



# Advanced Homebrew Lab Skills

May 13, 2022

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Zymology Labs, LLC 2022



# Agenda

- How do we define quality?
- Record your way to better beer
  - document control and equipment
- Count your way to better yeast health
- Wash and grow your way to cleaner yeast
- Collect and grow your way to wild beer
- Identify spoilage concerns
- Taste your way to better quality







The UNIVERSITY of VERMONT



BREWING SCHOOL



Zymology Labs LLC Fermented Beverage Analysis

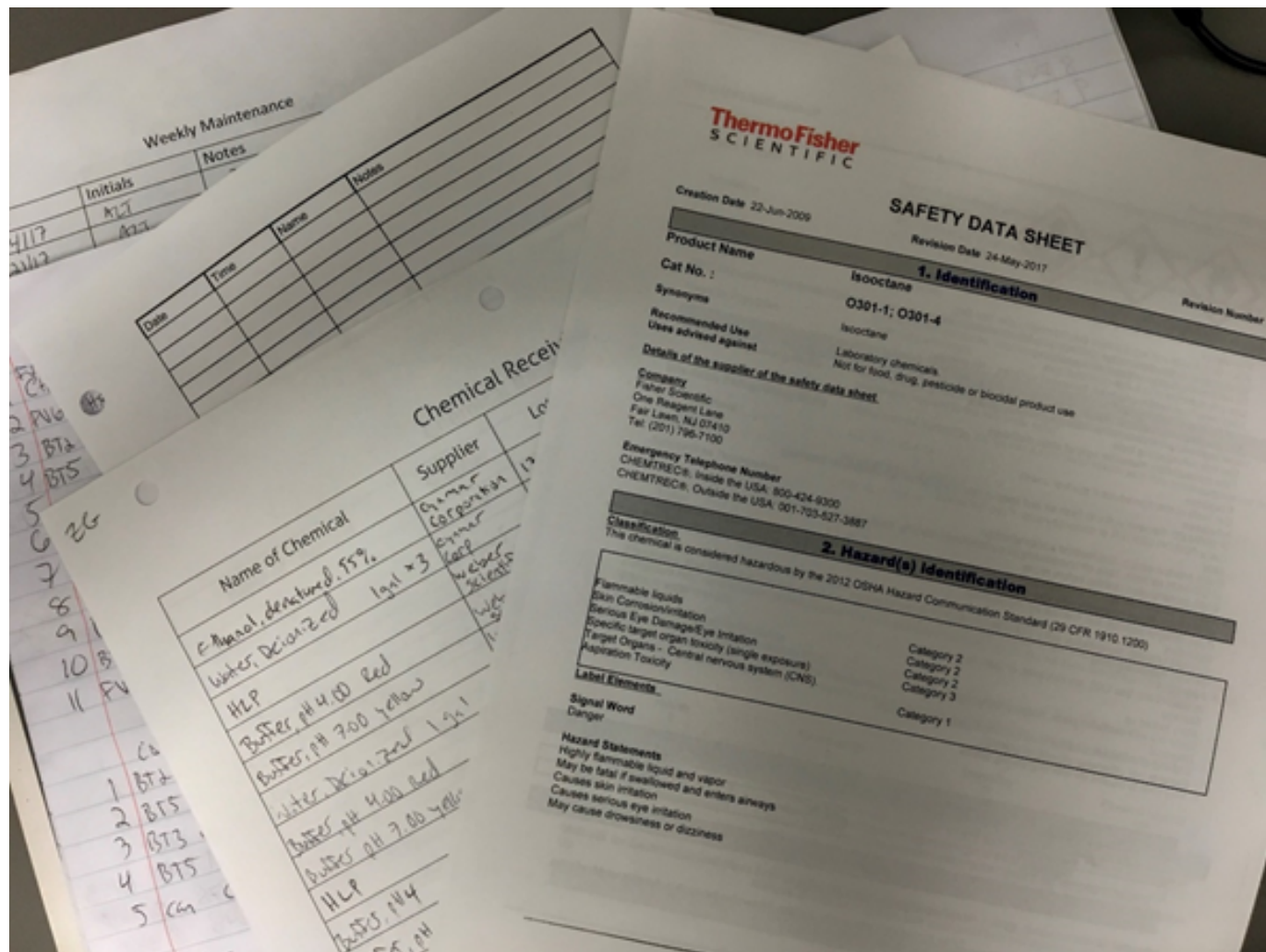








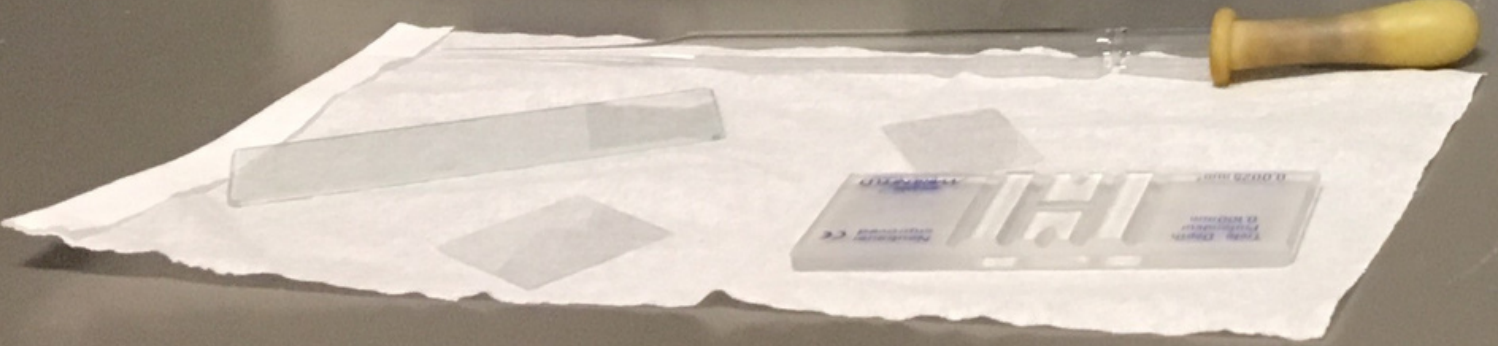
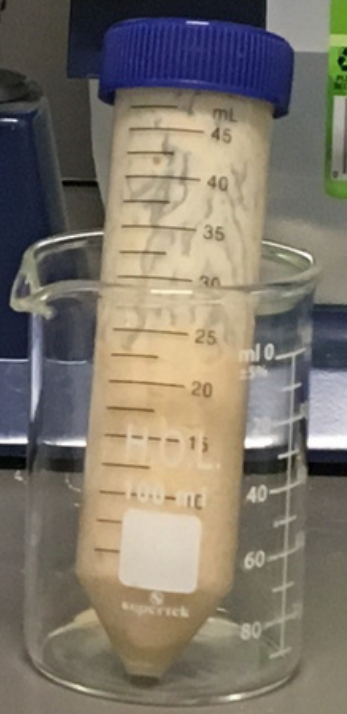
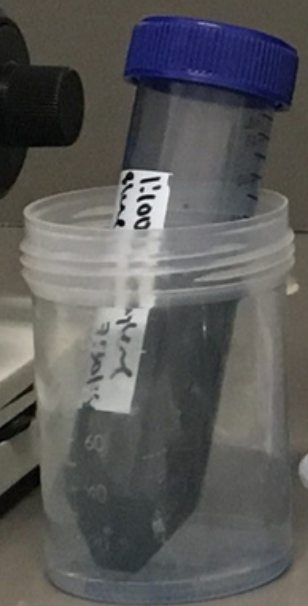
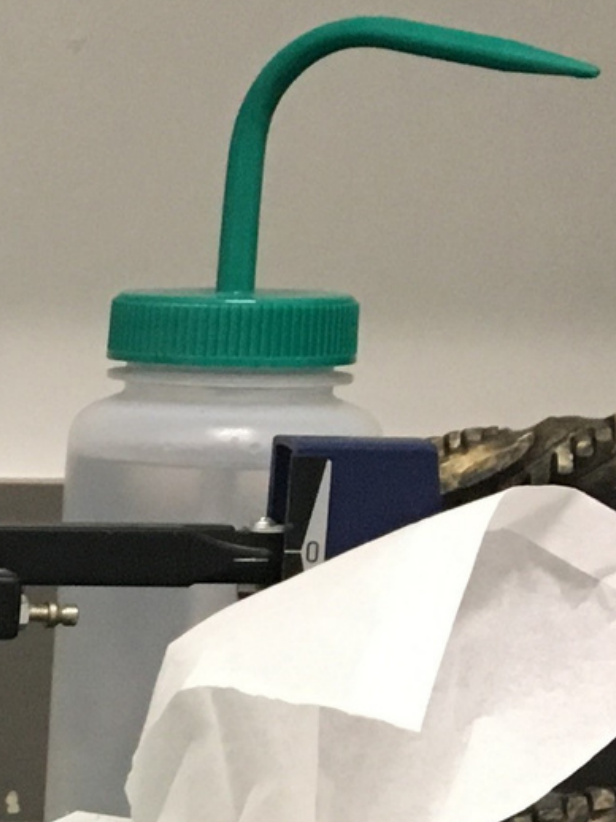
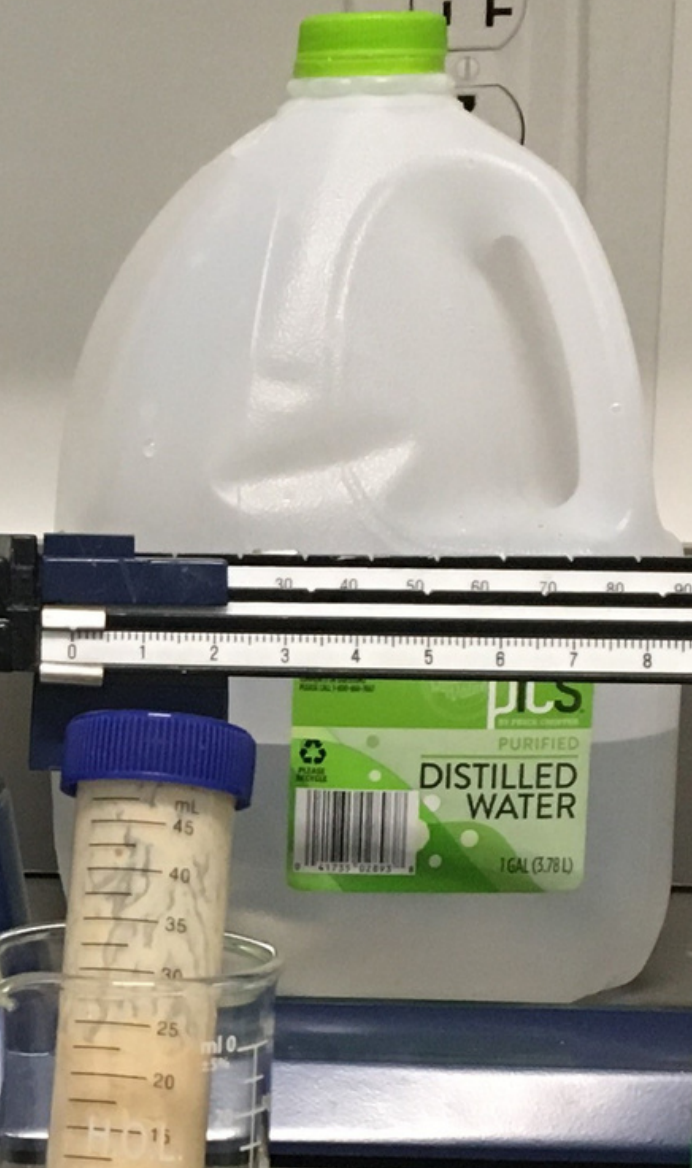
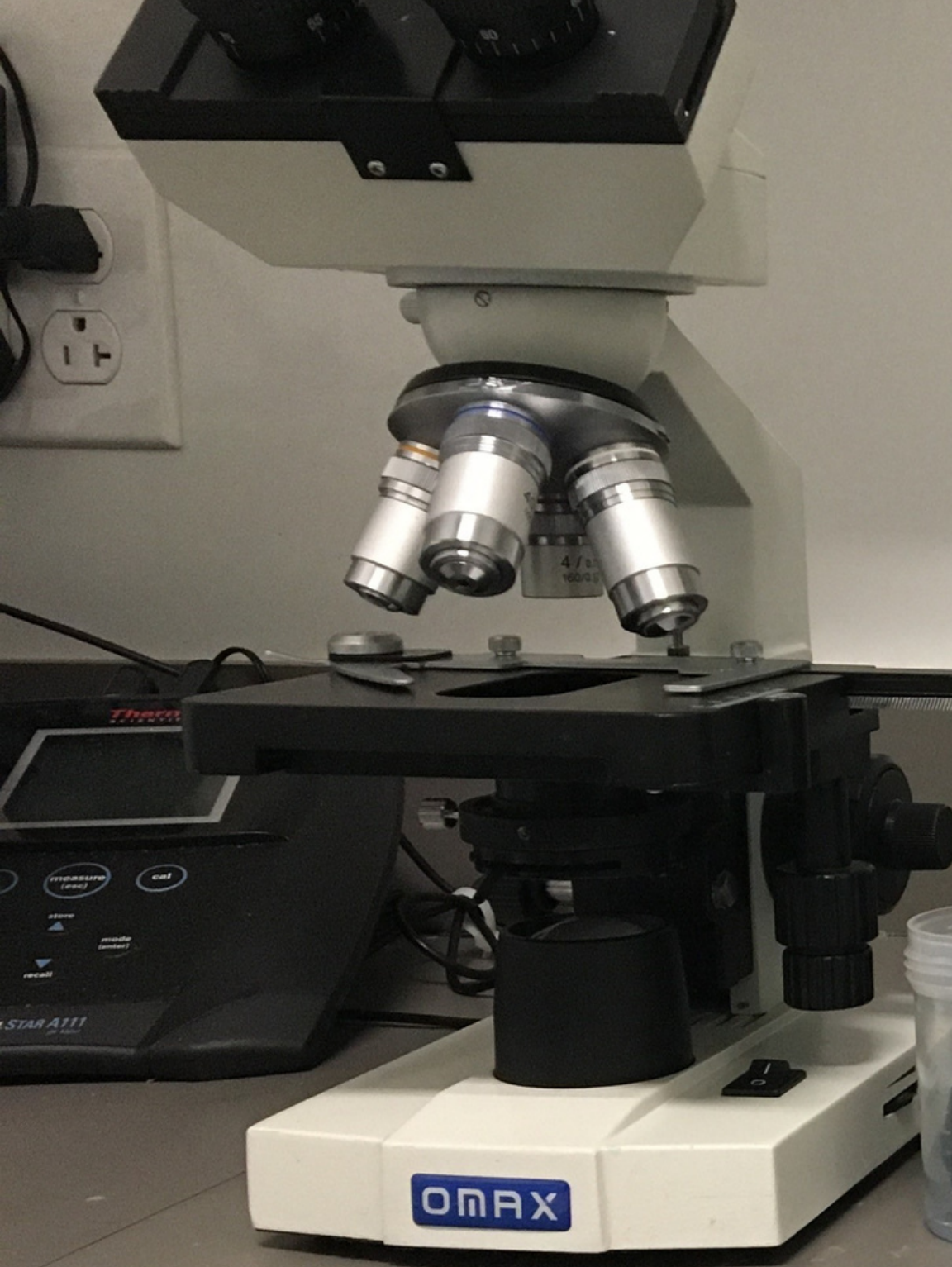
# document control and equipment



- brew day logs
- ingredients
- equipment
  - pH meter
  - hydrometer
  - thermometer
- fermentation notes
- sensory notes
- recipe development
- calculations



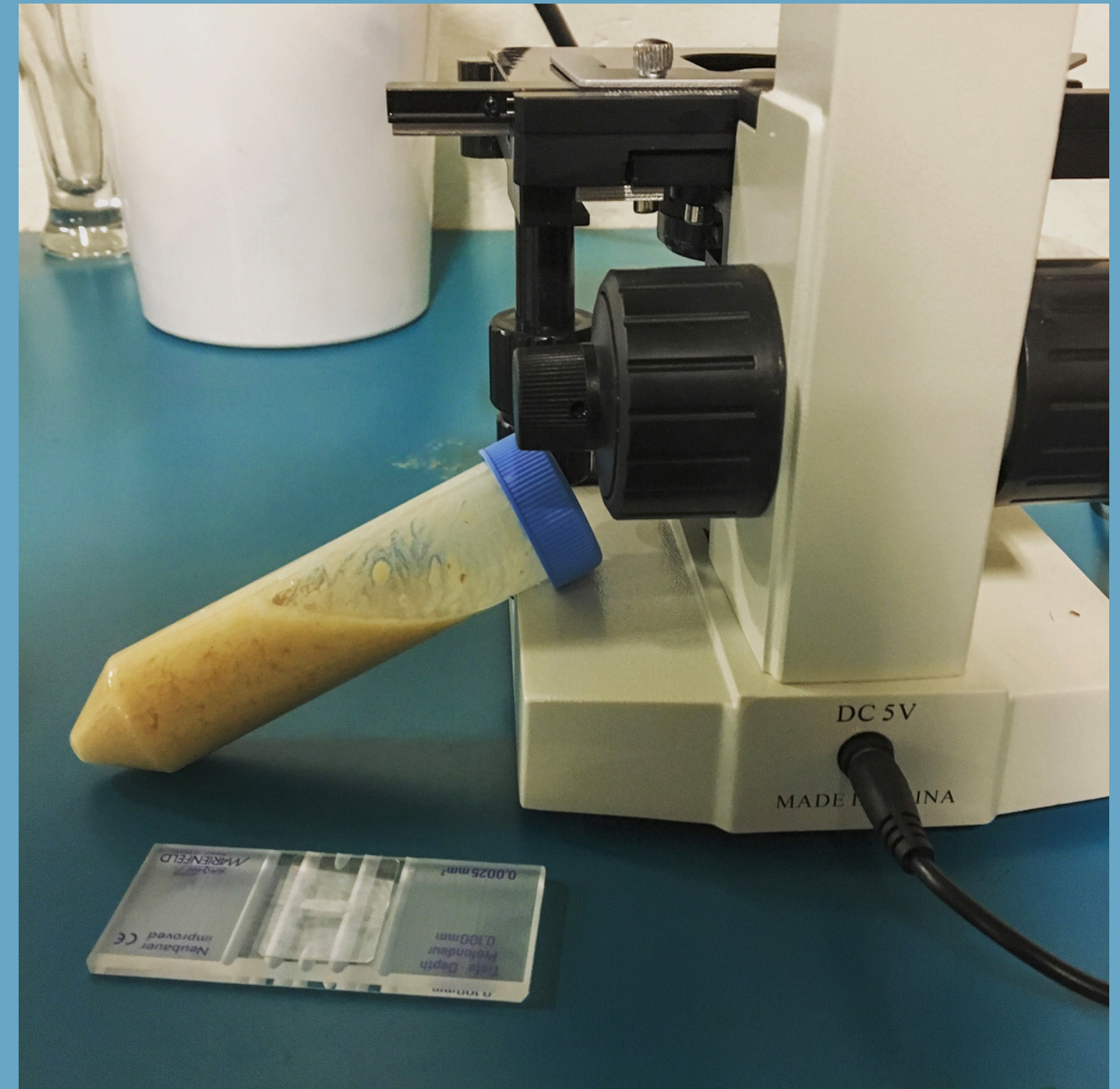




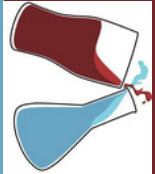
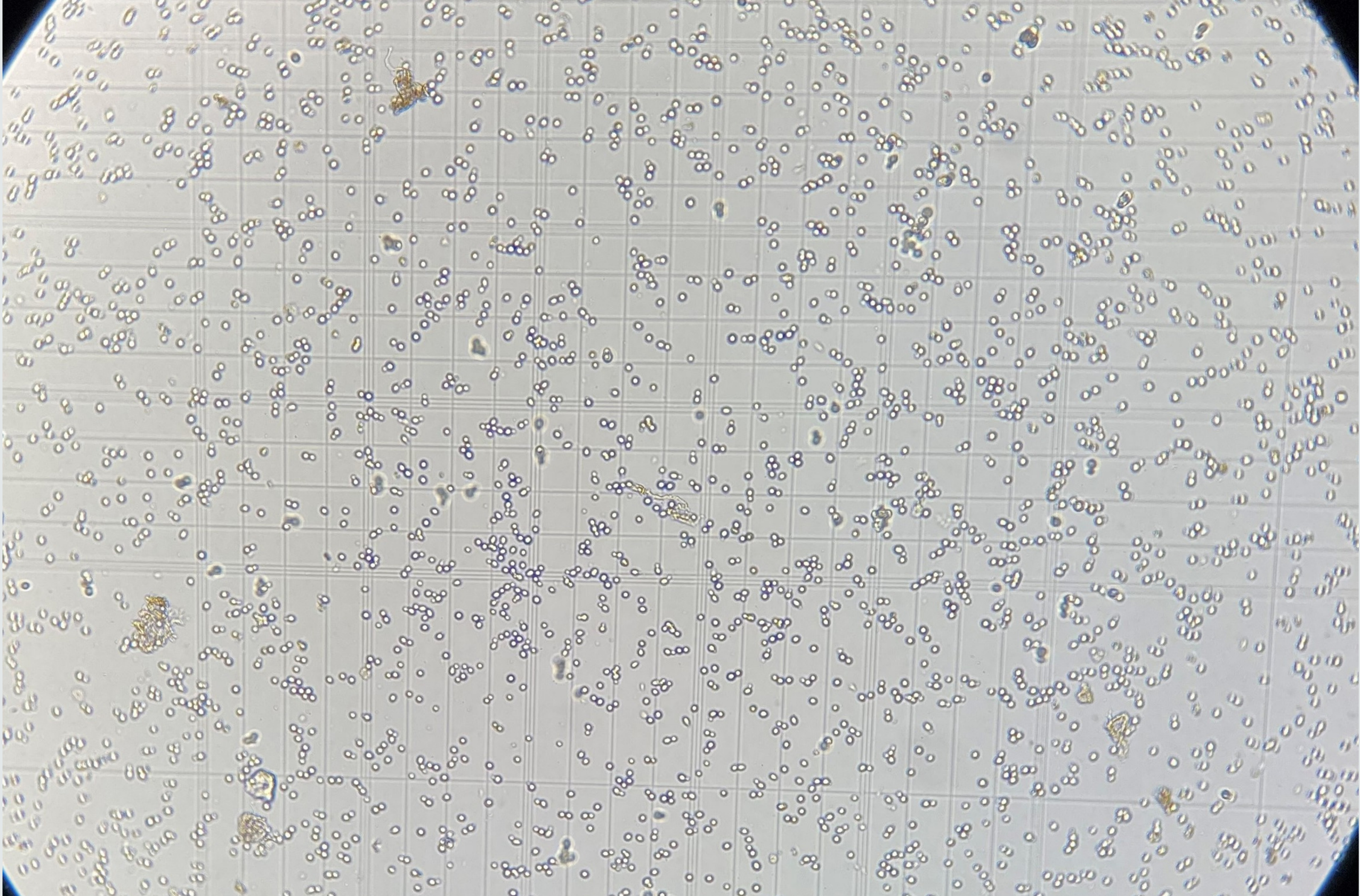


# Procedure

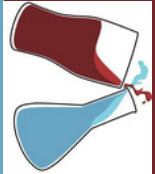
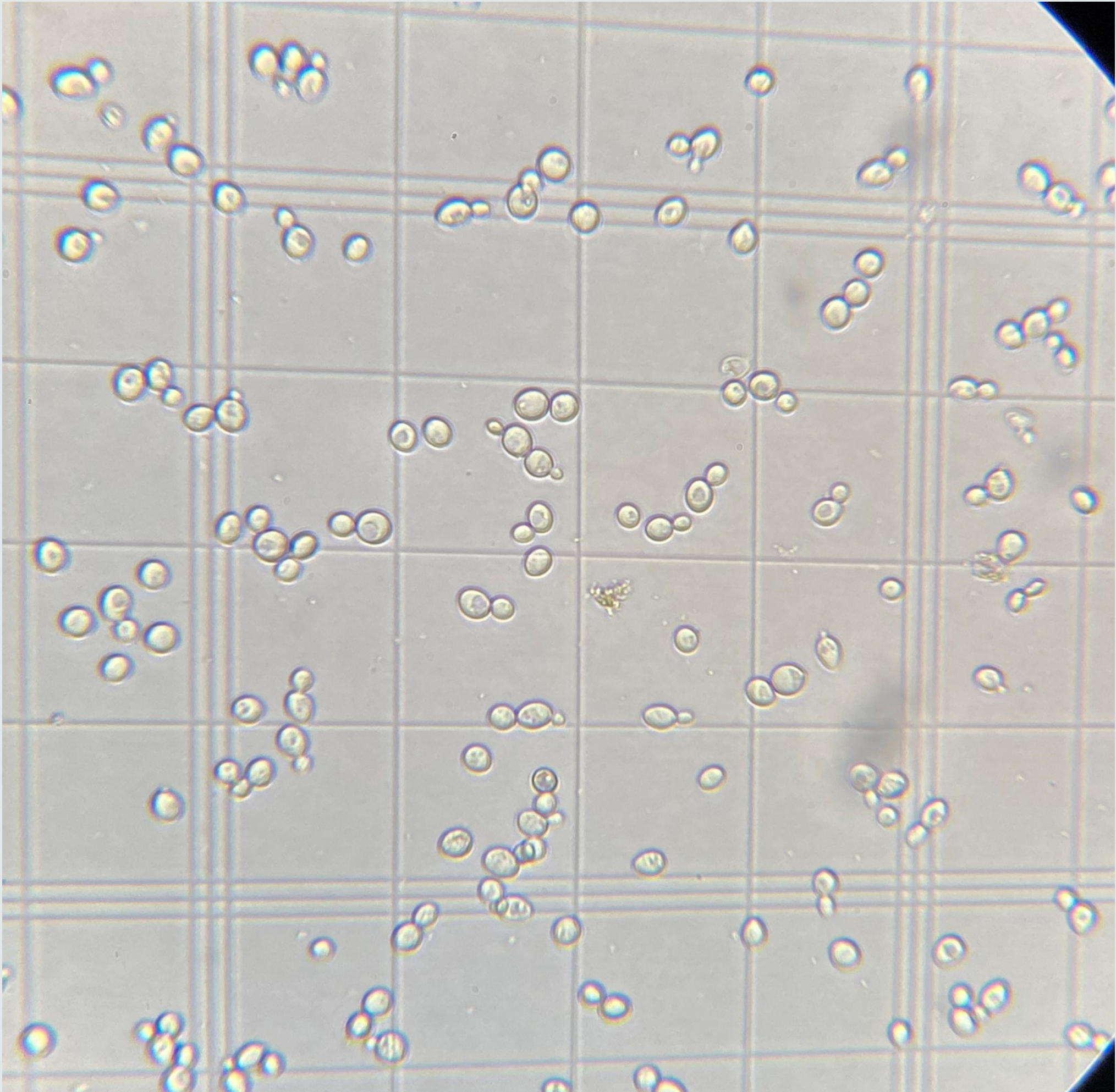
- collect homogenous yeast sample
- 1:100 dilution
- load hemocytometer
- count cells
- optional methylene blue stain
- calculate
- pitch
- repeat













# Calculations

Number of cells needed – Pitch rate

Starting point - 1 million cells per mL per degree Plato for ales  
1.5 to 2 million cells per degree Plato for lager

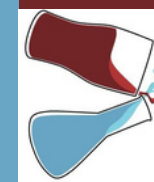
$$\text{Degree Plato wort} \times \frac{1 \times 10^6 \text{ viable cells/mL wort}}{1^\circ \text{ Plato}} = \text{viable cells/mL wort}$$

Example: For a 12°P beer you would need 12,000,000 viable cells/mL wort

$$12 \times \frac{1 \times 10^6 \text{ viable cells/mL wort}}{1^\circ \text{ Plato}} = 12,000,000 \text{ viable cells/mL wort}$$

$$\text{plato} = (-1 * 616.868) + (1111.14 * \text{sg}) - (630.272 * \text{sg}^2) + (135.997 * \text{sg}^3)$$

$$\text{SG} = 1 + (\text{plato} / (258.6 - ((\text{plato}/258.2) * 227.1)))$$





# Calculations

Total cells needed:

$$\text{gallons wort} \times \frac{3.785\text{L wort}}{1 \text{ gal wort}} \times \frac{1000\text{mL wort}}{1\text{L wort}} \times \frac{\text{viable cells}}{\text{mL wort}} = \text{total cells needed}$$

Example: If you are brewing 5 gal you need  $2.27 \times 10^{11}$  cells

$$5\text{gal wort} \times \frac{3.785\text{L wort}}{1\text{gal wort}} \times \frac{1000\text{mL wort}}{1\text{L wort}} \times \frac{1.2 \times 10^7}{\text{mL wort}} = 2.27 \times 10^{11} \text{ cells}$$

Yeast cells/mL or g slurry:

$$\frac{(\text{cell count})(5)(\text{dilution})}{\text{Chamber volume (0.0001mL)}} = \text{yeast cells/mL or g slurry}$$





# Calculations

Example: if you count 350 viable cells in 5 squares on the hemocytometer

$$\frac{(350\text{cells})(5)(100)}{(0.0001\text{mL})} = 1.75 \times 10^9 \text{ yeast cells/mL or g slurry}$$

Amount of yeast needed:

$$\frac{\text{Total cells needed}}{\text{Yeast cells/mL slurry}} = \text{volume of yeast slurry required (mL)}$$

Example:  $\frac{2.27 \times 10^{11} \text{ cells}}{1.75 \times 10^9 \text{ yeast cells/mL slurry}} = 129 \text{ mL}$

$$\frac{\text{Total cells needed}}{\text{Yeast cells/g slurry}} = \text{volume of yeast slurry required (g)}$$





# Calculations

Example: if you count 303 viable cells in 5 squares on the hemocytometer

$$\frac{(303\text{cells})(5)(10)}{(0.0001\text{mL})} = 1.515 \times 10^8 \text{ yeast cells/mL or g slurry}$$

Amount of yeast needed:

$$\frac{\text{Total cells needed}}{\text{Yeast cells/mL slurry}} = \text{volume of yeast slurry required (mL)}$$

Example:  $\frac{2.27 \times 10^{11} \text{ cells}}{1.515 \times 10^8 \text{ yeast cells/mL slurry}} = 1,498 \text{ mL}$

$$\frac{\text{Total cells needed}}{\text{Yeast cells/g slurry}} = \text{volume of yeast slurry required (g)}$$

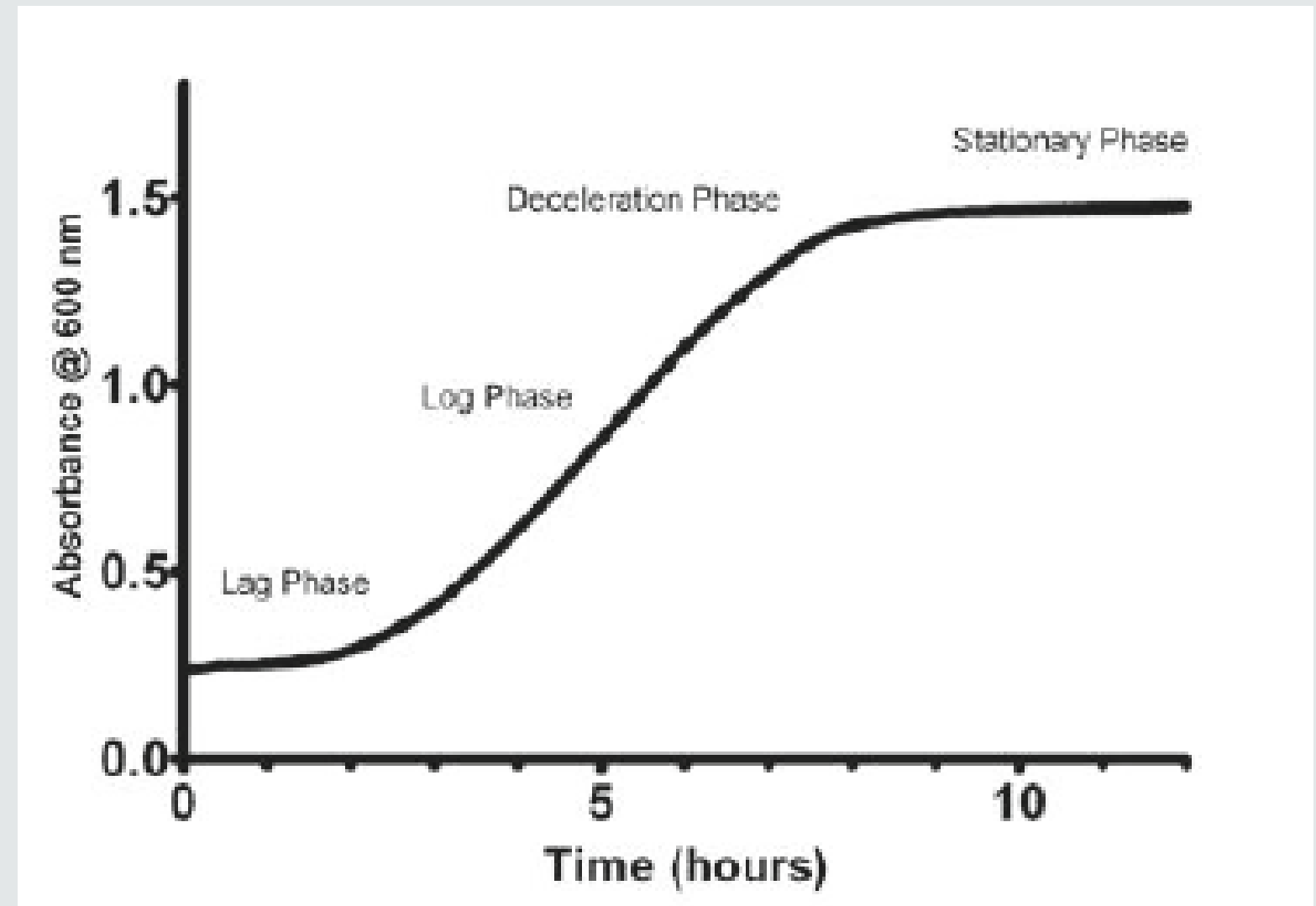




# Yeast Starter

- 1-2 L Erlenmeyer flask
- DME or 1.020-1.040 strength wort
- water
- stir plate/stir bar (optional)
- tinfoil or airlock
- yeast
- time

<https://byo.com/resource/build-a-yeast-starter/>



Alsuhaime, H. & Vojisavljevic, Vuk & Pirogova, Elena. (2013). Effects of Non-thermal Microwave Exposures on the Proliferation Rate of *Saccharomyces Cerevisiae* Yeast. 10.1007/978-3-642-29305-4\_14.

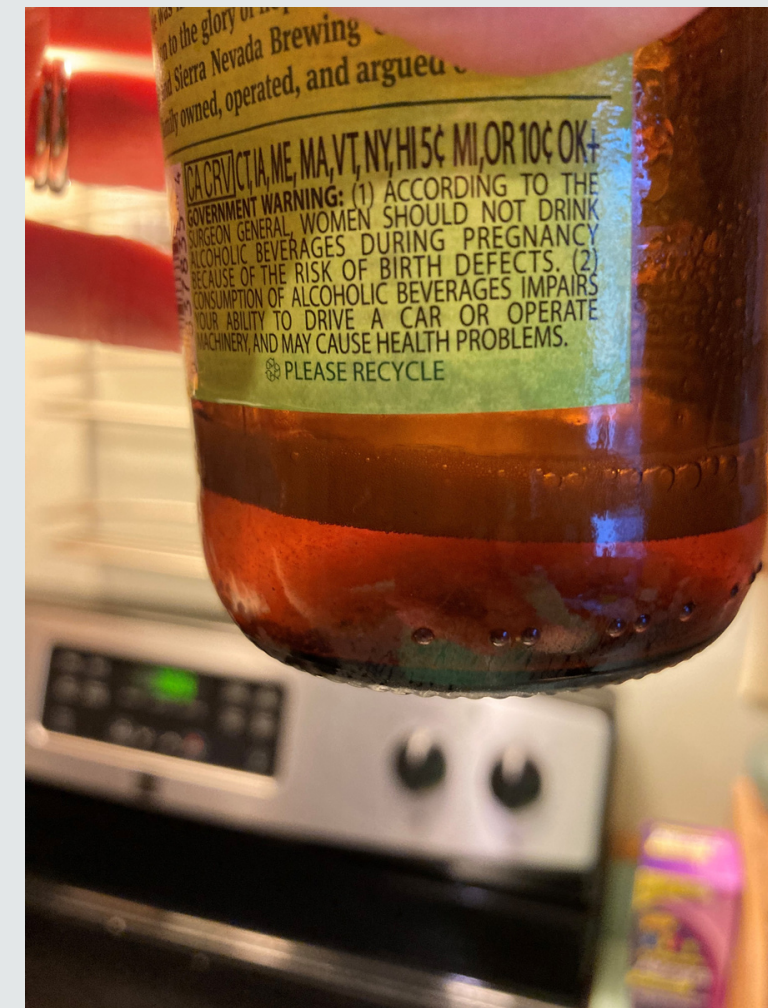
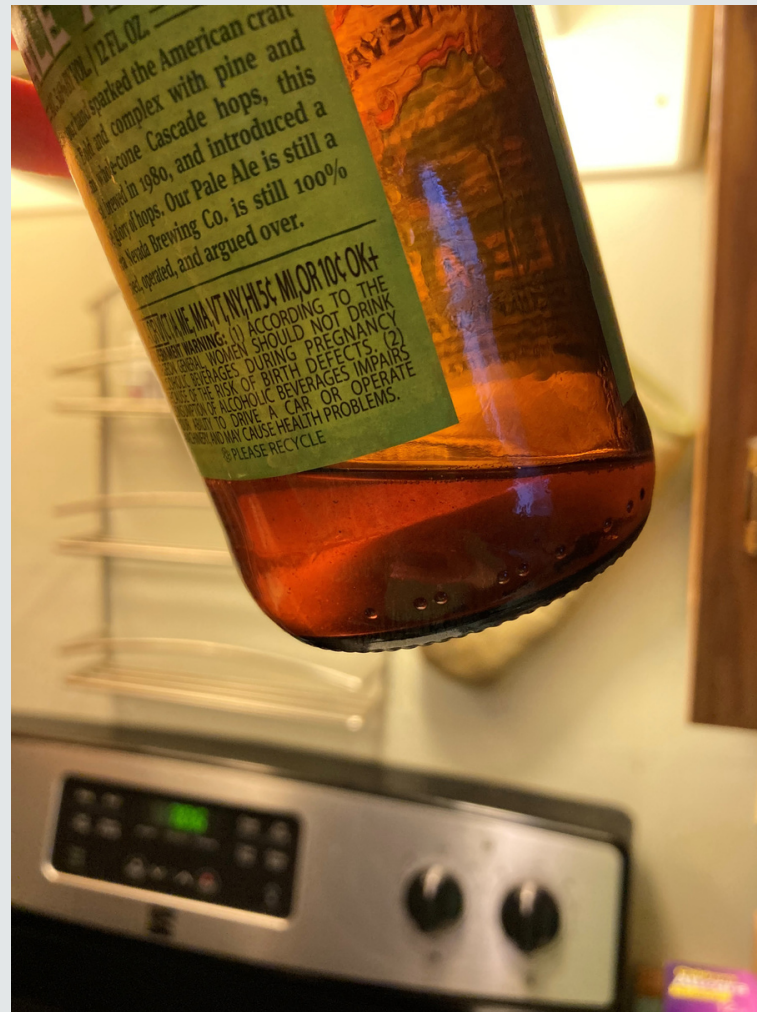
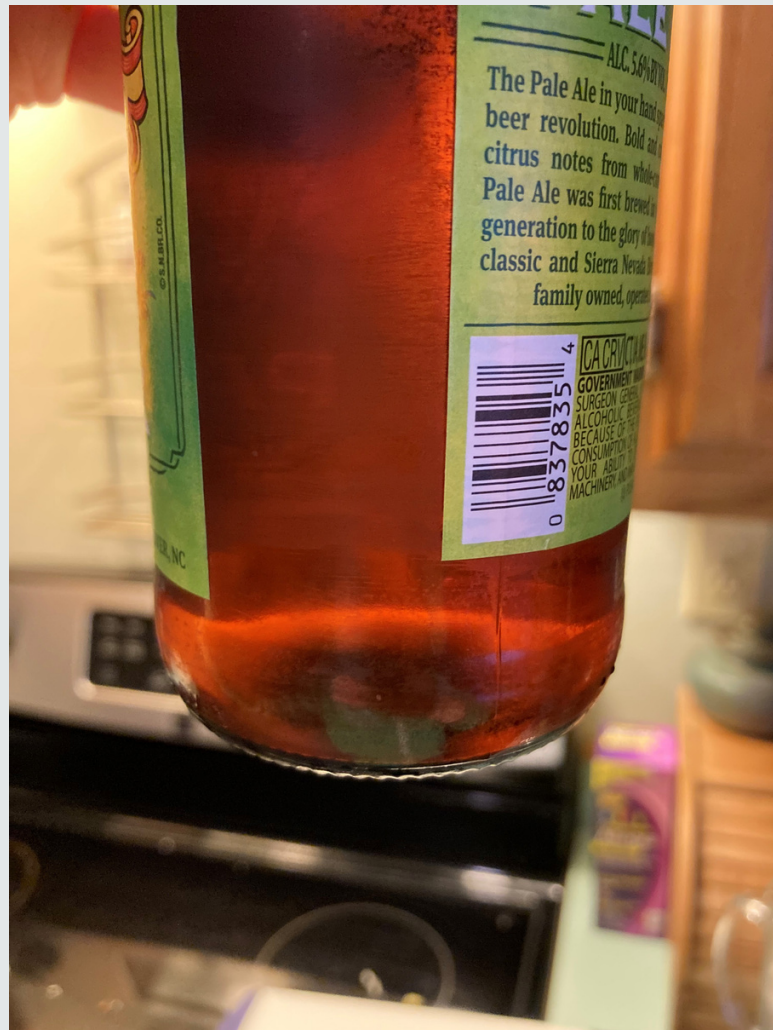




# Yeast starter from bottle dregs

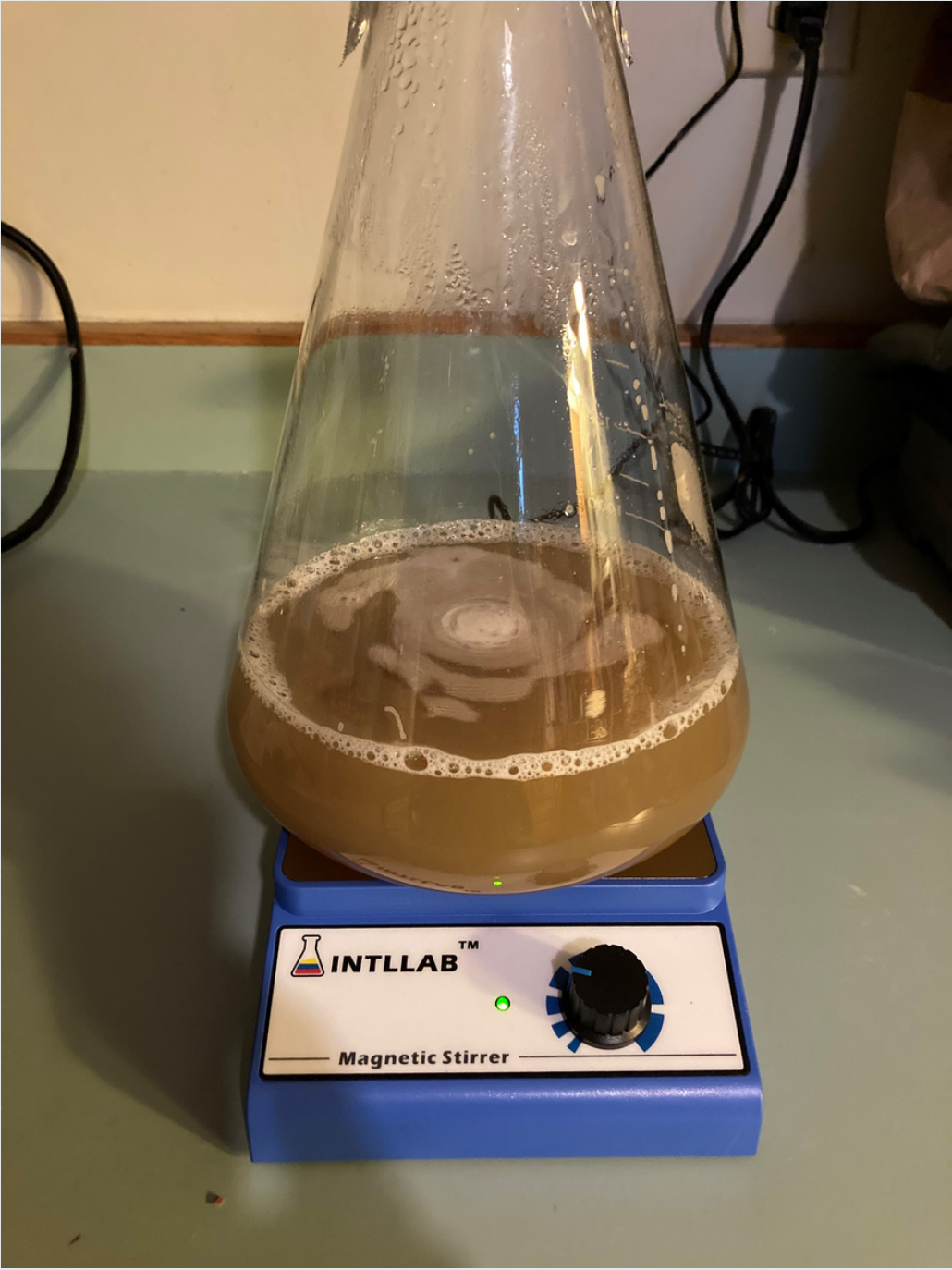
What you need

- yeast starter items
- unfiltered and unpasteurized beer

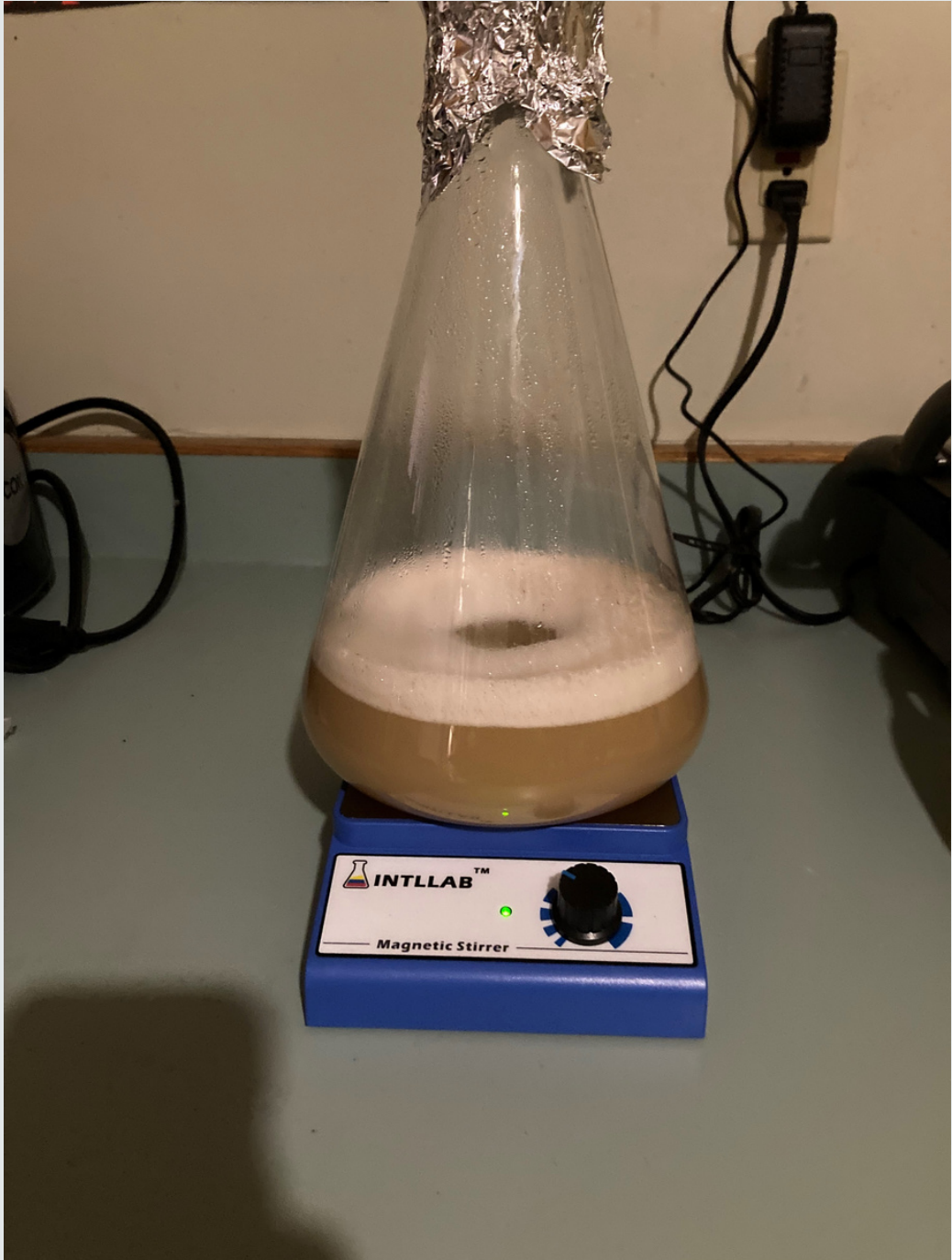




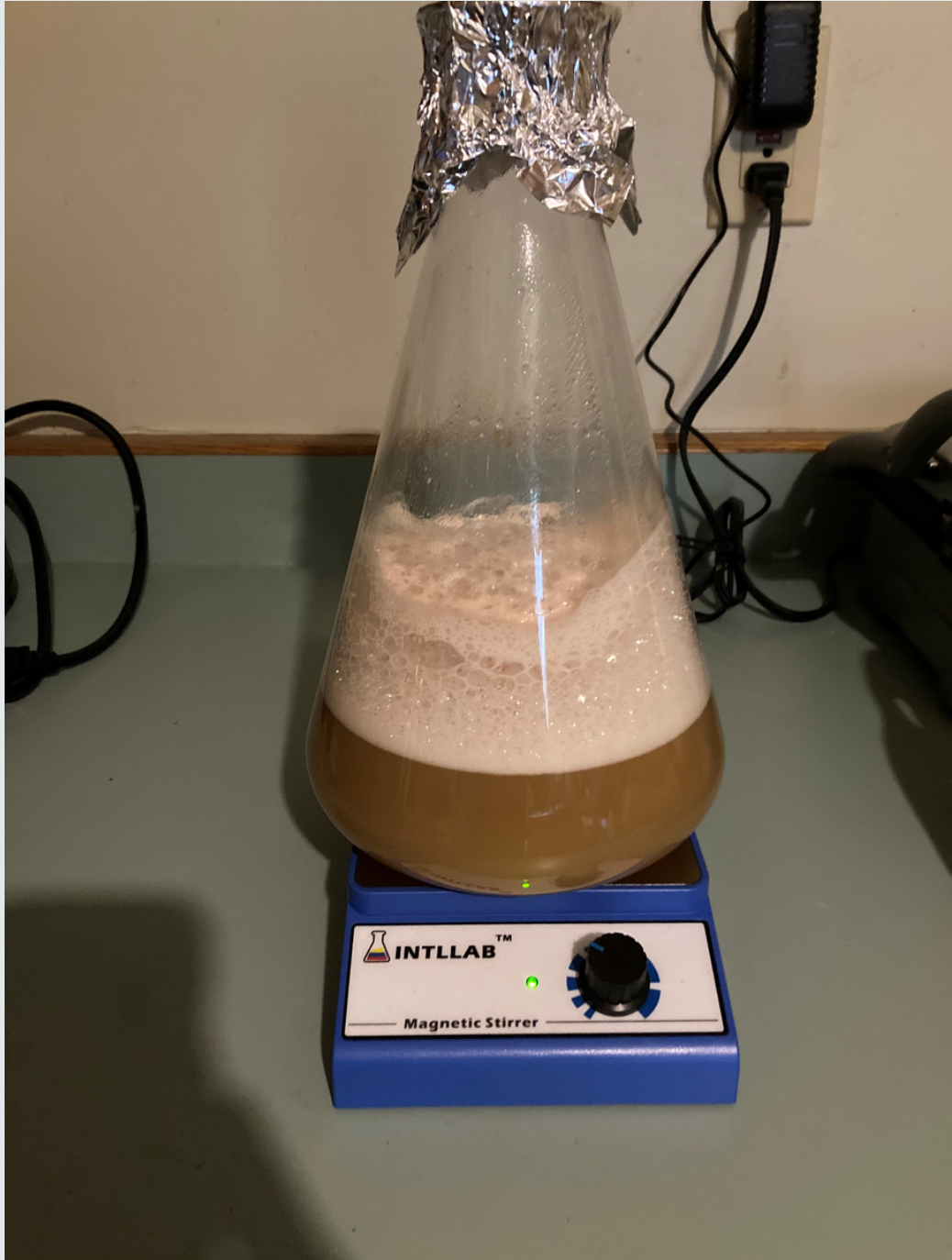
# Yeast starter from bottle dregs



Day 1



Day 2



Day 4





# Yeast Rinsing

- boil water/mason jars
- rack beer
- add cool, boiled water
- collect beer/trub/water
- let settle 30 min
- collect top yeast/water layer and leave trub behind

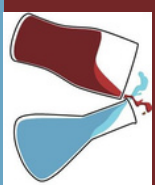
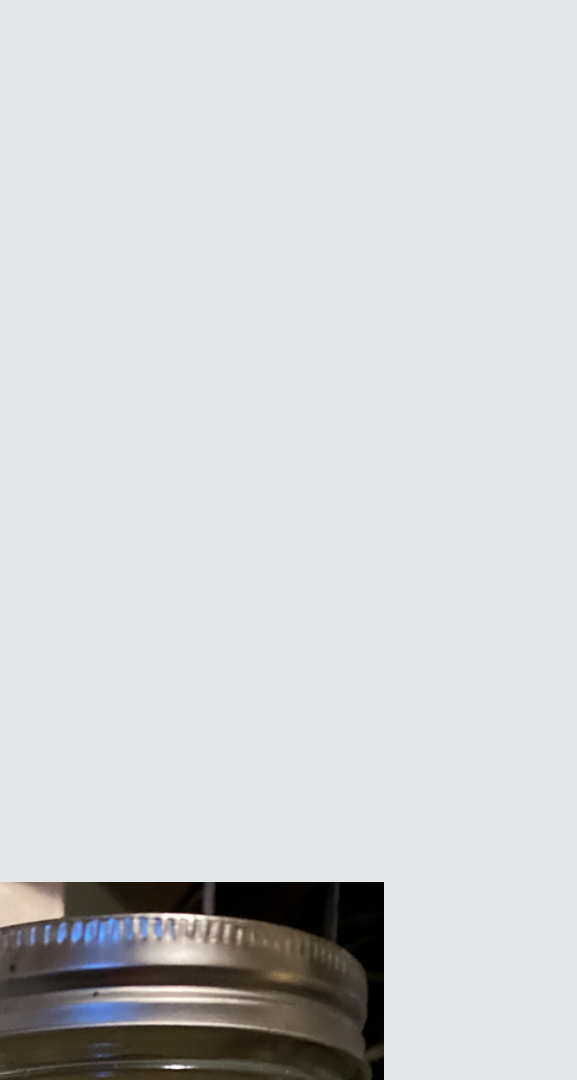
or

- collect off secondary
- date and generation

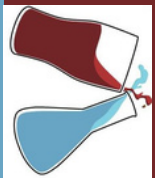
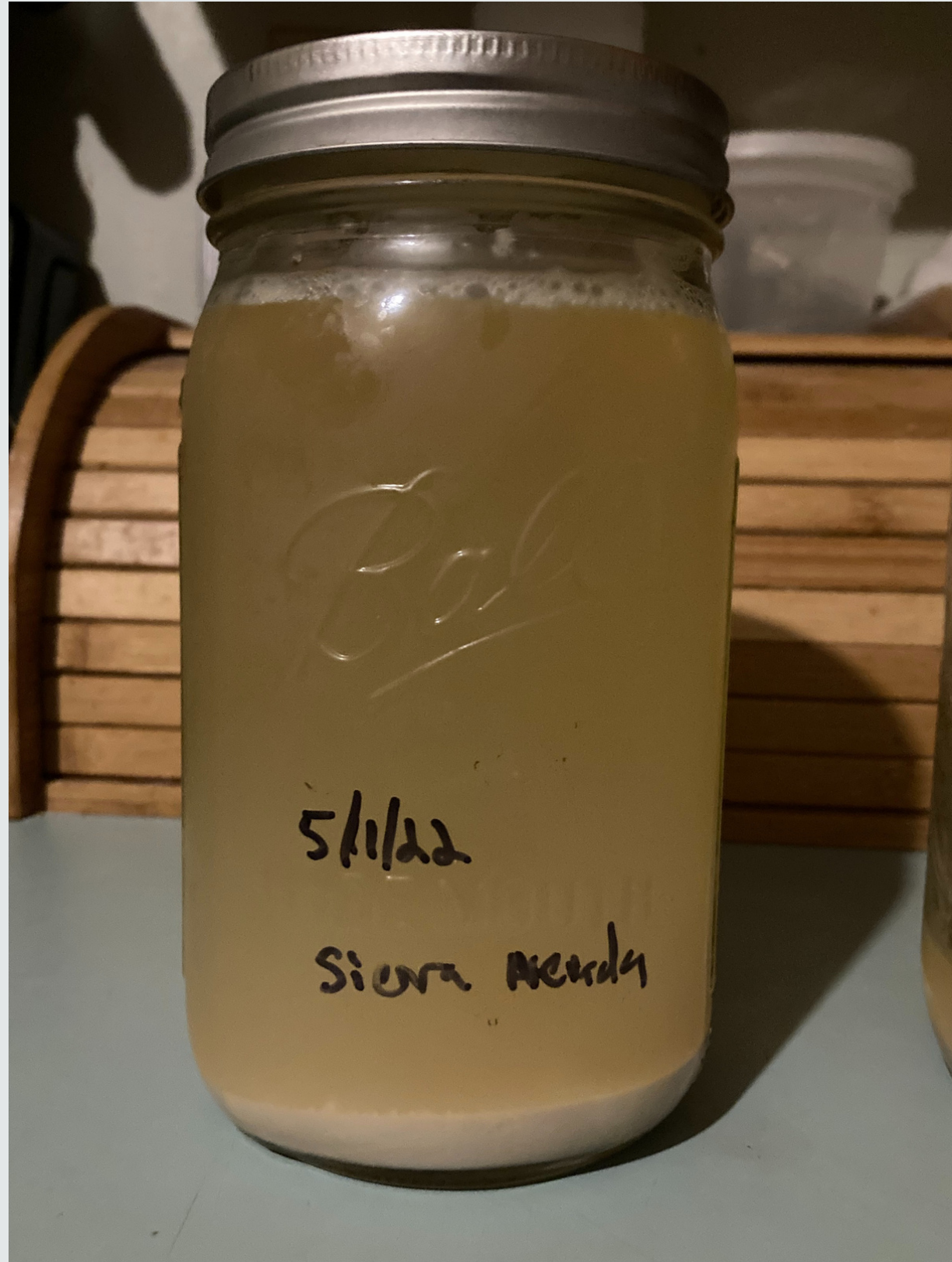
storage













# Yeast Washing

From American Homebrewers Association "Yeast Washing & Yeast Rinsing: What's the Difference?"

- Cool yeast to 36-40°F (2-4°C). This temperature must be maintained throughout the process.
- Determine how much yeast is needed to ferment your homebrew recipe, and place it in a suitable, sanitized container.
- Once you are two hours from pitching the yeast, start the the acid washing process.
- Thoroughly mix in food-grade phosphoric acid until the pH of the slurry is between 2.0-2.5 pH.
- Hold the yeast at this pH and temperature for 60-90 minutes, while stirring continuously.
- Pitch entire mixture into the the fermenter with wort.





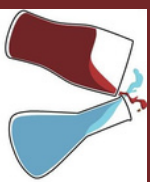
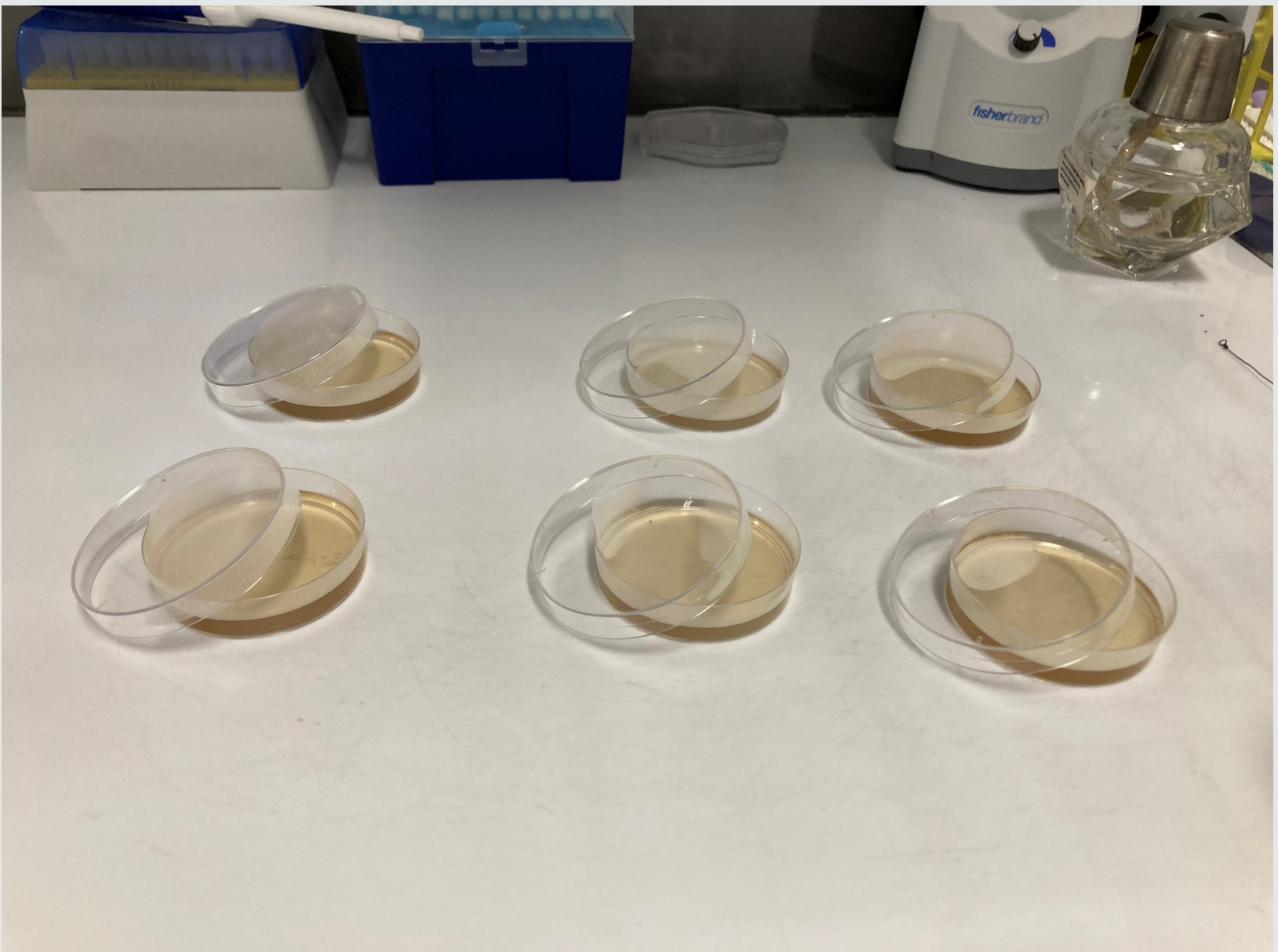
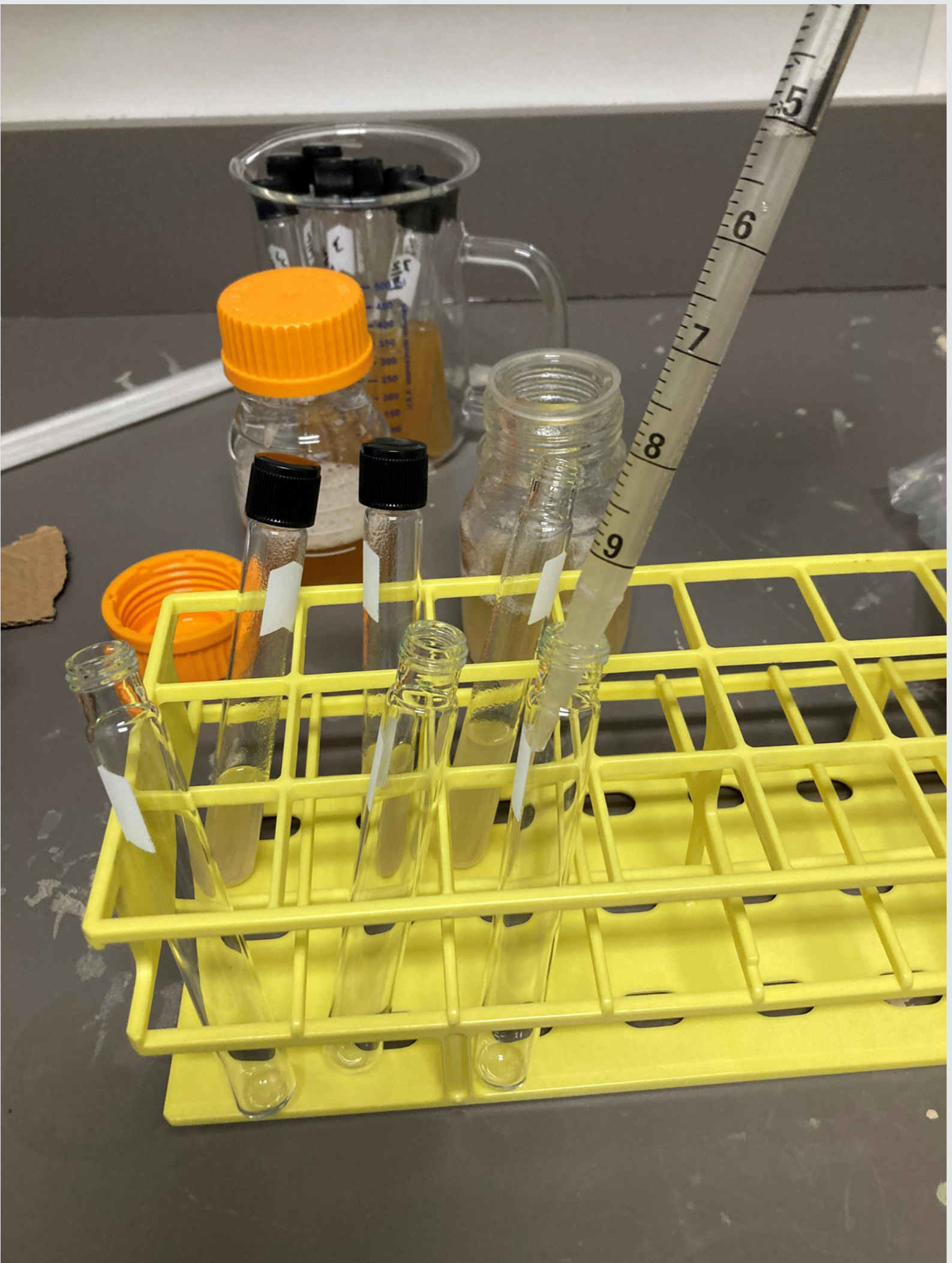
# Agar plates and slants

## What you need

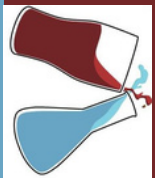
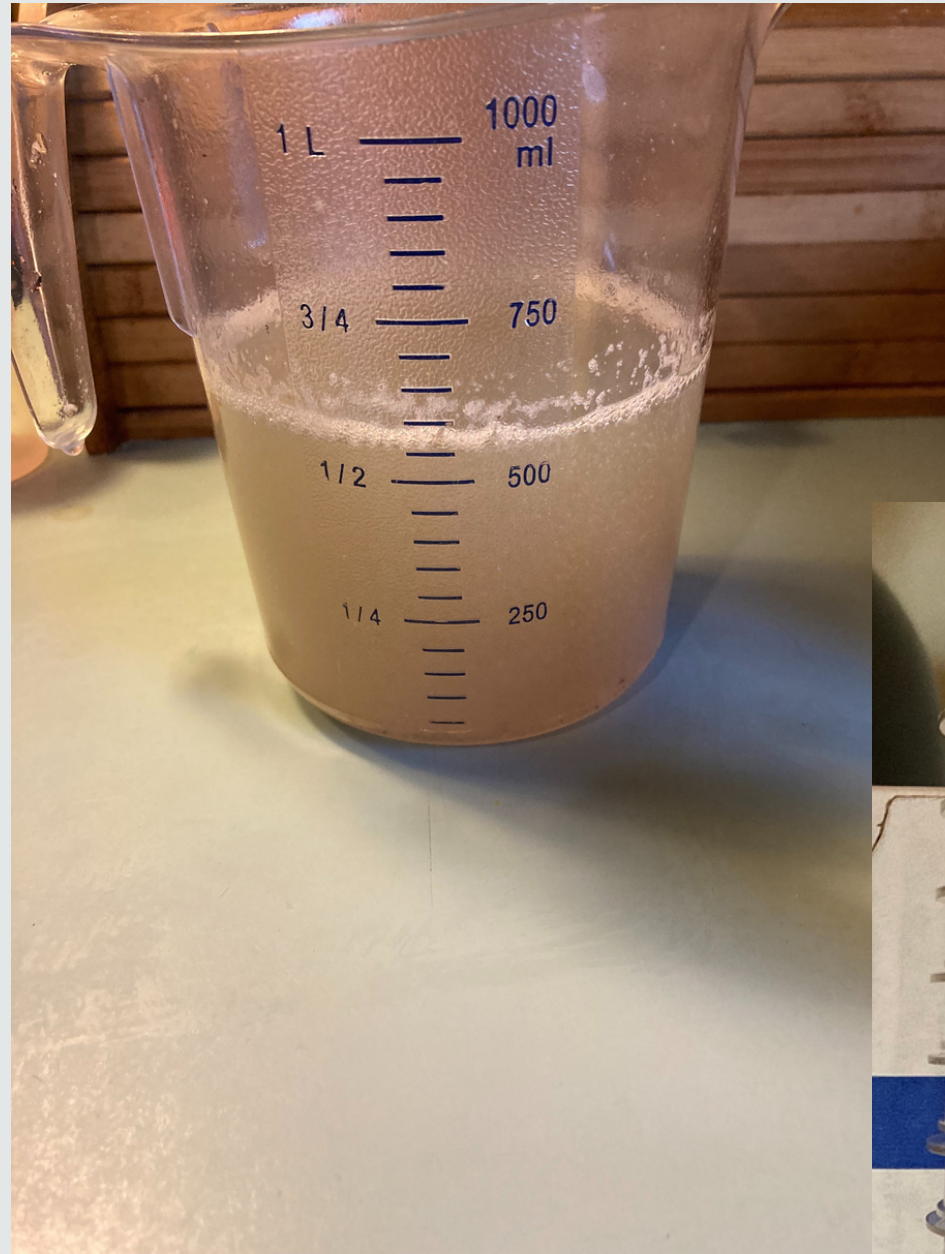
- 2-3g agar
- 2-3 grams DME or dilute wort (wort agar)
- 100mL water
- small flask
- 50 grams potato (potato dextrose)
- 1 cup water (potato dextrose)
- 2 grams sugar (potato dextrose)
- 2 gram agar (potato dextrose)
- sterile plates and test tubes
- alcohol lamp or bunsen burner
- autoclave (slants)





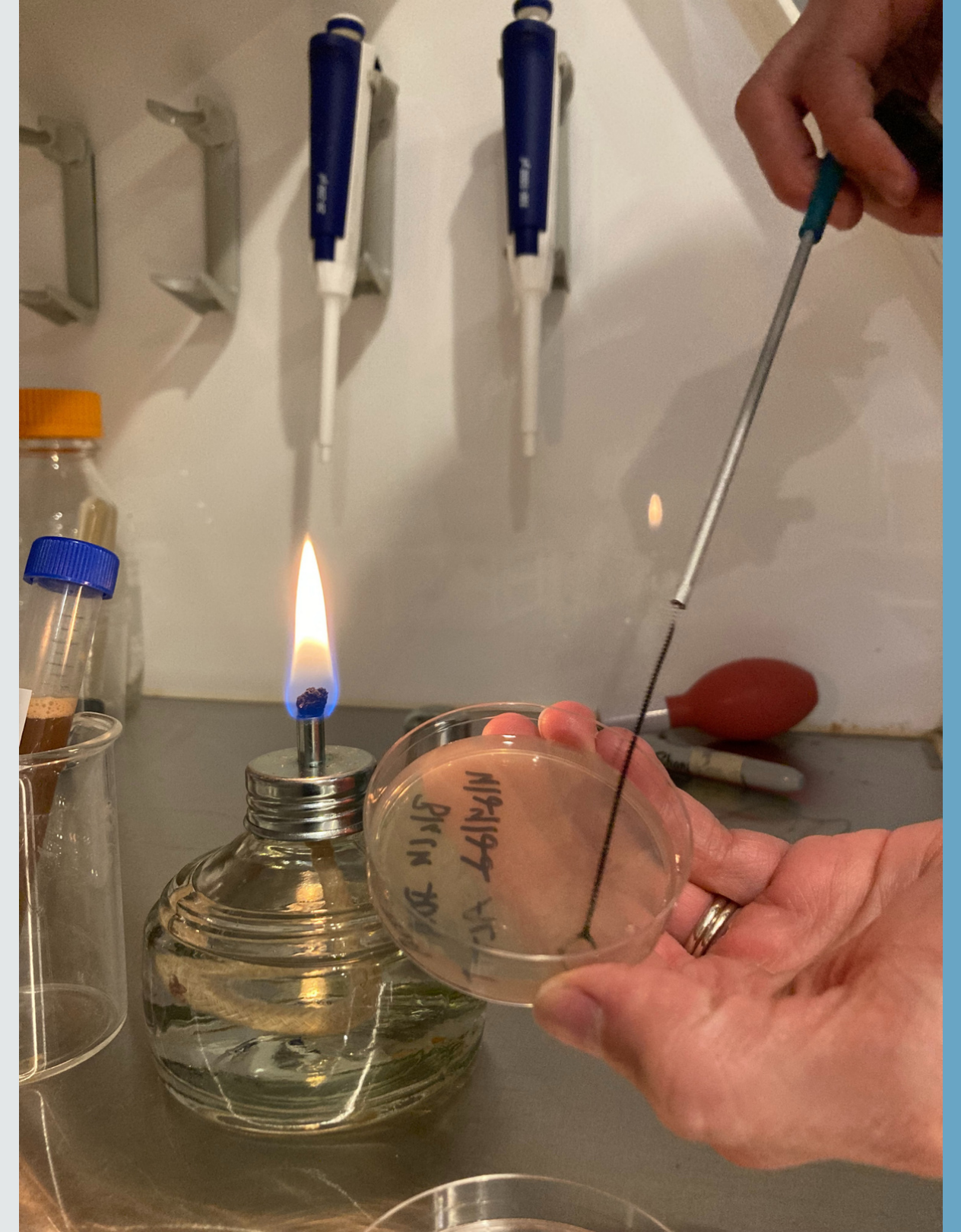
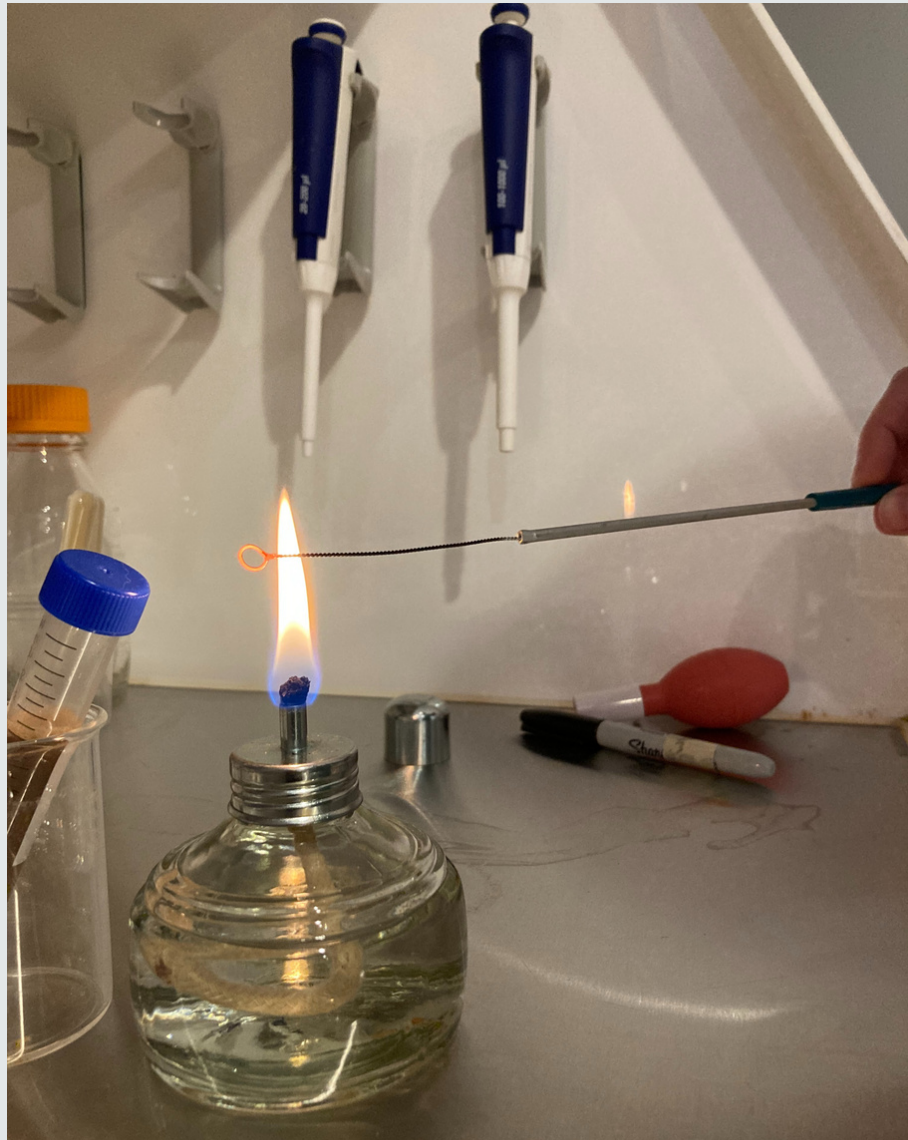






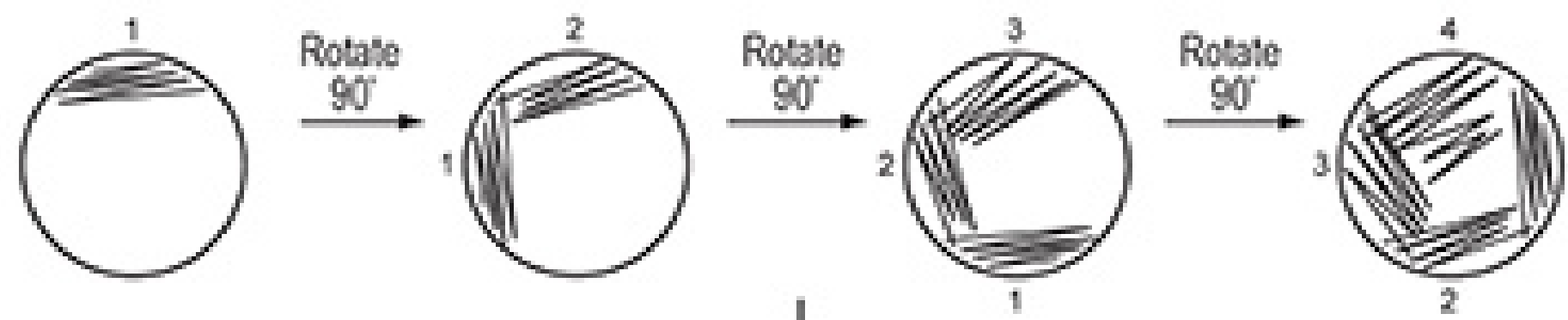


# Yeast starter from a slant or plate





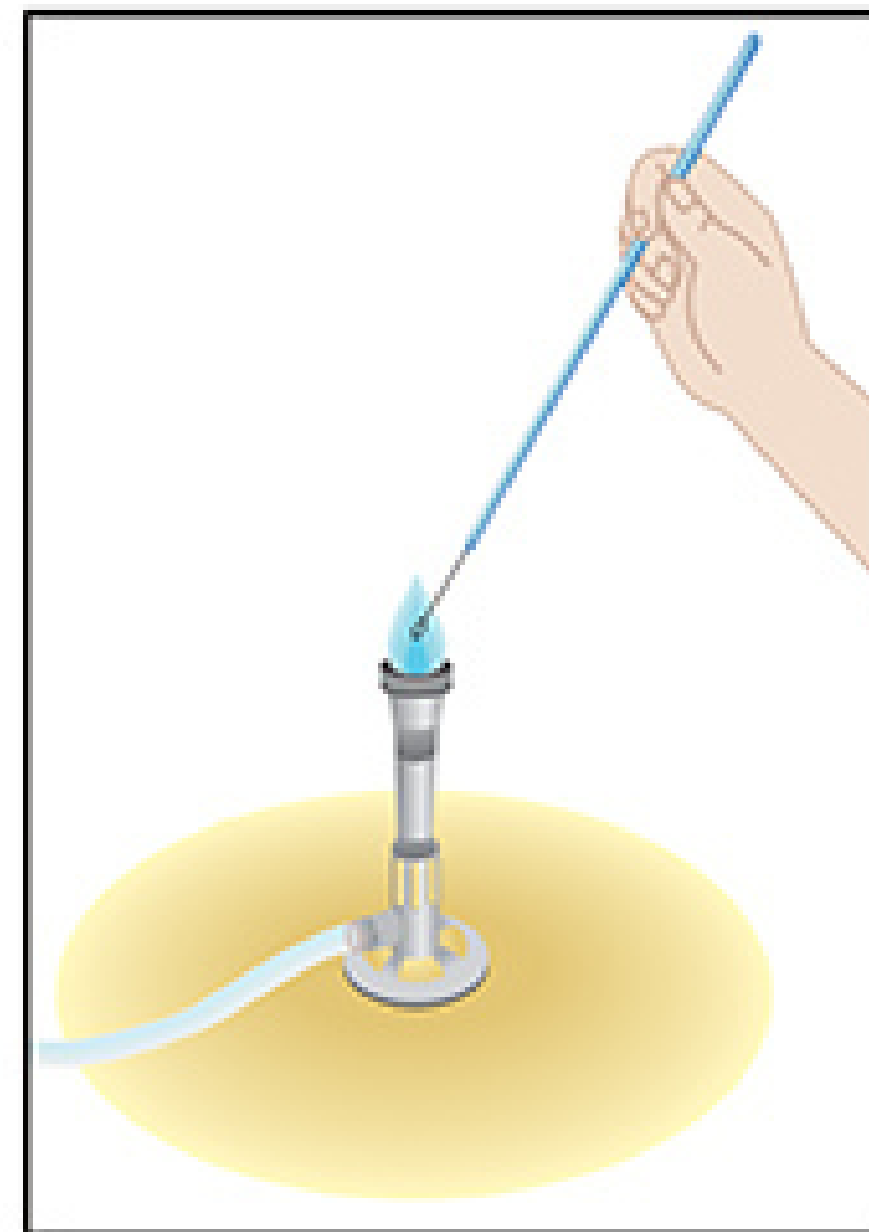
**A** Quadrant Method  
Streak Pattern:



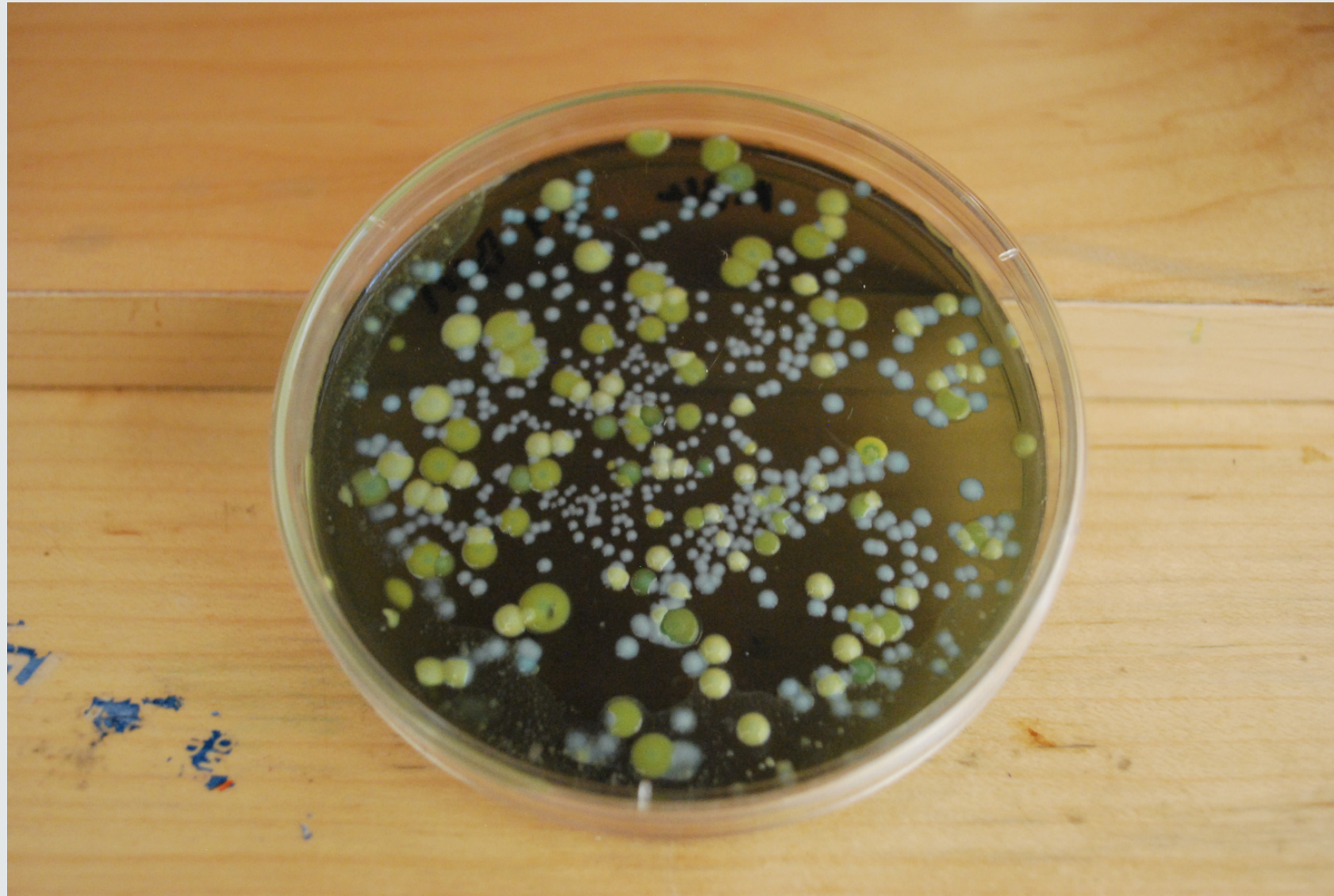
**Results:**



**B**







Malt with Bromocresol Green Agar. Mix of *Saccharomyces*, *Brett*, and *Pediococcus*. 3 colony morphologies.

<https://bkyeast.wordpress.com/2012/02/19/selective-media-part-i/>

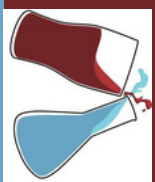
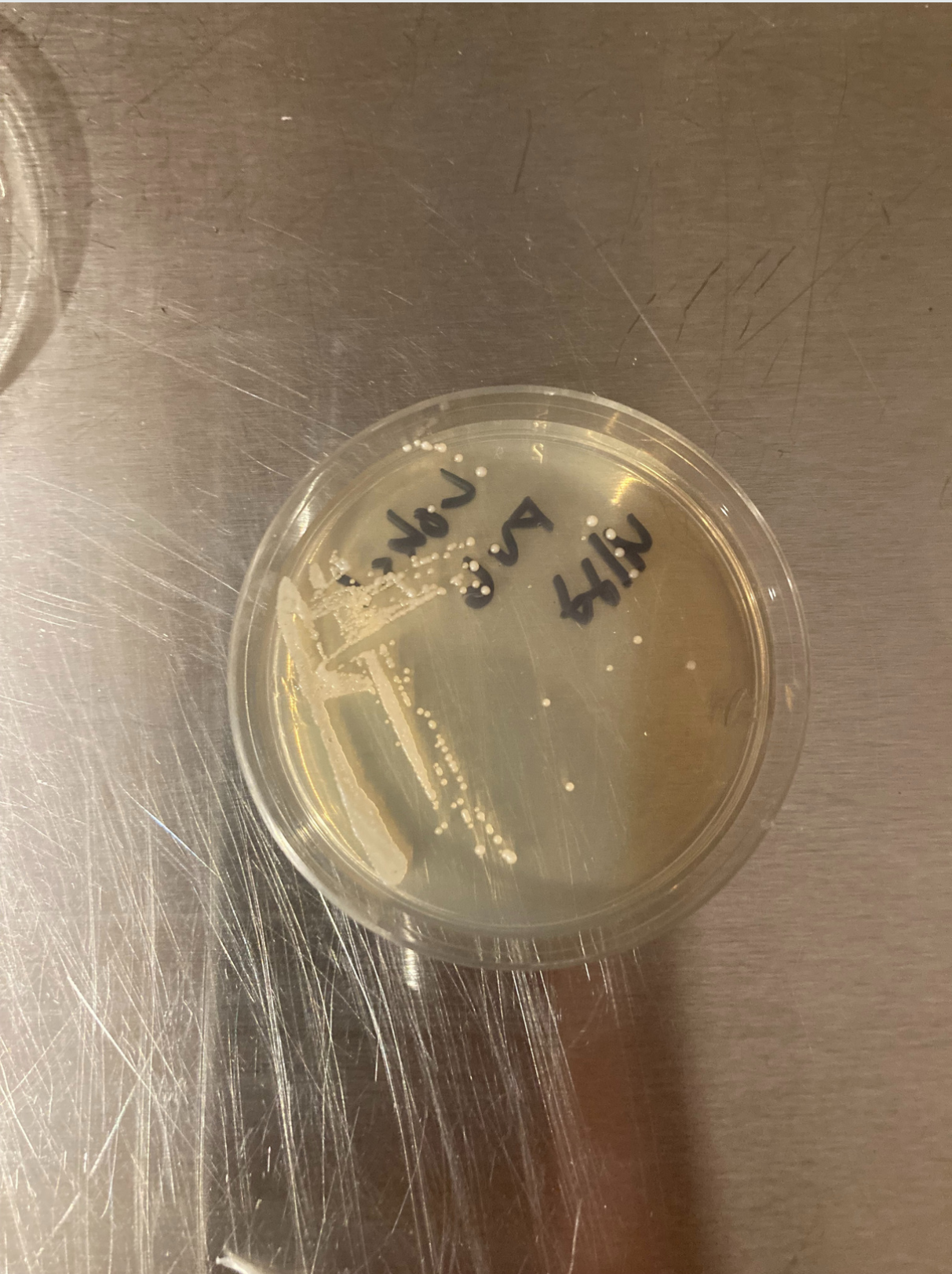


Potato Lactose UI Agar. Mix of *Saccharomyces*, *Brettanomyces* and *Pediococcus*. 3 colony morphologies seen.

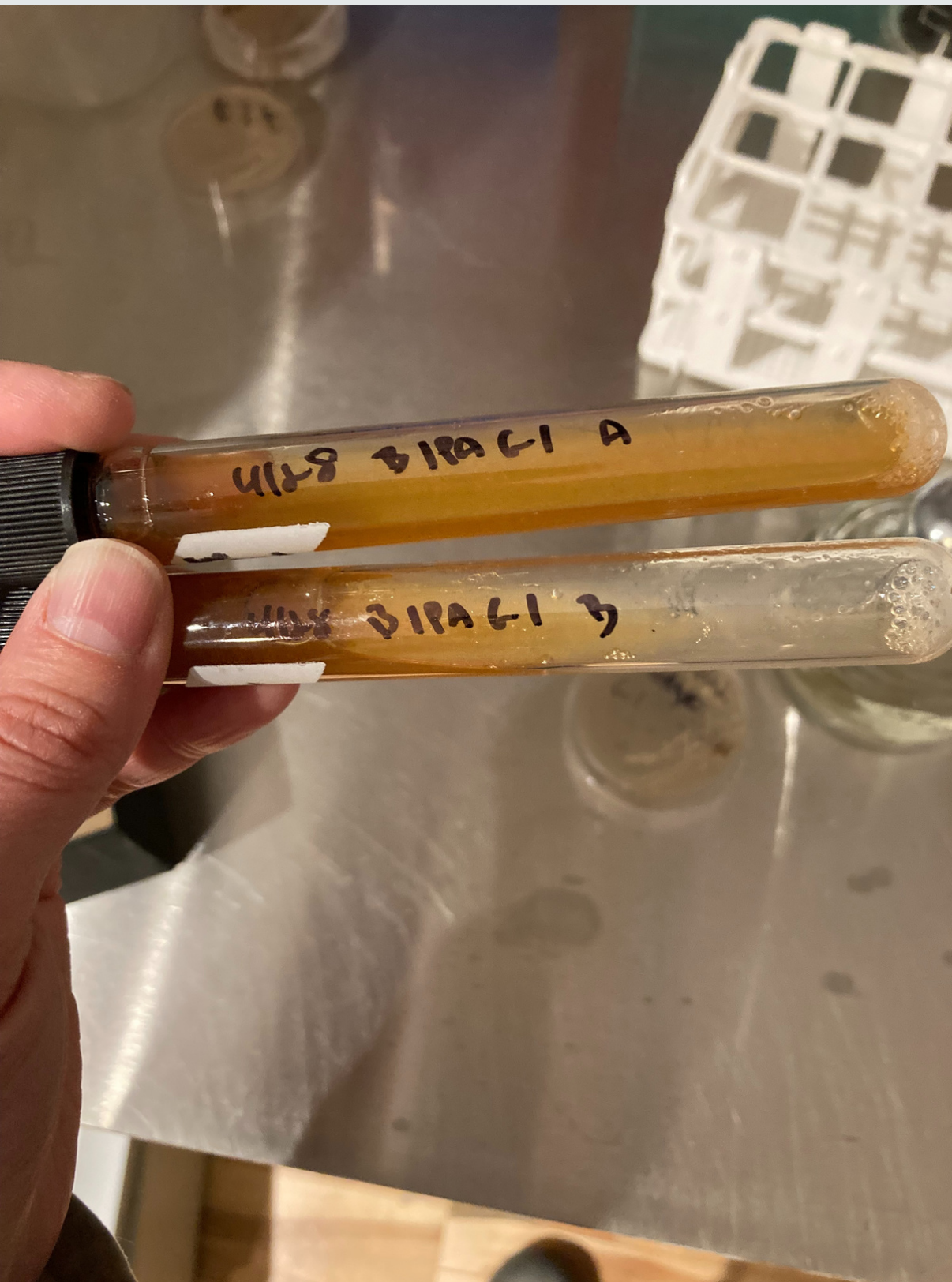
<https://bkyeast.wordpress.com/2012/02/19/selective-media-part-i/>



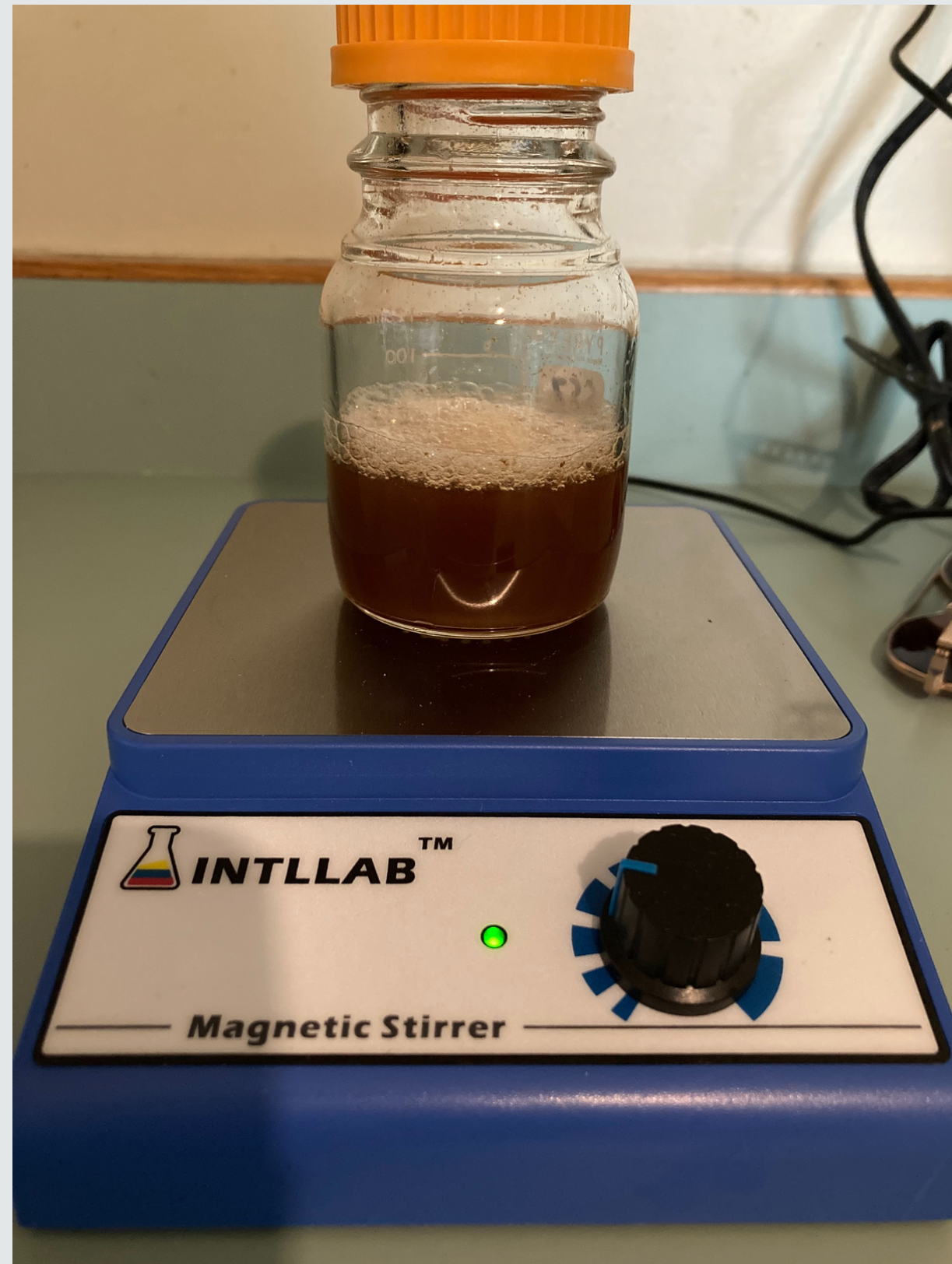








5mL



50mL



500mL





# Collecting Wild Yeast

Yeast starter jars, fruit/vegetables, environmental swabs

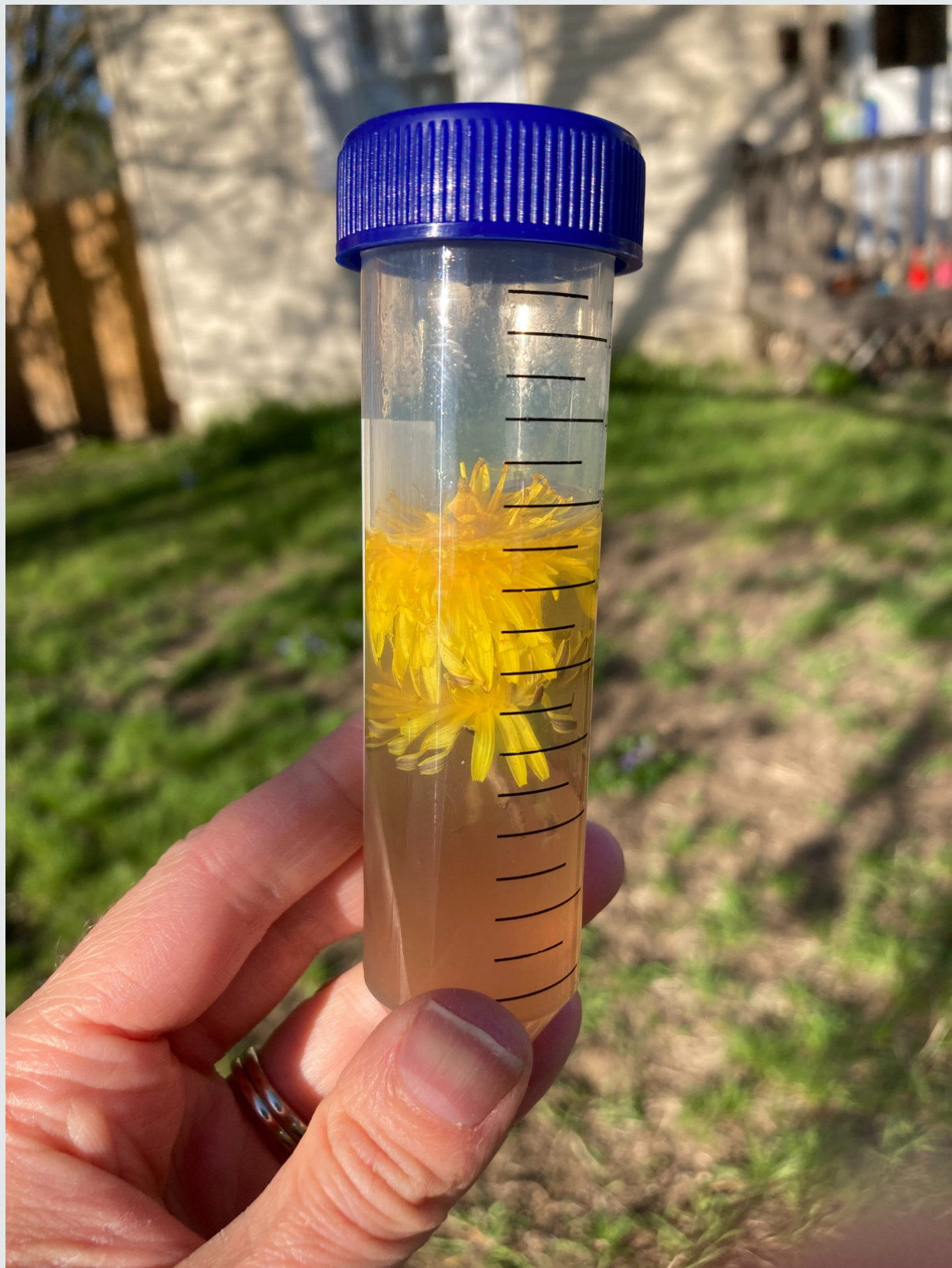
What you need

Wild yeast starter jar

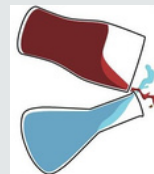
- sterile mason jar
- cheesecloth or mesh lining
- 1-1.25L water
- 100grams DME or wort
- ~1gram of hops
- 1/4 tsp lactic acid
- 1/8 volume cheap vodka/whiskey/run



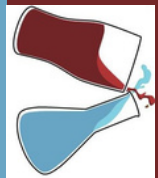




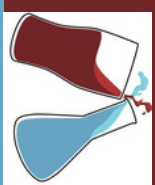
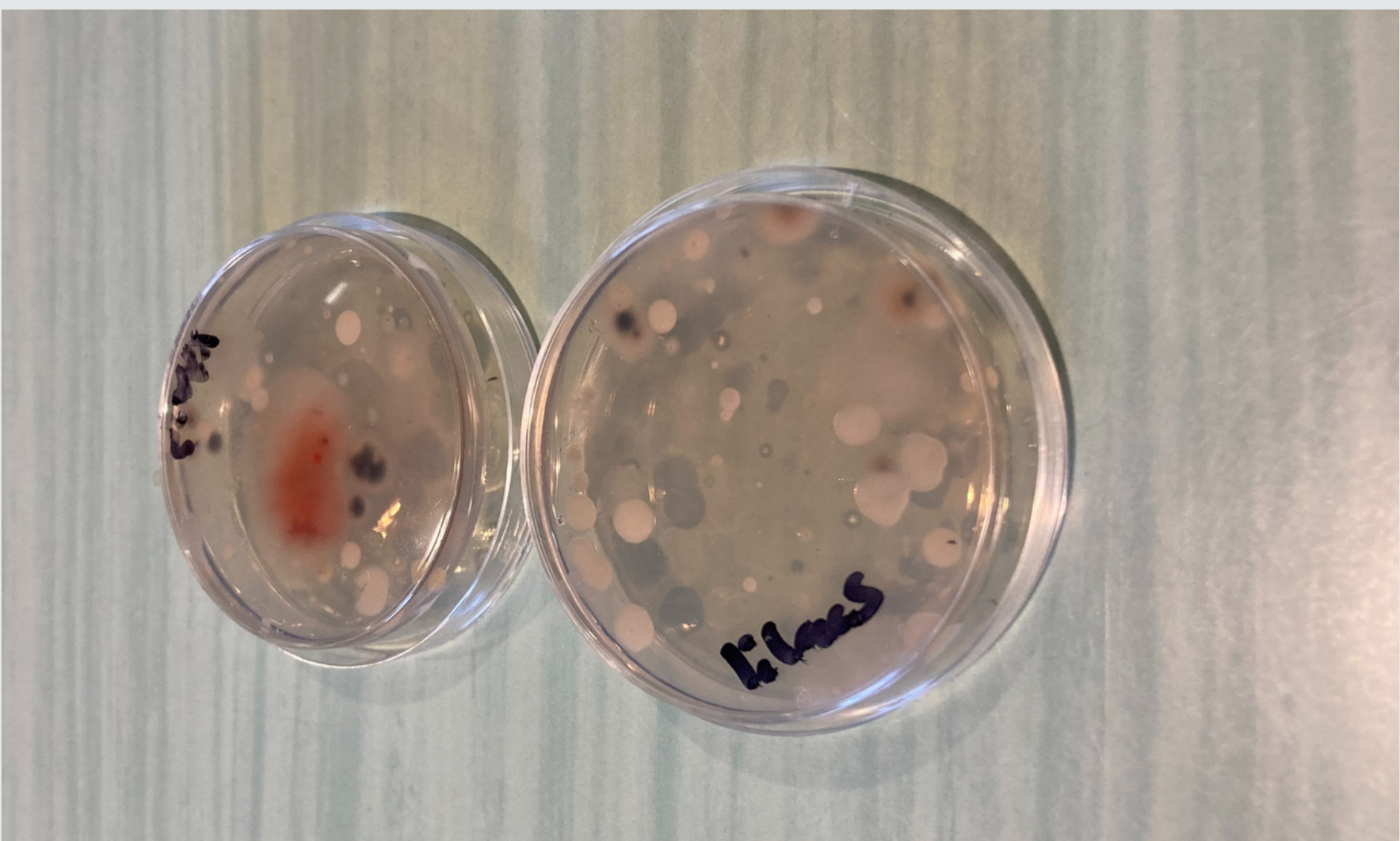
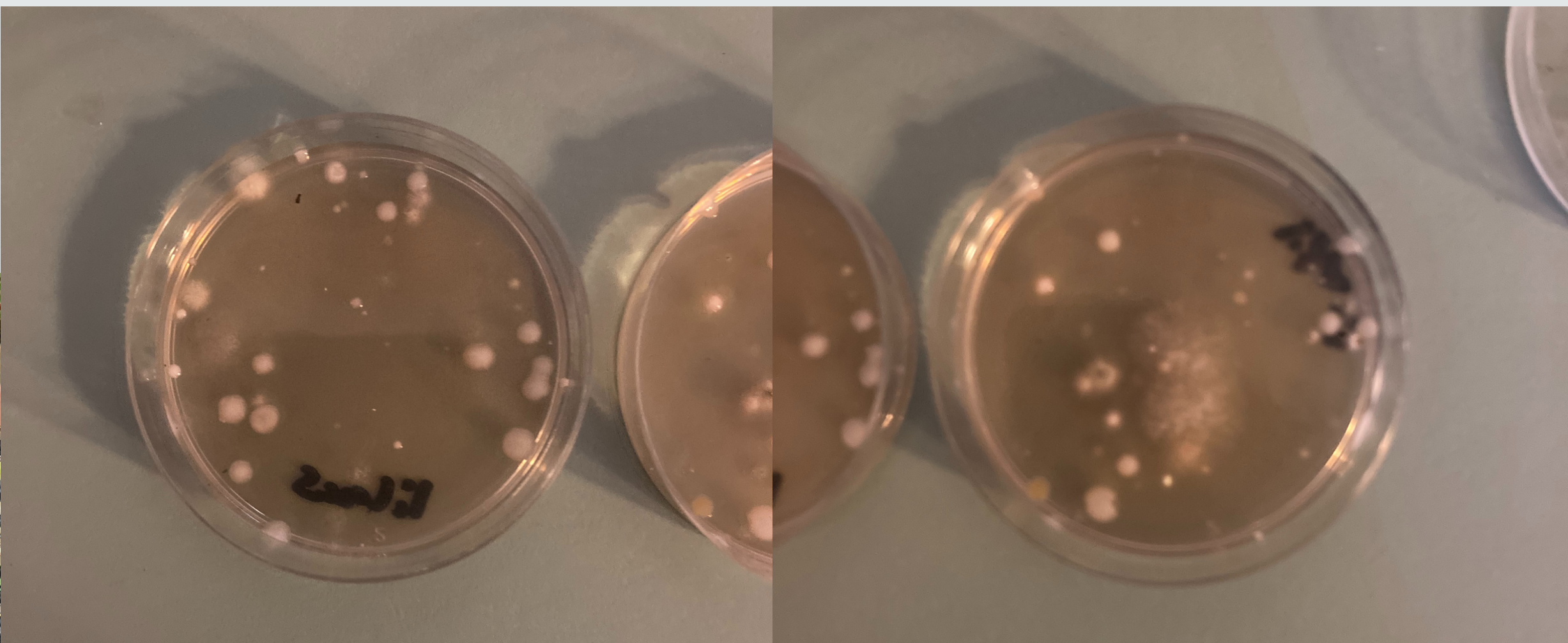














# Is it safe?

- clear or white good
- pink/red bad
- yellow/golden avoid
- filamentous/fuzzy - avoid
- mucoid avoid
- wait at least 2 weeks before trying to plate
- Sui Generis Brewing
- isolating strains

<https://www.youtube.com/watch?v=abQNI6WIdc8&t=2s>

## Colony Morphology - Colony Shape

Lowest-Risk



Circular



Ovoid



Spindle

Some Potential  
& Risk



Irregular



Punctiform

AVOID



Filamentous



Rhizoid



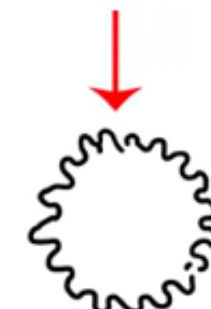
## Colony Morphology - Margins



Entire



Undulate



Erose



Lobate



Filamentous



Curled

Lowest Risk

Some Potential  
& Risk

AVOID



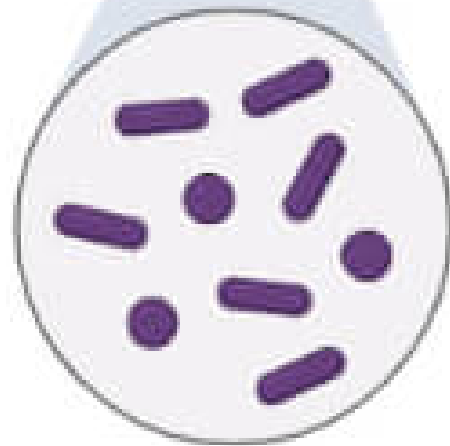
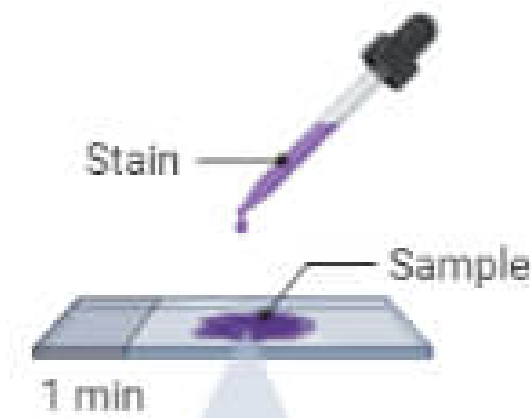


# Gram Stain

## Step 1

### Crystal violet

Primary stain added to specimen smear.

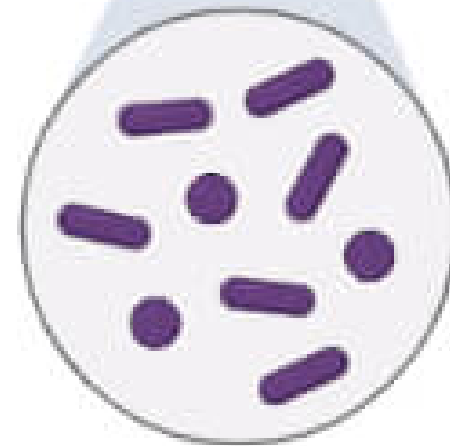
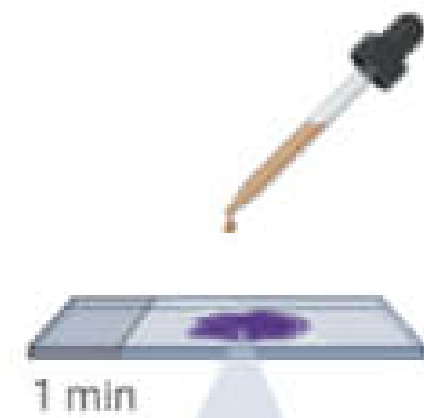


● Gram (+): purple  
▬ Gram (-): purple

## Step 2

### Iodine

Mordant makes dye less soluble so it adheres to cell walls.

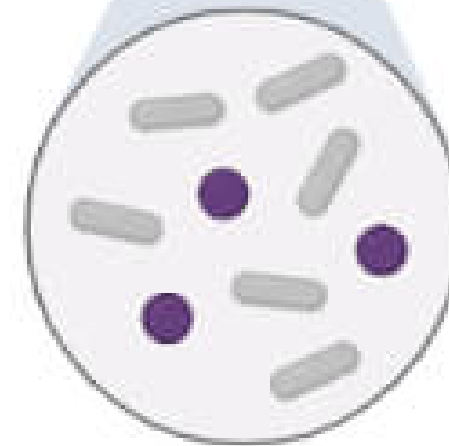
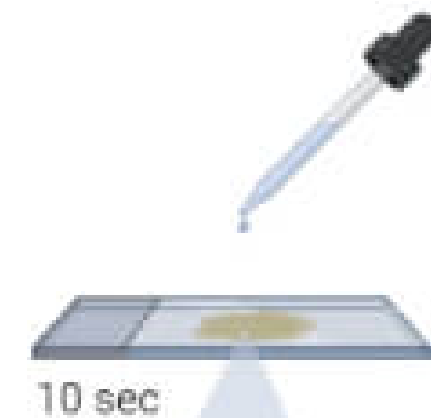


● Gram (+): purple  
▬ Gram (-): purple

## Step 3

### Alcohol

Decolorizer washes away stain from gram (-) cell walls.

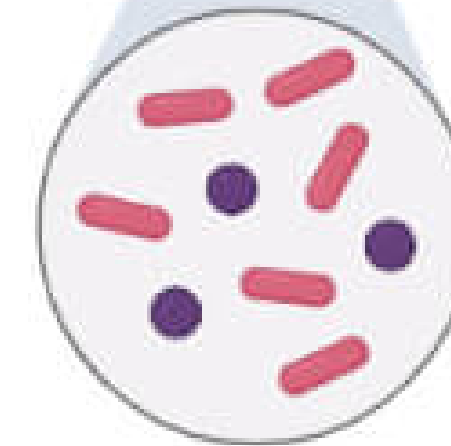
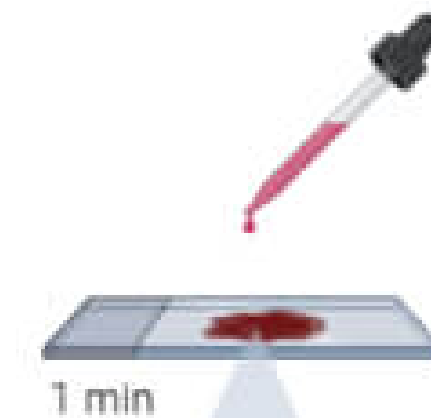


● Gram (+): purple  
▬ Gram (-): colorless

## Step 4

### Safranin

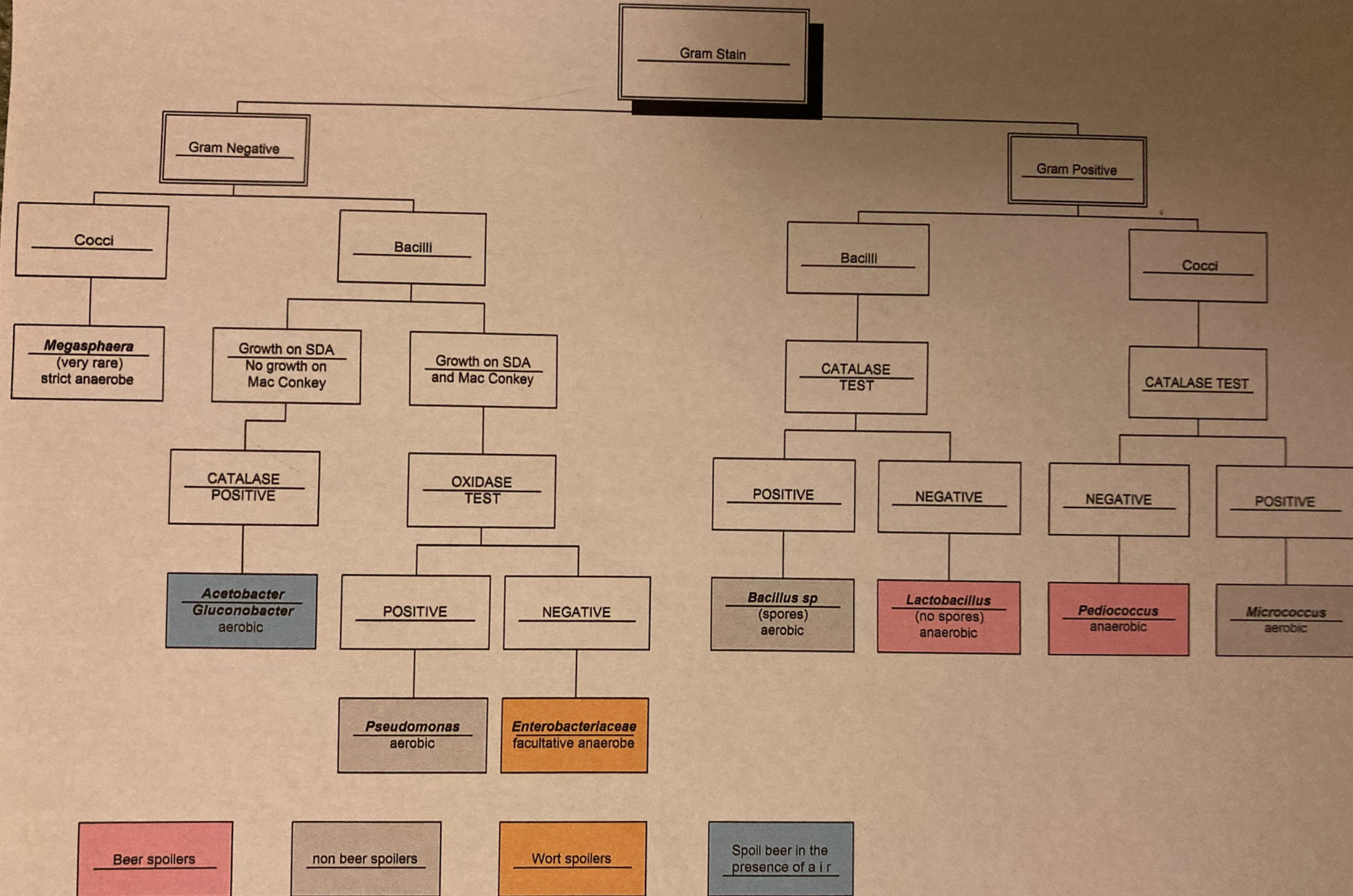
Counterstain allows dye adherence to gram (-) cell walls.



● Gram (+): purple  
▬ Gram (-): red









# Forced Fermentation

What you need


- 1L Erlenmeyer flask
- Tinfoil
- Stir/hot plate
- Magnetic Stir bar
- Hydrometer/graduated cylinder
- ~500mL of wort
- Yeast 25-50mL– use the same yeast that the main fermentation uses






# Sensory

- BJCP
- Cicerone
- Under the Jenfluence
- True to style/target
- R&D
- Bottle release
- Evaluation
- Training
  - BJCP Guidelines for Doctoring Beer
  - Grocery store
  - Spike kits
  - Triangle/tetrad



## BEER SCORESHEET

http://www.bjcp.org      AHA/BJCP Sanctioned Competition Program      http://www.homebrewersassociation.org



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Judge Name (print) \_\_\_\_\_

Judge BJCP ID \_\_\_\_\_

Judge Email \_\_\_\_\_

*Use Avery label # 5160*

Category # \_\_\_\_\_ Subcategory (a-f) \_\_\_\_\_ Entry # \_\_\_\_\_

Subcategory (spell out) \_\_\_\_\_

Special Ingredients: \_\_\_\_\_

Bottle Inspection:  Appropriate size, cap, fill level, label removal, etc.

Comments \_\_\_\_\_

**BJCP Rank or Status:**

Apprentice       Recognized       Certified  
 National       Master       Grand Master  
 Honorary Master       Honorary GM       Mead Judge  
 Provisional Judge       Rank Pending       Cider Judge

**Non-BJCP Qualifications:**

Professional Brewer       Beer Sommelier       GABF/WBC  
 Certified Cicerone       Adv. Cicerone       Master Cicerone  
 Sensory Training       Other \_\_\_\_\_

**Descriptor Definitions (Mark all that apply):**

**Acetaldehyde** – Green apple-like aroma and flavor.

**Alcoholic** – The aroma, flavor, and warming effect of ethanol and higher alcohols. Sometimes described as *hot*.

**Astringent** – Puckering, lingering harshness and/or dryness in the finish/aftertaste; harsh graininess; huskiness.

**Diacetyl** – Artificial butter, butterscotch, or toffee aroma and flavor. Sometimes perceived as a slickness on the tongue.

**DMS (dimethyl sulfide)** – At low levels a sweet, cooked or canned corn-like aroma and flavor.

**Estery** – Aroma and/or flavor of any ester (fruits, fruit flavorings, or roses).

**Grassy** – Aroma/flavor of fresh-cut grass or green leaves.

**Light-Struck** – Similar to the aroma of a skunk.

**Metallic** – Tinny, coinny, copper, iron, or blood-like flavor.

**Musty** – Stale, musty, or moldy aromas/flavors.

**Oxidized** – Any one or combination of stale, winy/vinous, cardboard, papery, or sherry-like aromas and flavors.

**Phenolic** – Spicy (clove, pepper), smoky, plastic, plastic adhesive strip, and/or medicinal (chlorophenolic).

**Solvent** – Aromas and flavors of higher alcohols (fusel alcohols). Similar to acetone or lacquer thinner aromas.

**Sour/Acidic** – Tartness in aroma and flavor. Can be sharp and clean (lactic acid), or vinegar-like (acetic acid).

**Sulfur** – The aroma of rotten eggs or burning matches.

**Vegetal** – Cooked, canned, or rotten vegetable aroma and flavor (cabbage, onion, celery, asparagus, etc.)

**Yeasty** – A bready, sulfury or yeast-like aroma or flavor.

**Aroma** (as appropriate for style) \_\_\_\_\_ /12  
Comment on malt, hops, esters, and other aromatics

---

**Appearance** (as appropriate for style) \_\_\_\_\_ /3  
Comment on color, clarity, and head (retention, color, and texture)

---

**Flavor** (as appropriate for style) \_\_\_\_\_ /20  
Comment on malt, hops, fermentation characteristics, balance, finish/aftertaste, and other flavor characteristics

---

**Mouthfeel** (as appropriate for style) \_\_\_\_\_ /5  
Comment on body, carbonation, warmth, creaminess, astringency, and other palate sensations

---

**Overall Impression** \_\_\_\_\_ /10  
Comment on overall drinking pleasure associated with entry, give suggestions for improvement

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**Total** \_\_\_\_\_ /50

SCORING GUIDE	<b>Outstanding</b> (45 - 50): World-class example of style.	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%;"><b>Classic Example</b></td> <td style="width: 25%;"><input type="checkbox"/></td> <td style="width: 25%;"><b>Stylistic Accuracy</b></td> <td style="width: 25%;"><input type="checkbox"/></td> <td style="width: 25%;"><input type="checkbox"/></td> <td style="width: 25%;"><input type="checkbox"/></td> <td style="width: 25%;"><input type="checkbox"/></td> <td style="width: 25%;"><b>Not to Style</b></td> </tr> <tr> <td><b>Very Good</b> (30 - 37): Generally within style parameters, some minor flaws.</td> <td><input type="checkbox"/></td> <td><b>Technical Merit</b></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><b>Significant Flaws</b></td> </tr> <tr> <td><b>Good</b> (21 - 29): Misses the mark on style and/or minor flaws.</td> <td><input type="checkbox"/></td> <td><b>Intangibles</b></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><b>Lifeless</b></td> </tr> <tr> <td><b>Fair</b> (14 - 20): Off flavors/aromas or major style deficiencies. Unpleasant.</td> <td><input type="checkbox"/></td> <td><b>Flawless</b></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td></td> </tr> </table>	<b>Classic Example</b>	<input type="checkbox"/>	<b>Stylistic Accuracy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<b>Not to Style</b>	<b>Very Good</b> (30 - 37): Generally within style parameters, some minor flaws.	<input type="checkbox"/>	<b>Technical Merit</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<b>Significant Flaws</b>	<b>Good</b> (21 - 29): Misses the mark on style and/or minor flaws.	<input type="checkbox"/>	<b>Intangibles</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<b>Lifeless</b>	<b>Fair</b> (14 - 20): Off flavors/aromas or major style deficiencies. Unpleasant.	<input type="checkbox"/>	<b>Flawless</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
	<b>Classic Example</b>		<input type="checkbox"/>	<b>Stylistic Accuracy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<b>Not to Style</b>																									
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<b>Problematic</b> (00 - 13): Major off flavors and aromas dominate. Hard to drink.	<input type="checkbox"/>	<b>Wonderful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																												

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# resources

Alsuhaime, H. & Vojisavljevic, Vuk & Pirogova, Elena. (2013). Effects of Non-thermal Microwave Exposures on the Proliferation Rate of Saccharomyces Cerevisiae Yeast. 10.1007/978-3-642-29305-4\_14. - file:///C:/Users/a/Downloads/Hamad\_Elena\_WC2012.pdf

Aryal, Sagar "Potato Dextrose Agar (PDA)- Principle, Uses, Composition, Procedure and Colony Characteristics," Accessed 5/9/22 <https://microbiologyinfo.com/potato-dextrose-agar-pda-principle-uses-composition-procedure-and-colony-characteristics/>

BKYeast. "Selective Media Part I" BYeast.wordpress.com. <https://bkyeast.wordpress.com/2012/02/19/selective-media-part-i/>

"DIYeast: Capturing Yeast," Bootlegbiology.com. <https://bootlegbiology.com/diy/capturing-yeast/>

Gram staining - Dahal, Prashant. "Gram Staining- Principle, Reagents, Procedure, Steps, Results," Microbe notes. <https://microbenotes.com/gram-stain-principle-reagents-procedure-and-result-interpretation/>

Green, Tom. "Tame The Yeast: How To Capture Wild Yeast For Homebrewing," Bisonbrew.com. <https://bisonbrew.com/how-to-capture-wild-yeast/#:~:text=Adjust%20the%20pH%20of%20the,a%20better%20chance%20at%20growing>

Heit, Bryan "New Video! Casting Agar Plates," Surigenarisbrewing.com. <https://suigenarisbrewing.com/index.php/2015/03/18/new-video-casting-agar-plates/>

Heit, Bryan. "Capturing and Brewing with Local Canadian Yeasts," <https://canadahomebrews.ca/2018/10/01/capturing-and-brewing-with-local-canadian-yeasts/making-selective-media> - <https://bkyeast.wordpress.com/2012/02/19/selective-media-part-i/>

Heit Bryan. Suri Generis Brewing "Capturing Wild Yeast Part III - Identifying Usable Yeasts," YouTube.com. <https://www.youtube.com/watch?v=abQNI6Wldc8&t=2s>

"Make A Yeast Starter," BYO.com. <https://byo.com/resource/build-a-yeast-starter/>

Put, Dan. "The Life Cycle of a Yeast Cell," MoreBeer.com. <https://www.morebeer.com/articles/lifeofayeast>

<https://www.brewersfriend.com/plato-to-sg-conversion-chart/#>

\*all websites accessed within the last 3-6 months.



# resources

## Media/micro

Siebel media guide - <https://www.mbaa.com/districts/EasternCanada/events/Documents/2012-11-21Goineau.pdf>

Premade plates - <https://www.s2cm.com/> - premade plates

escarpment labs youtube - [https://www.youtube.com/channel/UCyCSmOUfkp\\_QPH1PCIAxVQ/videos](https://www.youtube.com/channel/UCyCSmOUfkp_QPH1PCIAxVQ/videos)

<https://www.sigmaaldrich.com/technical-documents/articles/biology/Introduction-yeast-media.html#:~:text=Yeast%20Nitrogen%20Base%20is%20a,amino%20acid%20and%20carbon%20requirements.>

aseptic sampling - <https://www.asbcnet.org/lab/webinars/Pages/MicrobiologyVideos.aspx>

plating methods - [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846335/#:~:text=Procedures%20described%20include%20\(1\)%20streak,plate%20to%20another%20in%20an](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846335/#:~:text=Procedures%20described%20include%20(1)%20streak,plate%20to%20another%20in%20an)

microwaving media - <https://oregonbrewlab.com/media/>

<https://eurekabrewing.wordpress.com/yeast-cultivation/>

## chemistry

<https://www.asbcnet.org/lab/webinars/Pages/BeerMethodsSeries.aspx>

## lab suppliers

<https://www.fishersci.com>

<https://www.weberscientific.com/>

<https://yoursciencehub.com/>

## sensory

Aroxa - <https://www.aroxa.com/>

FlavorActiv - <https://www.flavoractiv.com/beverage/beer/>

Siebel - <https://shop.siebelinstitute.com/sensory-training-kits>

<https://undertheinfluence.beer/>

Avoiding Off-Flavors - Brew Your Own (byo.com)

<https://dev.bjcp.org/exam-certification/program/studying/beer-exam-study-guide/doctoring-beer/>

Podcasts - "False Bottomed Girls," "Bean to Barstool," "Check Your Beer"

## Zymology Labs resources

<https://www.craftbrewingbusiness.com/featured/a-day-in-the-craft-brewery-lab-part-5-in-depth-guide-for-cell-counting/>

<https://zymologylabs.com/resources/f/forced-fermentation-test-how-to-perform-one-and-why>



# resources

- ASBC
- MBAA
- BYO
- Beer Quality Handbook series
- BA Quality Management
- Fellow brewers/homebrewers
- Amy@Zymologylabs.com
- BYO20 for 20%

@zymologylabs.com

