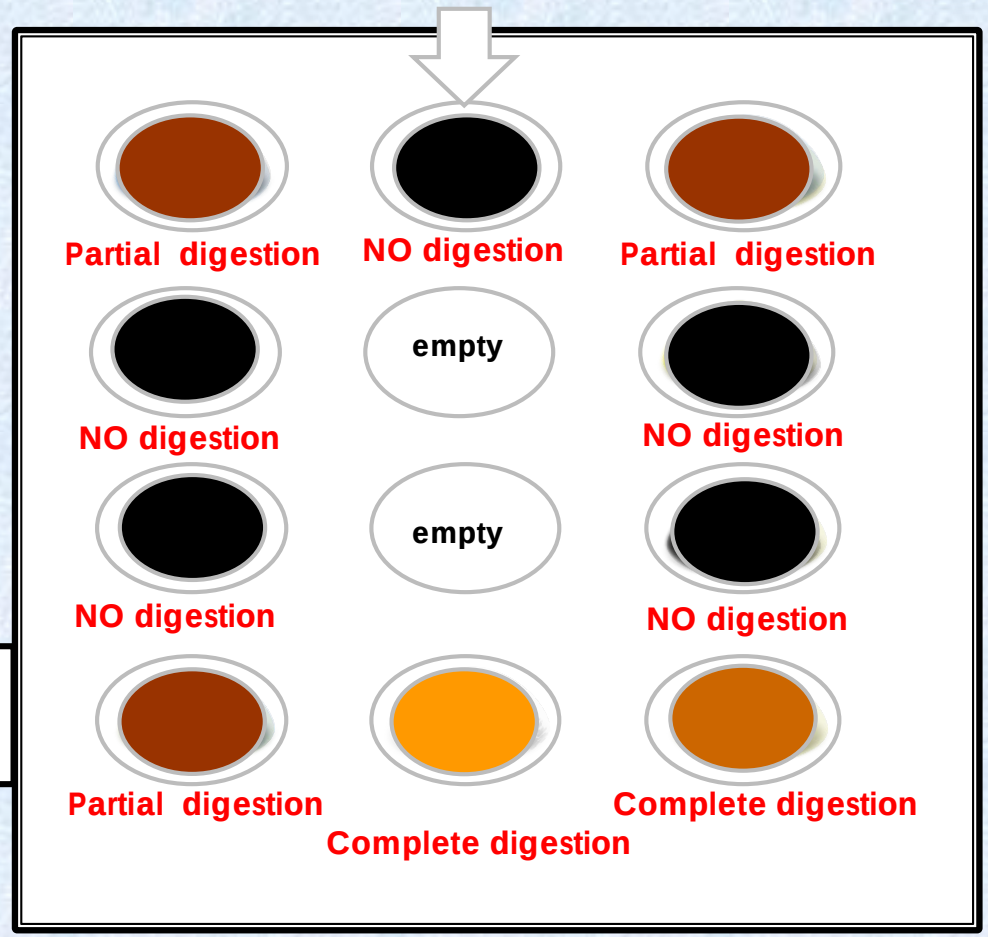
PARTIAL DIGESTION = Brown

COMPLETE DIGESTION = Yellow

ANALYZE YOUR RESULTS!

Identify the activity of the amylase (based on the level of digestion) in each sample based on the results.

Give an explanation for each of the results!



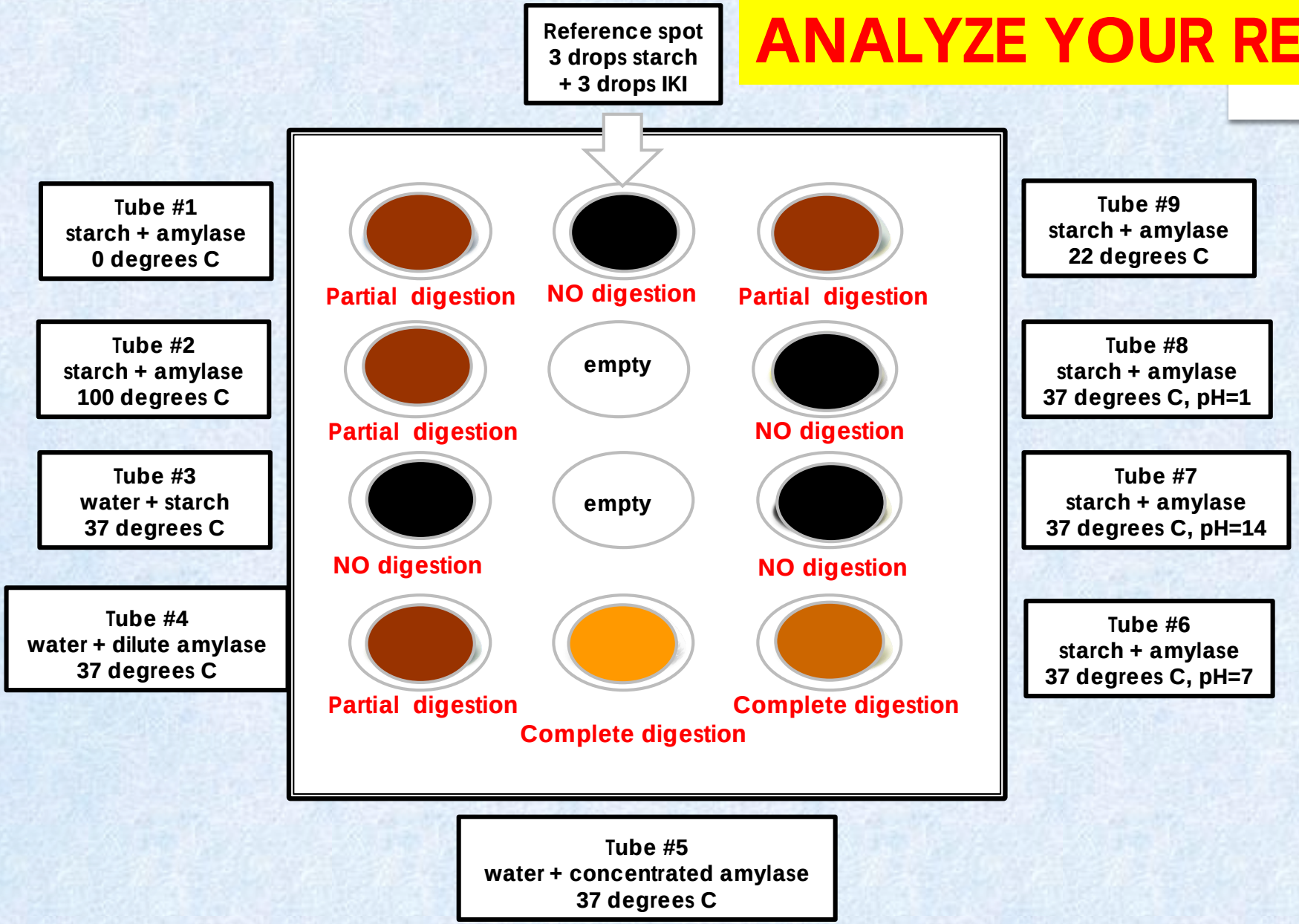
The samples that reacted strongly with the Lugol's Solution appear black or blue-black. In these samples, amylase was **unable** to convert starch into maltose (sugar).

There was no digestion (amylase was inactive or absent) in samples

2, 3, 7, and 8.

Let's look at **WHY?**

ANALYZE YOUR RESULTS!



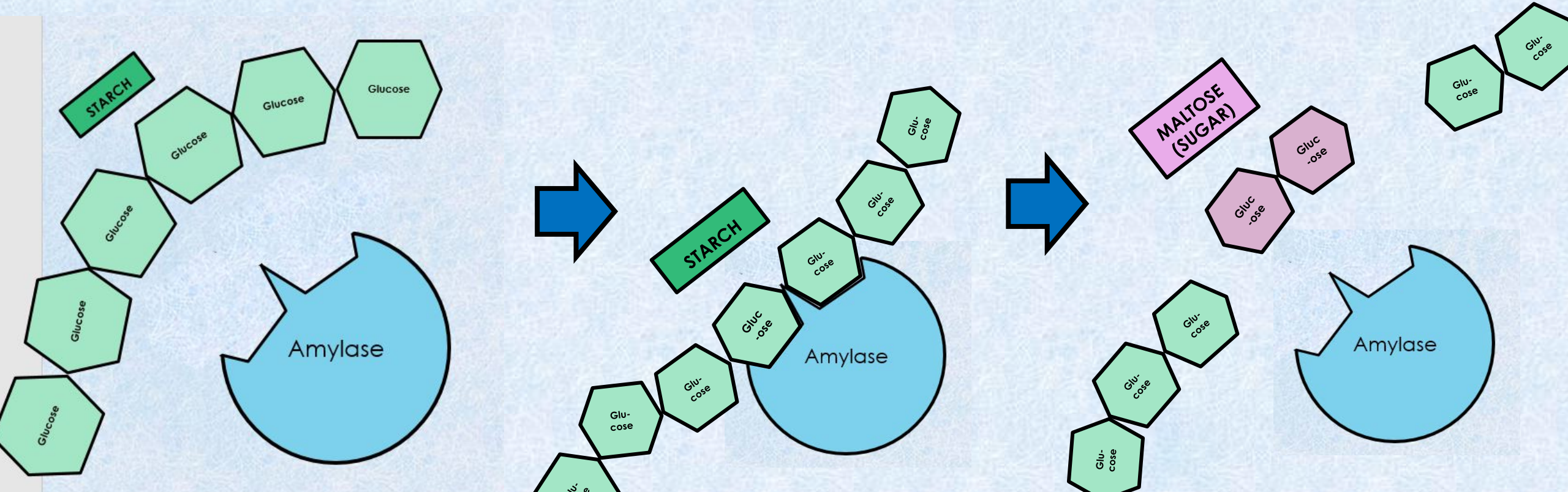
Starch is a polysaccharide that is made up of glucose monomers. Lugol's solution (IKI) turns dark blue-black in the presence of starch.

NEGATIVE CONTROL

NO digestion

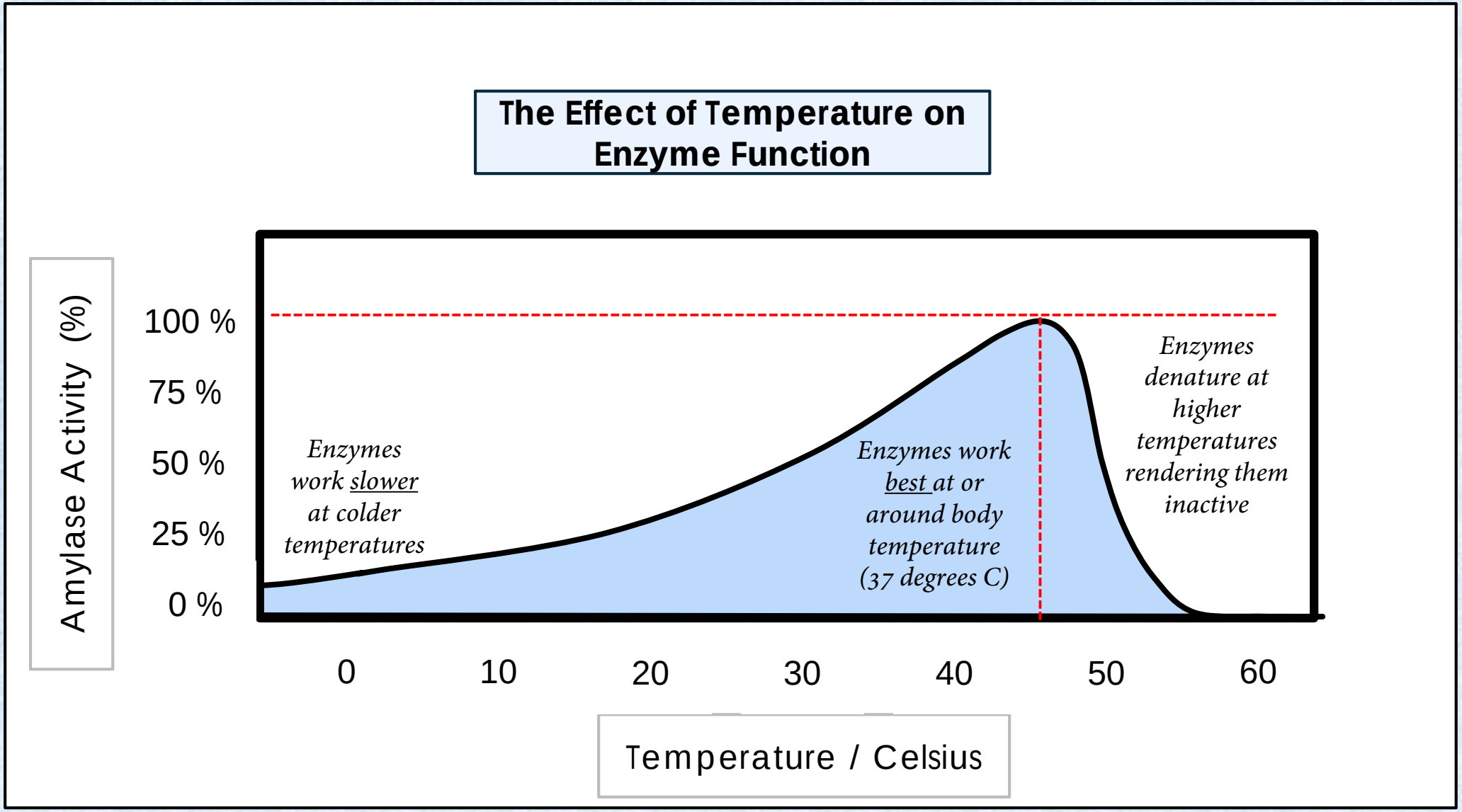
Reference spot
3 drops starch
+ 3 drops IKI

AMYLASE FUNCTION



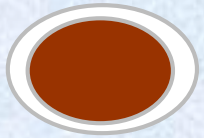
Tube #2
starch +
amylase 100
degrees C

- **Enzymes are sensitive to their environment. Amylase works best at around 37 degrees C, which is the same as our internal body temperature. When enzymes *(or any protein for that matter)* are heated too much *(anything over about 80 degrees C for amylase)* it will denature.**



Amylase Function vs. Temperature

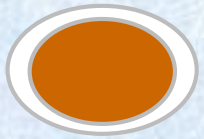
Tube #1
starch + amylase
0 degrees C



Partial digestion

In Test Tube #1 = Amylase was able to digest some of the starch in the sample, but at a much slower rate than observed at 37 degrees Celsius.

Tube #9
starch + amylase
22 degrees C



Partial digestion

In Test Tube #9 = Amylase was able to digest some of the starch in the sample, but at a slower rate than observed at 37 degrees Celsius.

Tube #6
starch + amylase
37 degrees C, pH=7

Complete digestion

In Test Tube #6 = Amylase was able to completely digest the starch in the sample, because it was at its optimal temperature of 37 degrees Celsius, which is the same as our body's internal temperature.

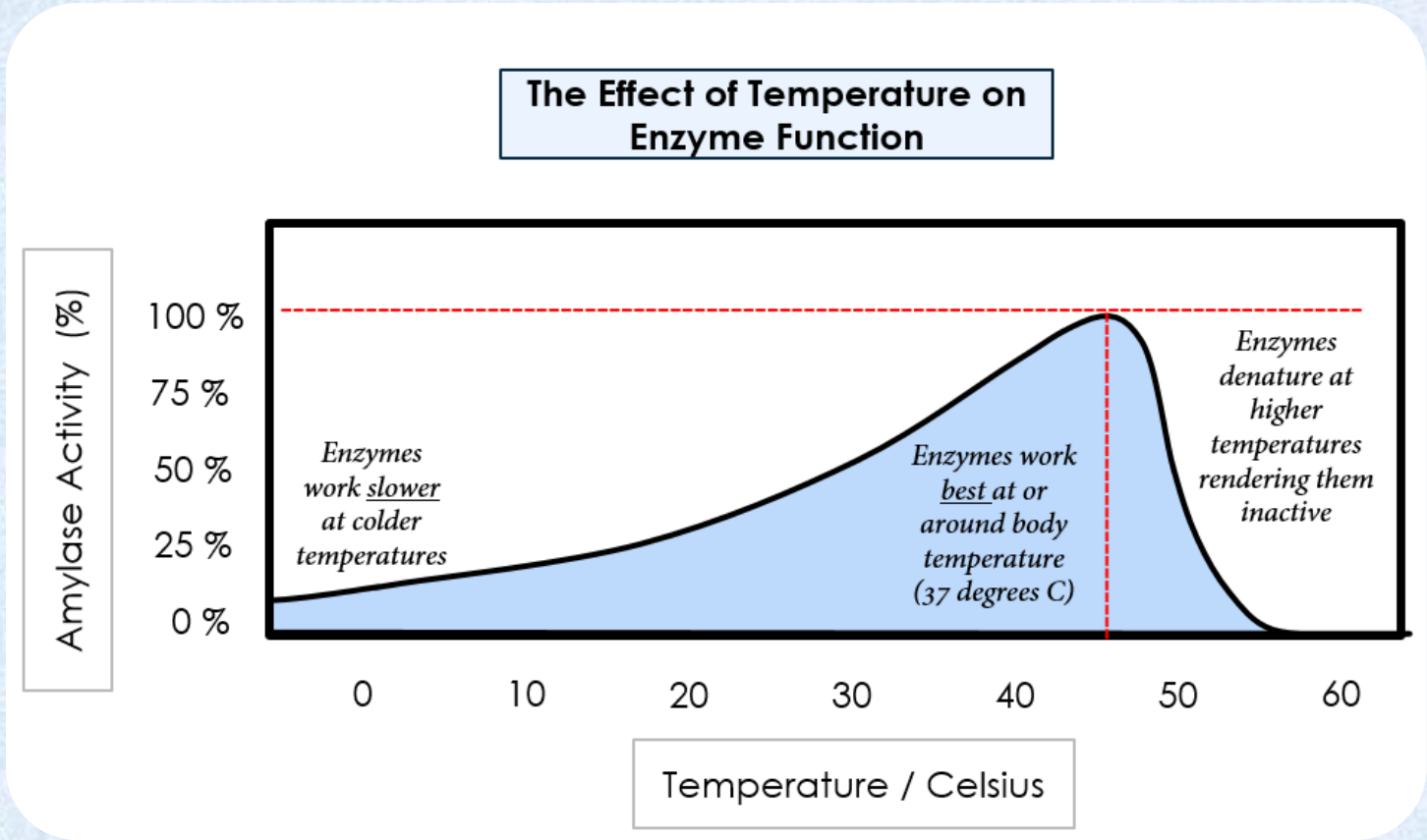
Tube #2
starch + amylase
100 degrees C

No digestion

In Test Tube #2 = Amylase was not to digest the starch in the sample, because the heat caused the enzyme to denature, rendering it inactive.

Amylase Function vs. Temperature

- Amylase works at a slower rate when it is cold, but cold temperatures do not act to denature it. However, at high temperatures (~100°C) amylase does become denatured causing irreversible damage to its molecular structure. This is due to the additional kinetic energy within the atoms that make up the enzyme.



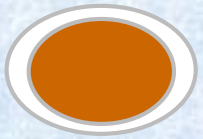
Amylase Function vs. Amylase Concentration

Tube #3
water + starch
37 degrees C

No digestion

In Test Tube #3 = Amylase was not present, therefore there was no means by which starch could have been digested into sugar. Therefore, no digestion was observed.

Tube #4
water + dilute amylase
37 degrees C



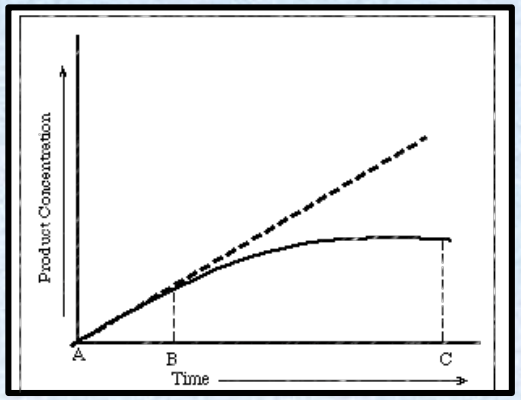
Partial digestion

In Test Tube #4 = Amylase was able to digest some of the starch in the sample, but at a slower rate because it was significantly diluted.

Tube #5
water + concentrated amylase
37 degrees C

Complete digestion

In Test Tube #6 = Amylase was able to completely digest the starch in the sample, because it was present in the sample at a high concentration.



The function of amylase will increase with increased concentration, until the substrate become the limiting factor.

Amylase Function vs. pH

Tube #8
starch + amylase
37 degrees C, pH=1

No digestion

In Test Tube #8 = Amylase was not present, therefore there was no means by which starch could have been digested into sugar. Therefore, no digestion was observed.

Tube #7
starch + amylase
37 degrees C, pH=14

Partial digestion

In Test Tube #7 = Amylase was able to digest some of the starch in the sample, but at a slower rate because it was significantly diluted.

Tube #6
starch + amylase
37 degrees C, pH=7

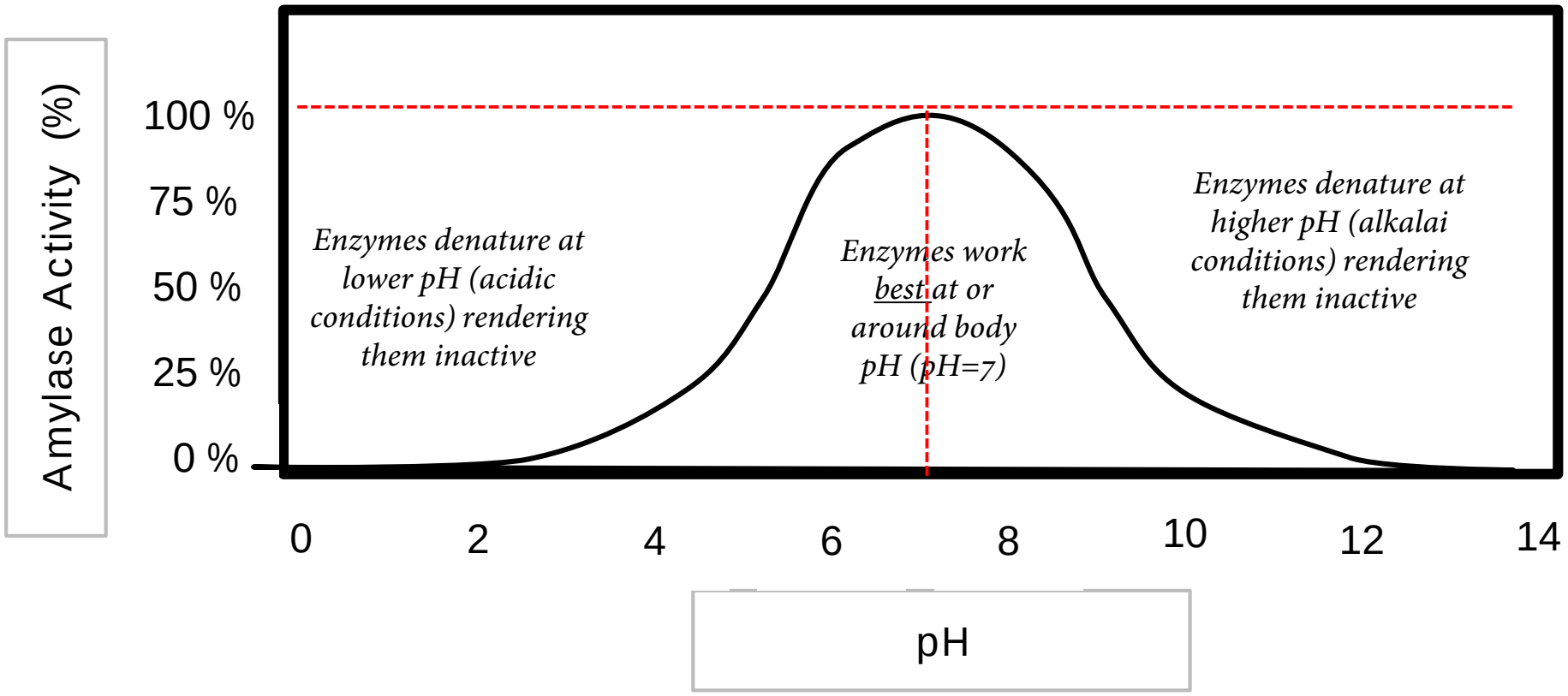
Complete digestion

In Test Tube #6 = Amylase was able to completely digest the starch in the sample, because it was present in the sample at a high concentration.

The function of amylase is best at pH=7 (neutral) which is the pH inside our bodies. The activity of the enzyme will decrease as the pH is moved further away from neutral, either higher or lower. Extreme pH (pH=1 and pH=14) will act to denature the enzyme rendering it inactive due to causing a significant structural change in the enzyme.

The function of amylase is best at pH=7 (neutral) which is the pH inside our bodies. The activity of the enzyme will decrease as the pH is moved further away from neutral, either higher or lower. Extreme pH (pH=1 and pH=14) will act to denature the enzyme rendering it inactive due to causing a significant structural change in the enzyme.

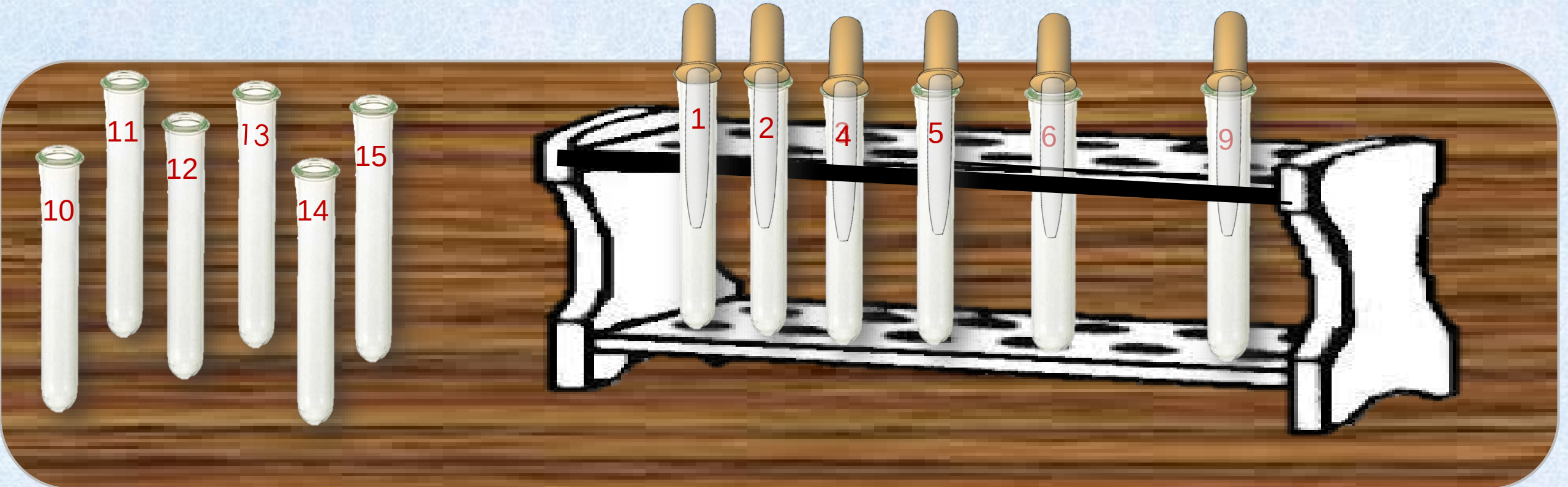
The Effect of pH on Enzyme Function



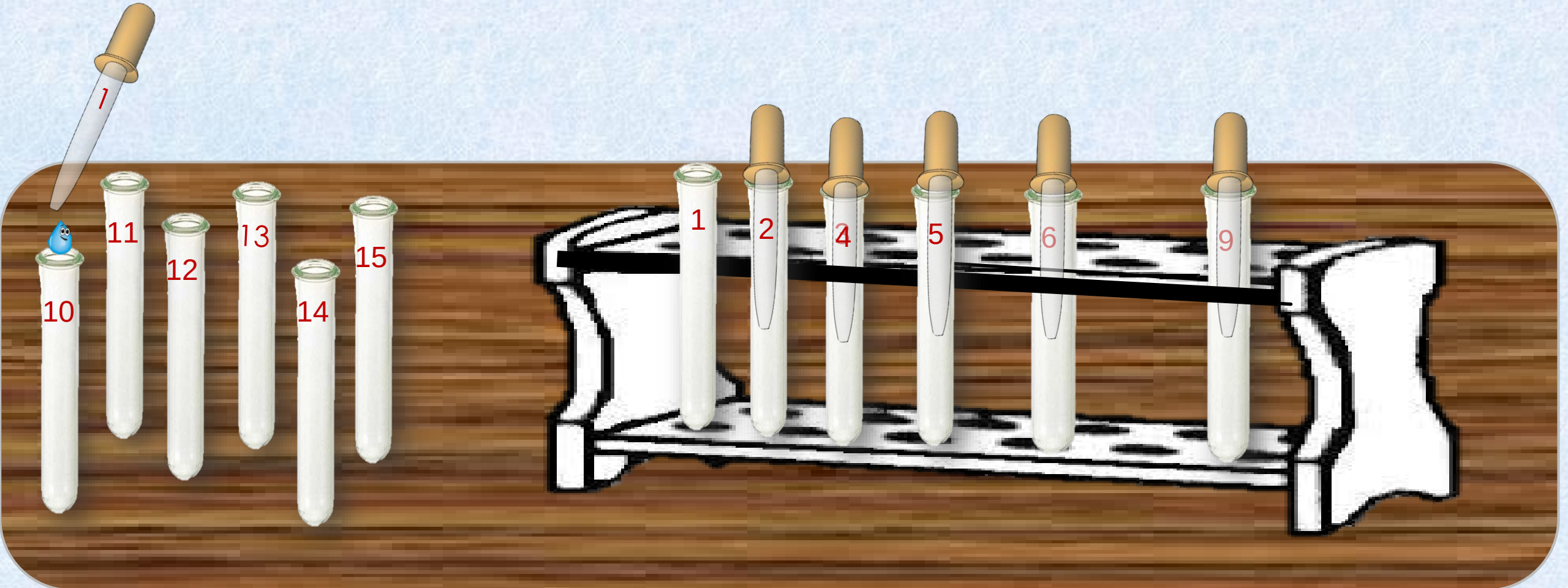
NEXT STEP

GET NEW CLEAN TUBES THAT WERE LABELLED 10,11,12, 13, 14 AND 15!

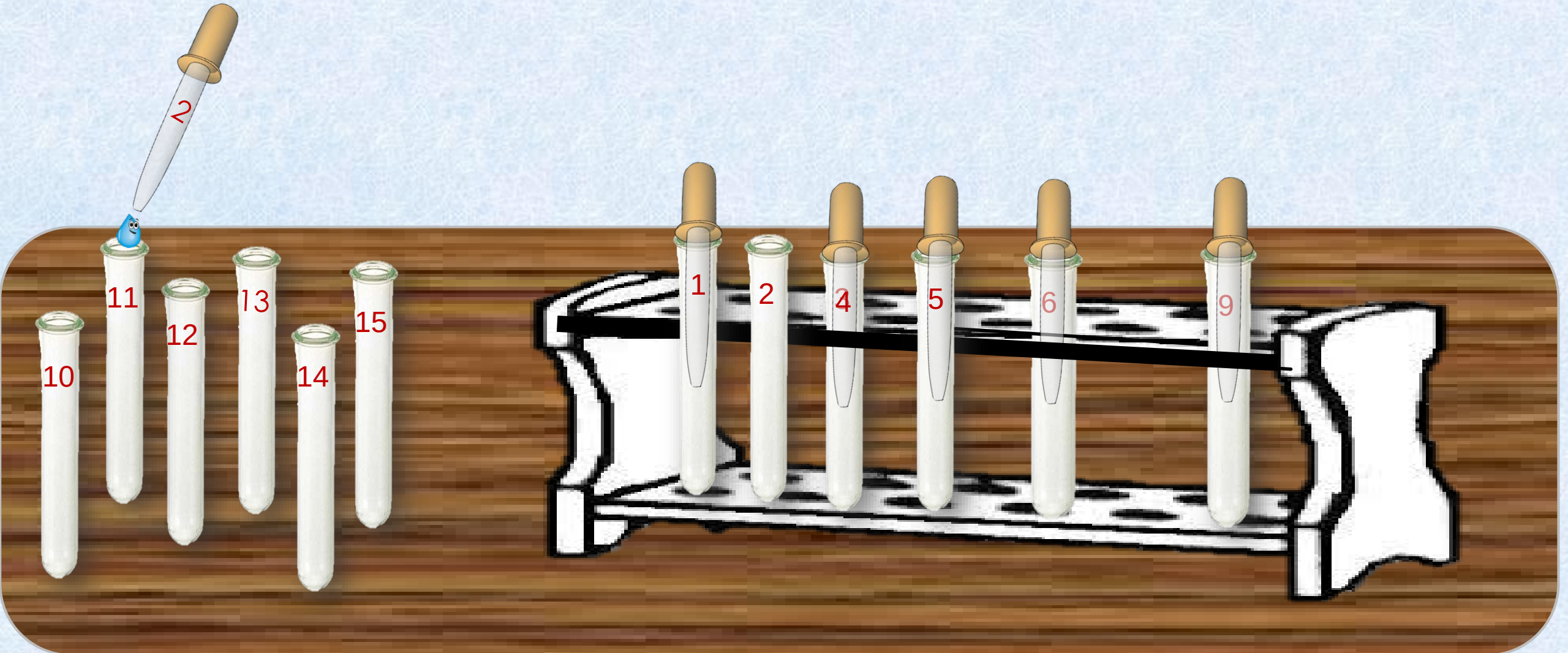
- We will now confirm the digestion of starch to sugar by performing the sugar test on tubes 1,2,4,5,6 and 9. We will test 1 through 6, because they showed either partial or complete digestion. We will test 9 to see if heat had an effect on the sample.



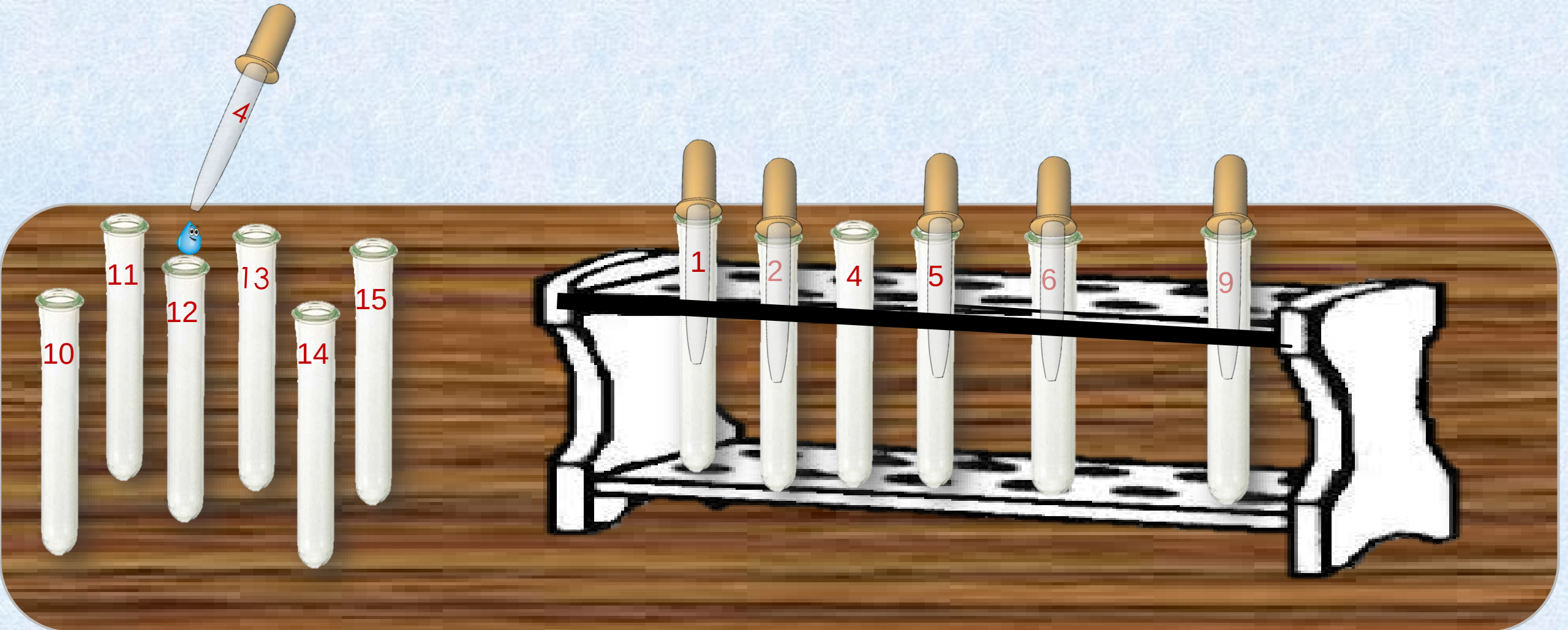
- Transfer 10 drops of liquid from test tube 1 into test tube 10.



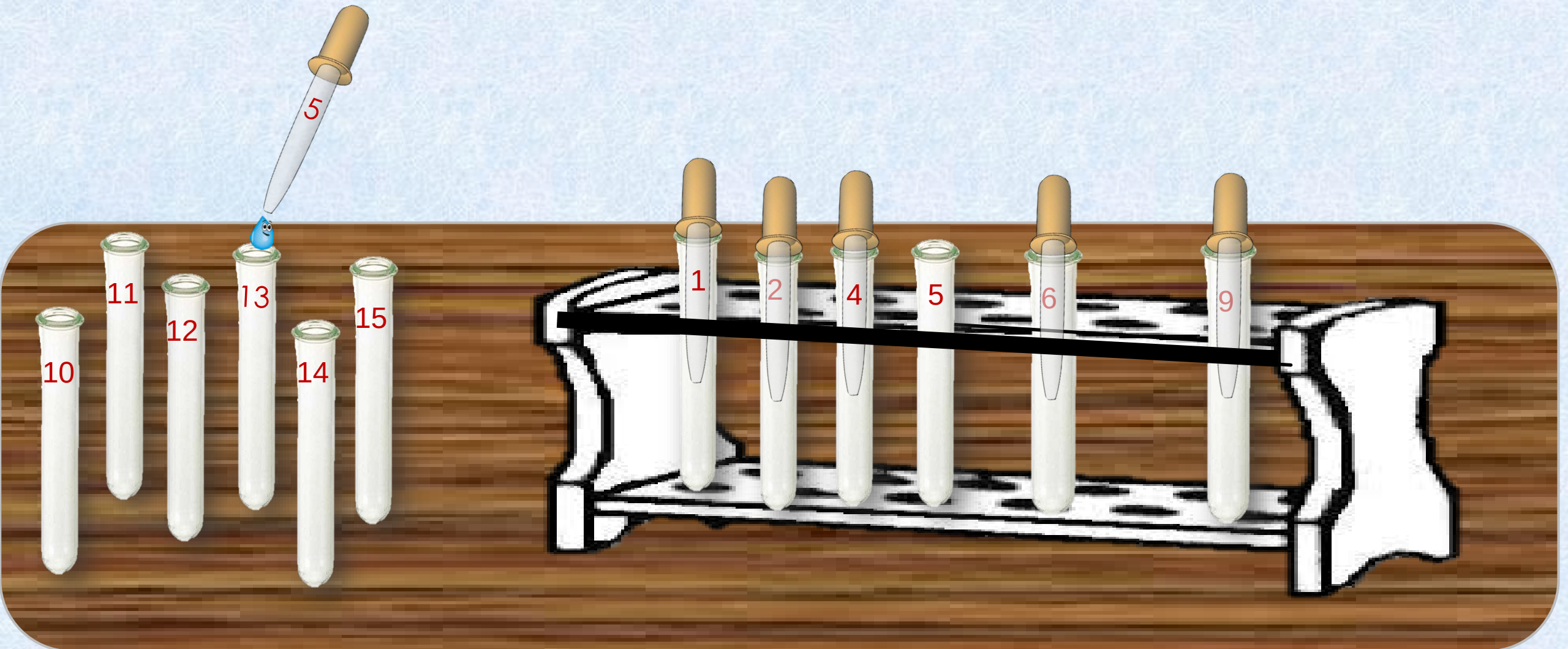
- Transfer 10 drops of liquid from test tube 2 into test tube 11.



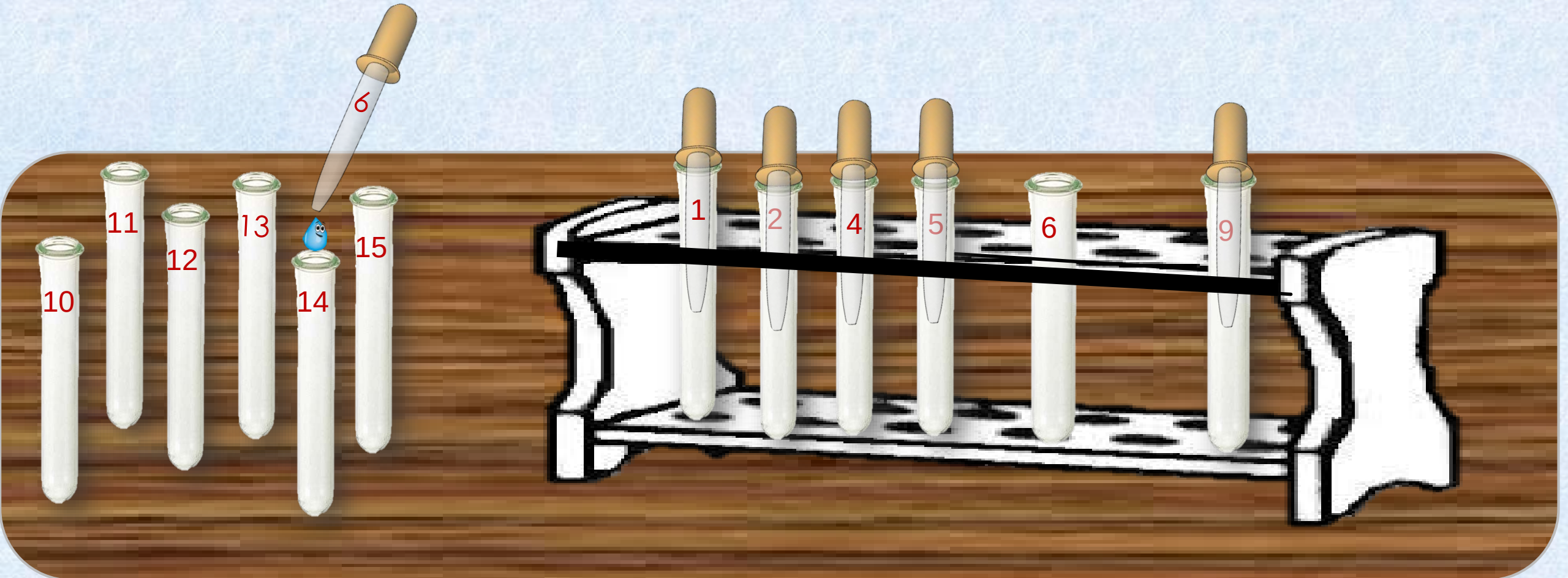
- Transfer 10 drops of liquid from test tube 4 into test tube 12.



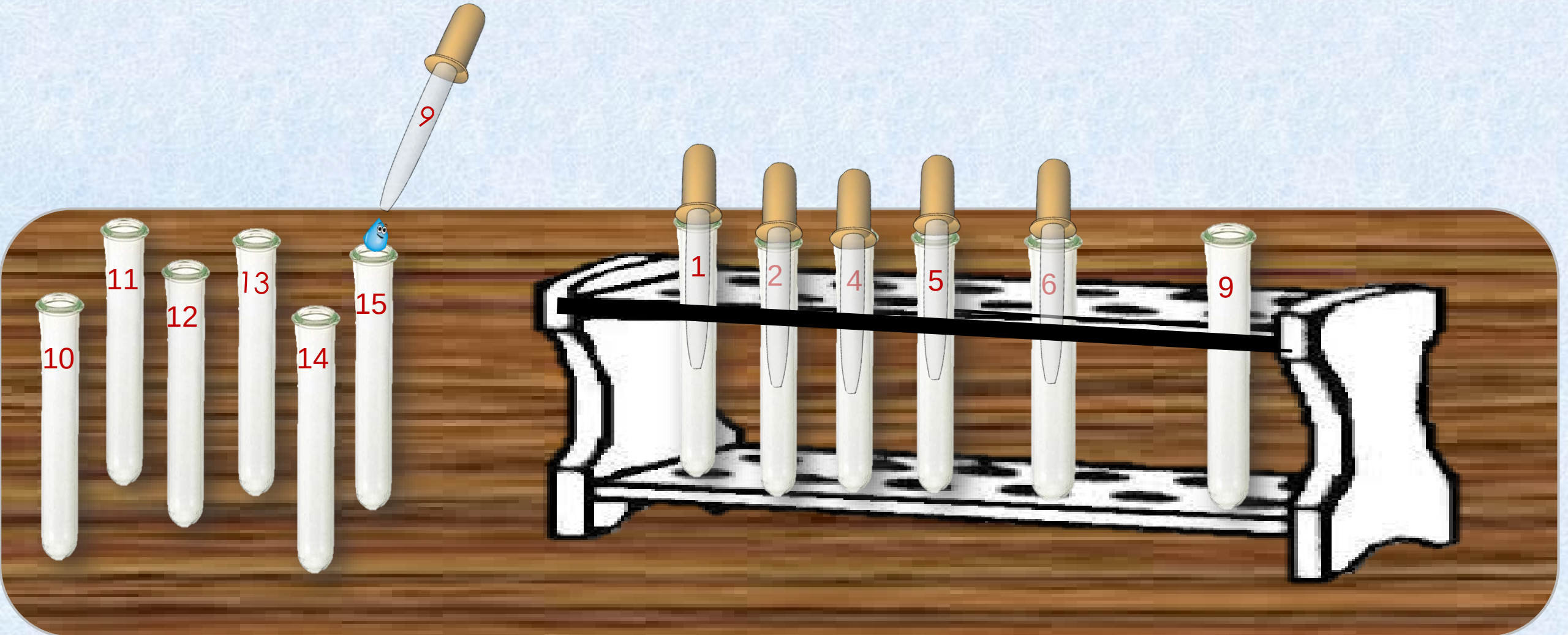
- Transfer 10 drops of liquid from test tube 5 into test tube 13.



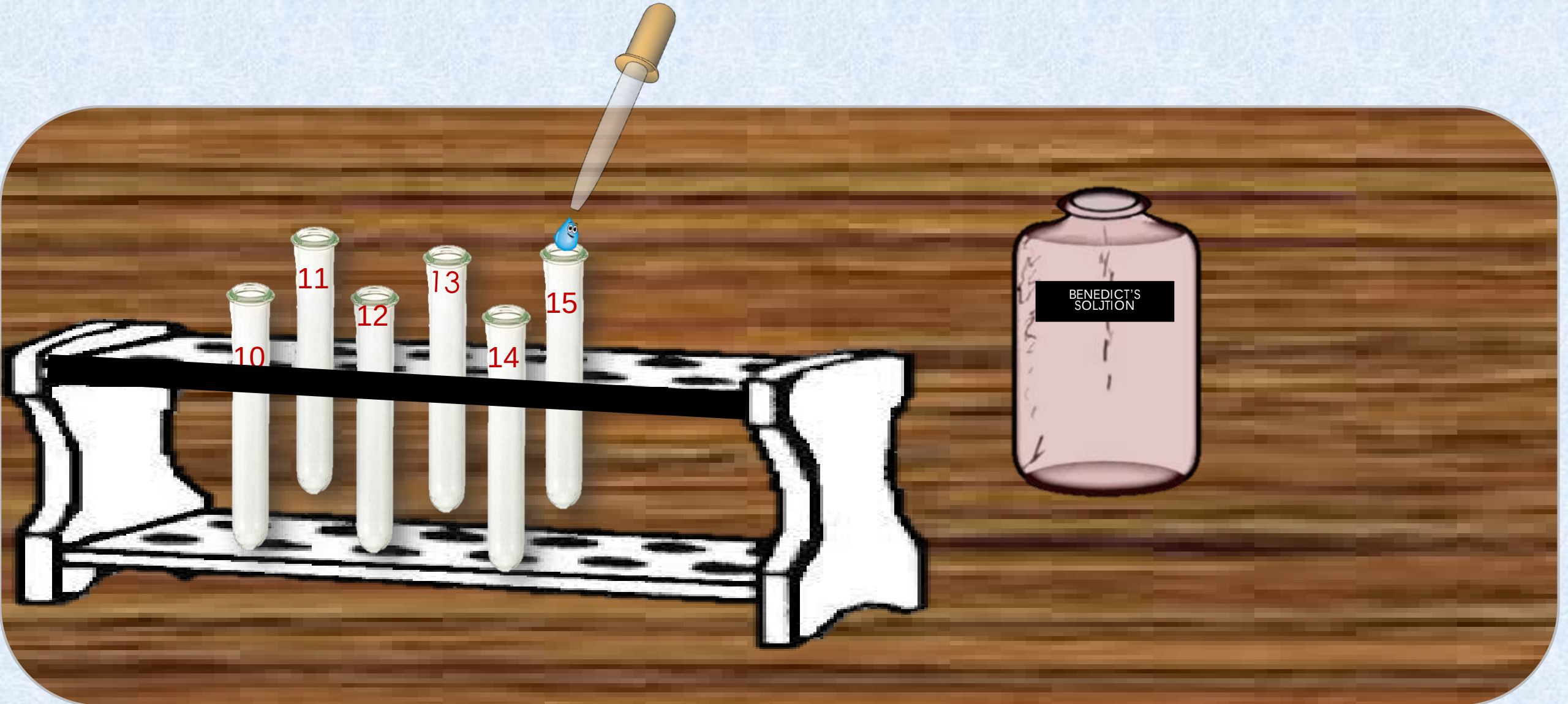
- Transfer 10 drops of liquid from test tube 6 into test tube 14.



- Transfer 10 drops of liquid from test tube 9 into test tube 15.

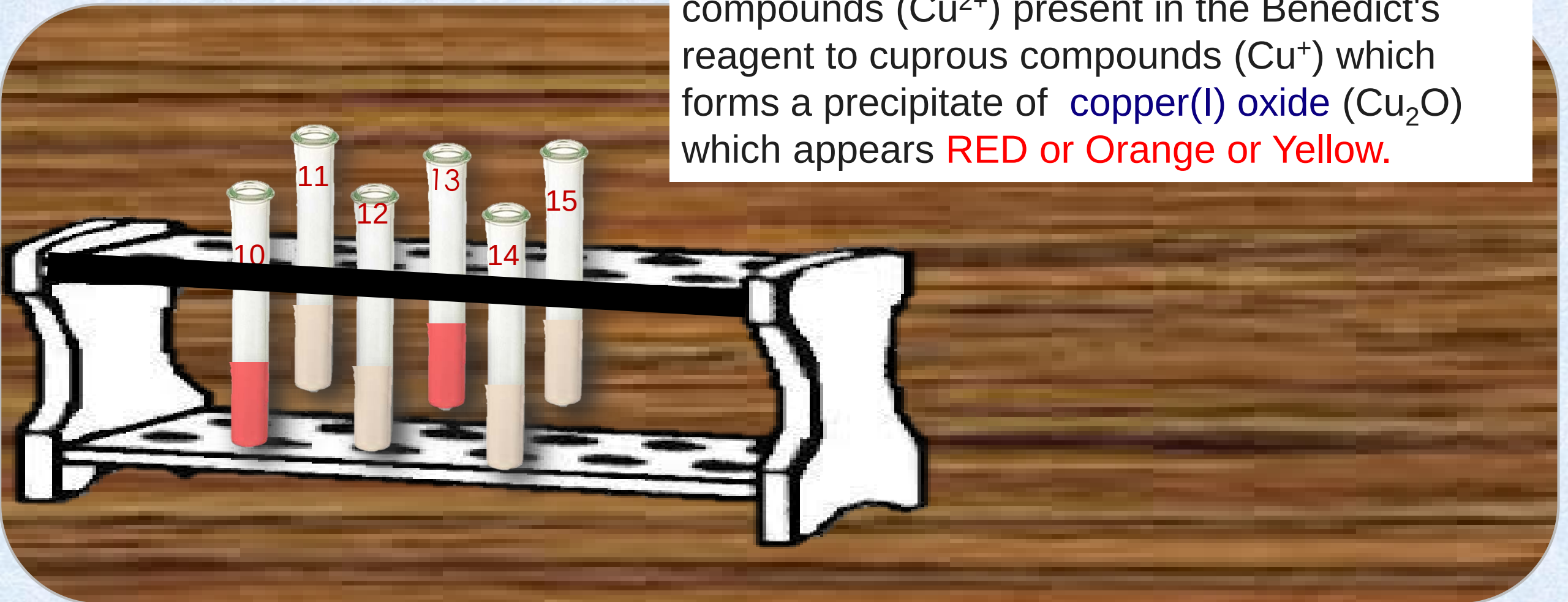


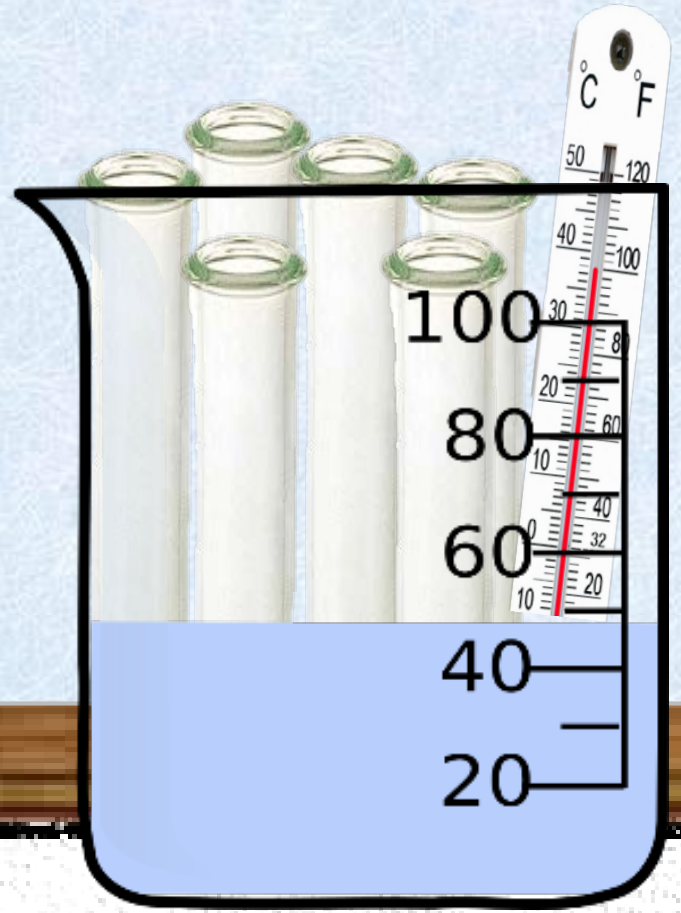
- ADD 10 drops of Benedict's Solution to each tube.



- A red (could also be orange or yellow) color indicates the presence of sugar.

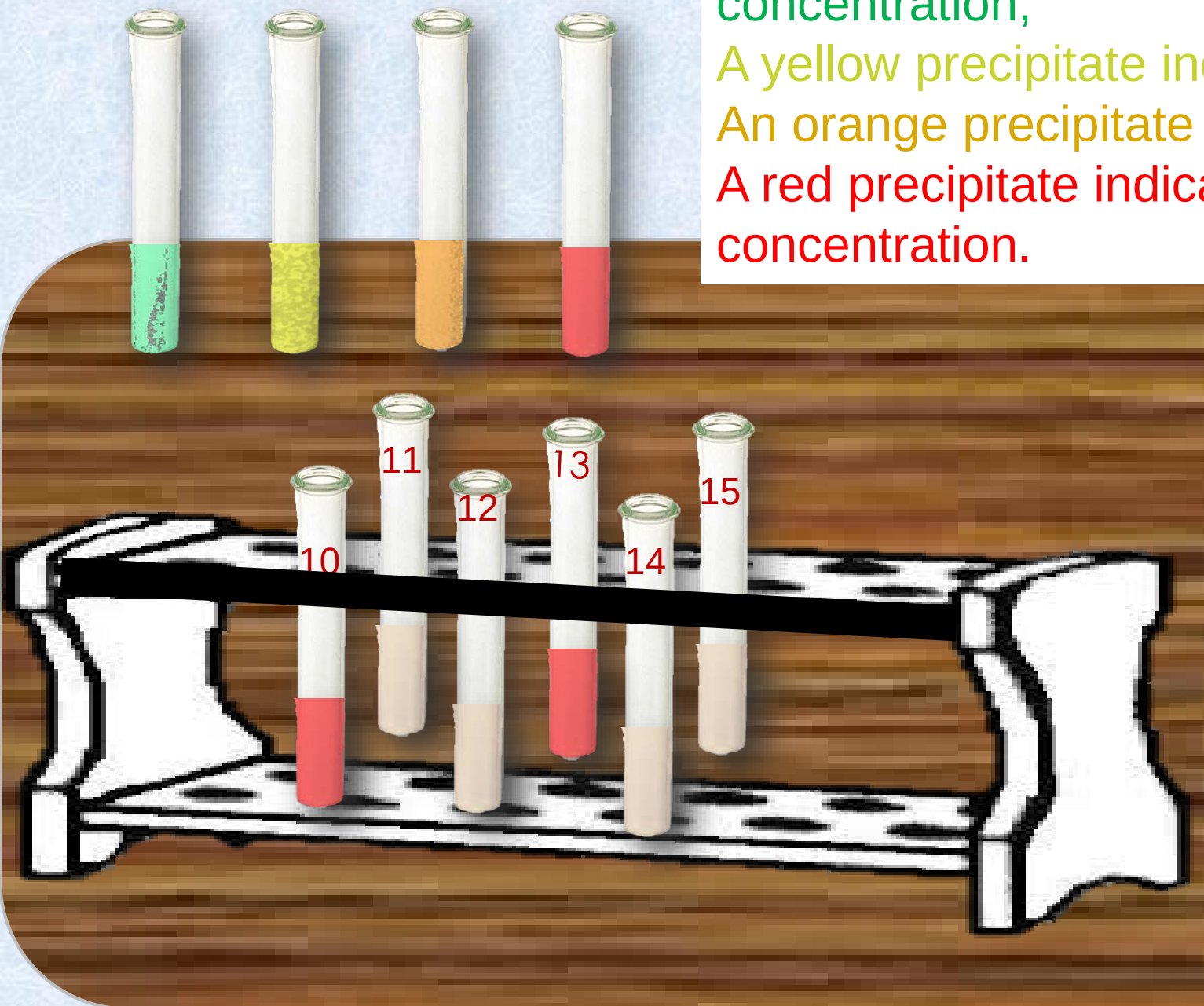
The Benedict's test indicates the presence of ‘reducing sugars’ when those sugars are heated in the presence of an alkali solution. These ‘reducing sugars’ get converted to reducing enediols. The enediols reduce the cupric compounds (Cu^{2+}) present in the Benedict's reagent to cuprous compounds (Cu^{+}) which forms a precipitate of copper(I) oxide (Cu_2O) which appears RED or Orange or Yellow.





Incubate the tubes at 100 degrees C on the hot plate for **15 minutes**.

A greenish precipitate indicates a 0.5 g% concentration;
A yellow precipitate indicates a 1 g% concentration;
An orange precipitate indicates a 1.5 g%
A red precipitate indicates a 2 g% or higher concentration.



The color of the obtained precipitate gives an idea about the quantity of sugar present in the solution, hence the test is semi-quantitative.