BYO Yeast Lab Course

Chris White, Ph. D CEO, President, and Founder
Kara Taylor, Head of Laboratory Operations

WHITE LABS
PURE YEAST & FERMENTATION

Outline

Intro
Yeast Handling Practices
Yeast Harvesting
Aseptic Technique
Yeast Storage Principles
HLP Lab
Yeast Starters
Yeast Streaking Lab
Forced Wort Testing
Forced Diacetyl Testing
Dilutions Lab
Cell Counting Lab
Pitch Rates
Q&A

What have we been up to?

White Labs in 2020

- New Products- Appalachian Tart
- New Canned Beers
- Kitchen & Tap
- New Nutrients

Why should you implement some of these techniques?

- Consistent beer from batch to batch
- Learn from your mistakes!
- Improve your skills

Where to start…

Budget

Time

Goals

Goals for your lab

- Goals
  - Yeast health & management
  - Clean brewing process
  - Predictable fermentations
  - No undesirable off flavors
  - Identify contaminants
- What you need to know
  - Viability
  - pH
  - Forced wort testing
  - Forced diacetyl testing
  - Contamination detection

Techniques we go over today will help you get there

Yeast Handling Principles

What should we consider on a smaller scale?

- How do we collect yeast ?
- Where do we store it?
- What do we look for when reusing?
- How do I know it's healthy?

Yeast Handling Principles

- How many strains are you using?
- How clean is your environment?
- Investment in accuracy
  - What lab techniques will you apply?

Yeast Handling Principles

Good yeast handling practices = Healthy yeast

- Better flavors and aromas
- Ability to reuse your yeast
- Predictable fermentation rates

It all starts with a good harvest…

Yeast Harvesting

- Ideally collecting 1-3 days from terminal gravity
  - Why?
- Purge dead yeast/trub from bottom
- Avoid top layer of yeast
- Collect creamy consistency

Yeast Collection Apparatuses

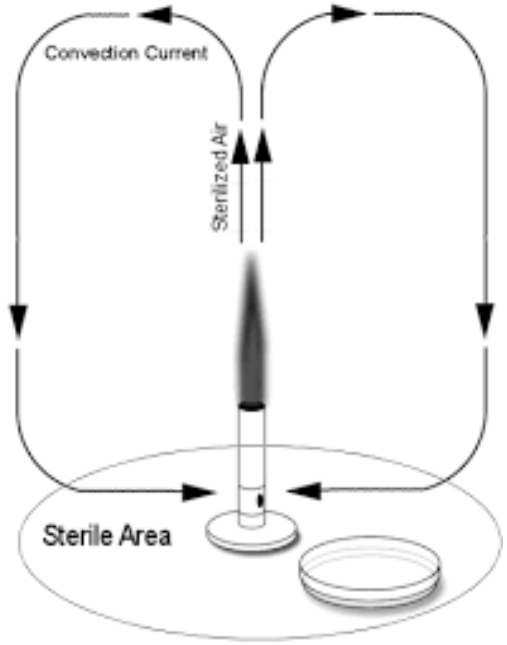
Modified Yeast Brink – Premier Stainless

BREAK

- 5 min

Aseptic Technique

- Clean Work Area
- Good personal hygiene
- Handling



Aseptic Technique Demo

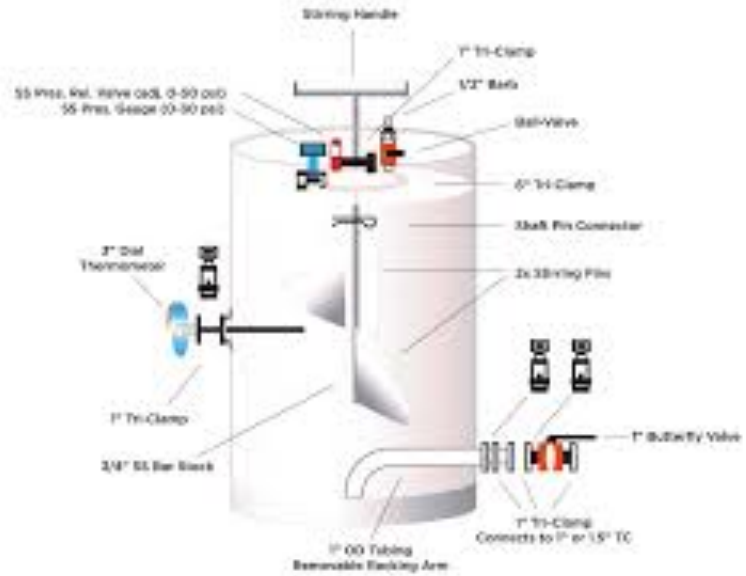
Yeast Storage Principles

How long?

- Each strain is different- some are hardier than others
- 2 months, potentially longer
- Make a starter if you're not sure

Yeast Storage

- Store yeast 2-4℃
- Avoid CO2 buildup by degassing
  - Install blowoff if necessary
- Avoid hotspots
- Use easy to clean, sanitary designe items
- Can you take a sample?



Yeast Washing and Rinsing

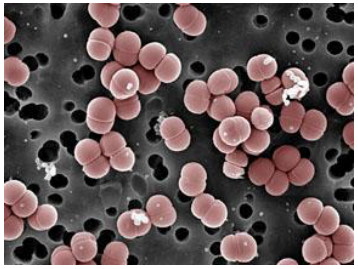
- Yeast washing not a ”White Labs approved” process
  - Only rids of bacteria and difficult to keep yeast healthy
- Rinsing can be helpful when:
  - You have collected too much dead yeast/ protein

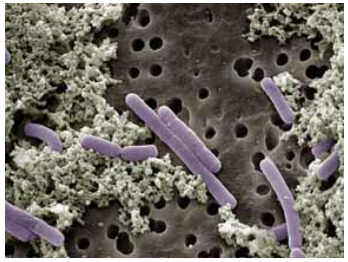
HLP Lab

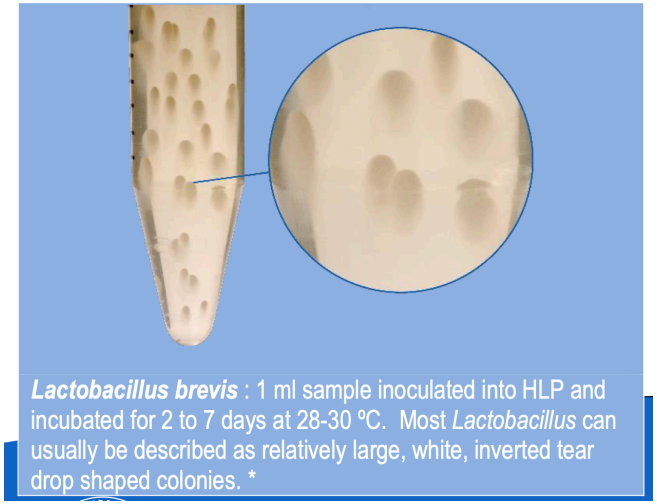
Hsu's Lactobacillus and Pediococcus media

Detects Lactic Acid Bacteria
Gram-positive rods or cocci

***Microwavable**







Lactobacillus brevis : 1 ml sample inoculated into HLP and incubated for 2 to 7 days at 28-30 ºC. Most *Lactobacillus* can usually be described as relatively large, white, inverted tear drop shaped colonies. *

HLP Lab

Positive = Hazy, opaque, or small white colonies
Negative = Clear

Sometimes wild yeast can grow

Don’t worry if it’s Pedio or Lacto- both are ”bad”

HLP Lab

Procedure HLP (prepared media):

1. Take beer or your 1:100 diluted yeast sample and aseptically place 1ml into an empty sterile tube. Label tube appropriately.
2. Once media has cooled to appropriate temperature (45C or 113F), mix each sample in the tubes thoroughly and then add HLP media up to the last mark on tubes - approximately 14ml. Close lid tightly and invert tube to mix.
3. Let tubes solidify.
4. Place closed tubes in tray and place tray in 30C incubator for 3-5 days.

BREAK

- 5 min

Yeast Starters

Why are yeast starters helpful?

- Can help big beers ferment fully
- Can help create a proper lager- cold fermented
- Ensuring yeast is active, revive lower viability
- Peace of mind

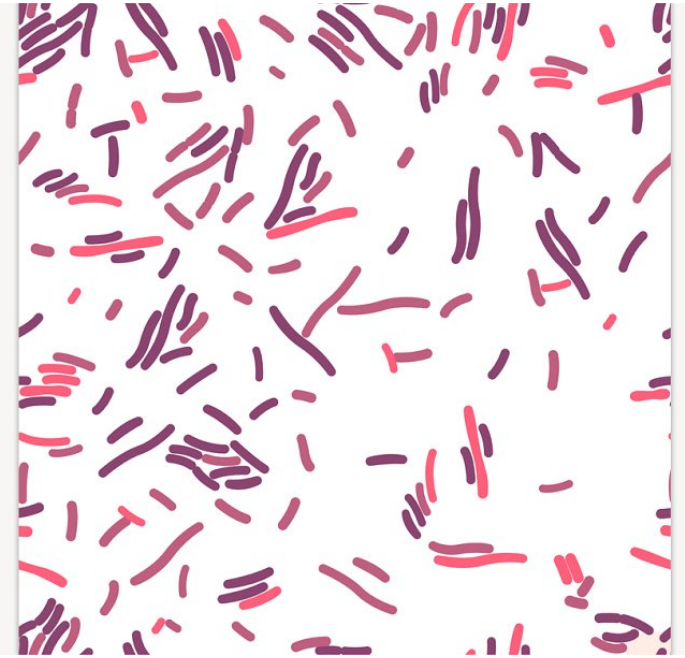
Yeast Starters

Why can starters be “harmful”?

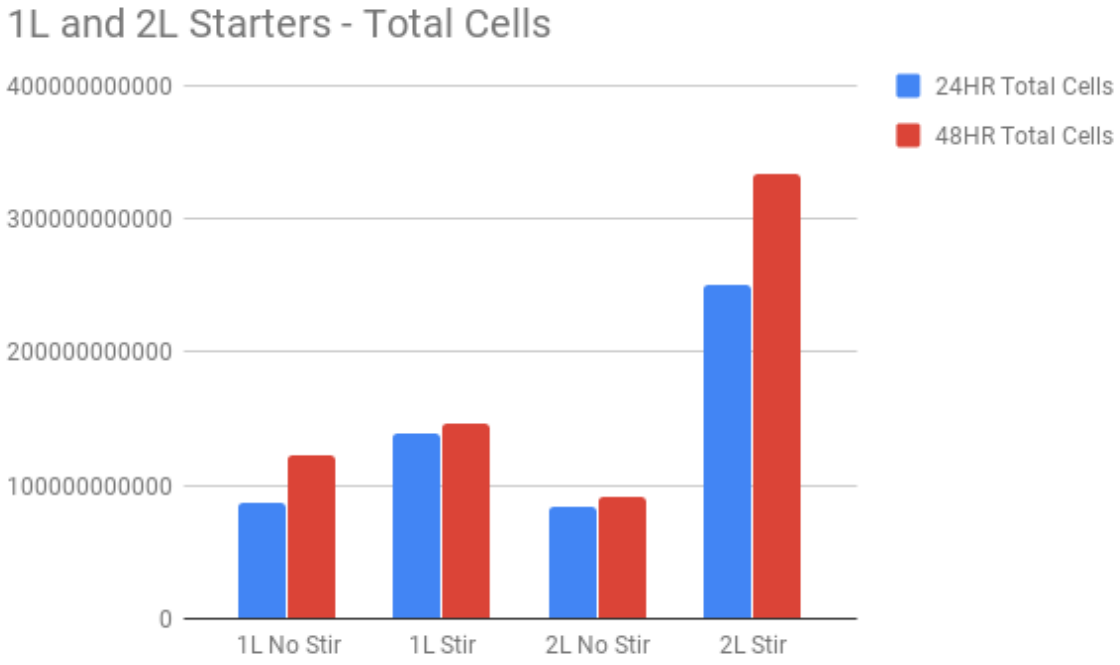
Main issue: Extra Transfer

- An additional step to potentially contaminate the beer

Extra time, resources/ingredients required in addition to brewing time



Yeast Starters



ADVANCING FERMENTATION. CULTIVATING COMMUNITY.

Yeast Starters

What recipe should I use?

- 1.032-1.040 Starting Gravity
- Ideally maltose based- DME/LME
- Boil!
- Keep at room temperature or warmer
- Pinch of yeast nutrient
- Pre-made starter liquid can be useful

Yeast Starters

- Overall 2L is better than 1L
- Stir/Aeration is best

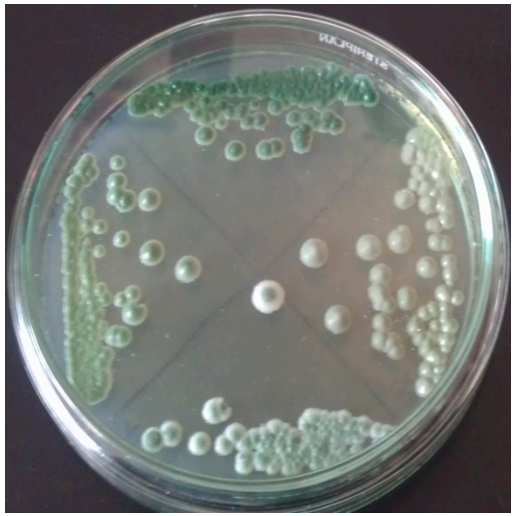
Applying Lab Techniques:

- Cell counting
- HLP

Yeast Streaking Lab

How is yeast streaking used?

- Isolate pure colonies
- Keep yeast slants in your own collection
- Ensure you have “good” colonies



Yeast Streaking Lab

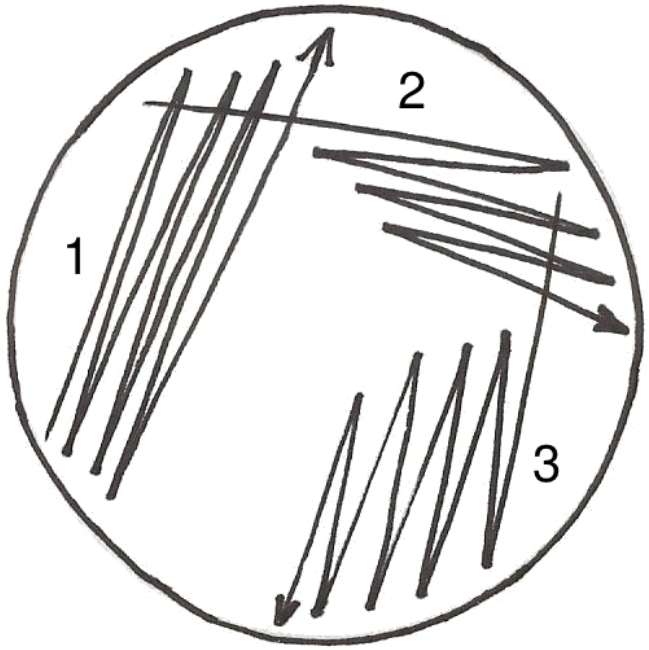
- YPD or YEPD media is the typical media used
- Media with wort can also be used
- WLN (Wallerstein's Nutrient Media) -blue stain

Growth will happen 48-72 hrs

Slants are also used for long term storage

Yeast Streaking

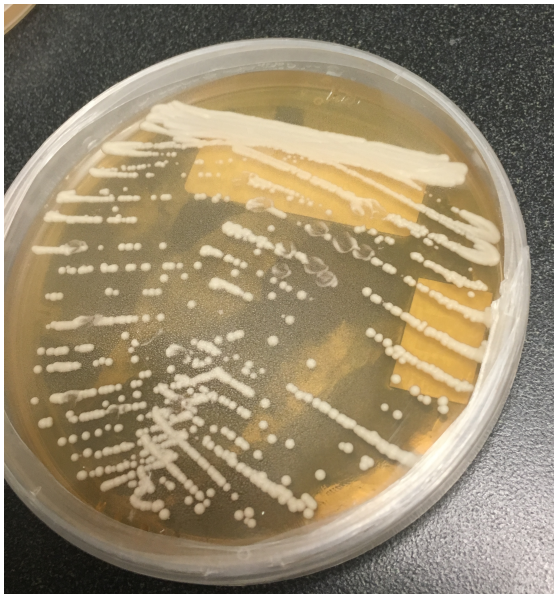
- Pick up 1 colony from working plate
- Streak in a zig zag on 1/3 of the plate
- Use new loop to streak through first zig zag
- Use new loop to streak through second zig zag.
- Incubate at 90F for 3-5 days



Yeast Streaking

What should this look like?

- Idea is to end up with individual colonies
- Pick colonies same size and shape



Forced Wort Testing: Sanitation

Definitions:

- Clean – soil reduced to an acceptable level. Usually done with a combination of water and detergent
- Sanitized – viable organisms reduced to an acceptable level on a clean surface.
- Sterile – all organisms including spores and viruses are completely destroyed.

Forced Wort Testing: Sanitation

Cleaning is necessary to sanitize something

Difficult to keep things sanitized when you brew outside
- Avoid air drafts
- Change sanitizer buckets often
- Make sure you're using the correct amounts of chemicals and temperatures

Forced Wort Testing

- Simple way to check cleanliness of the brewing process
- After you have cooled, oxygenated, and transferred the wort, collect a small amount prior to pitching the yeast
- Incubate this sample and look for evidence of contamination

Forced Wort Testing

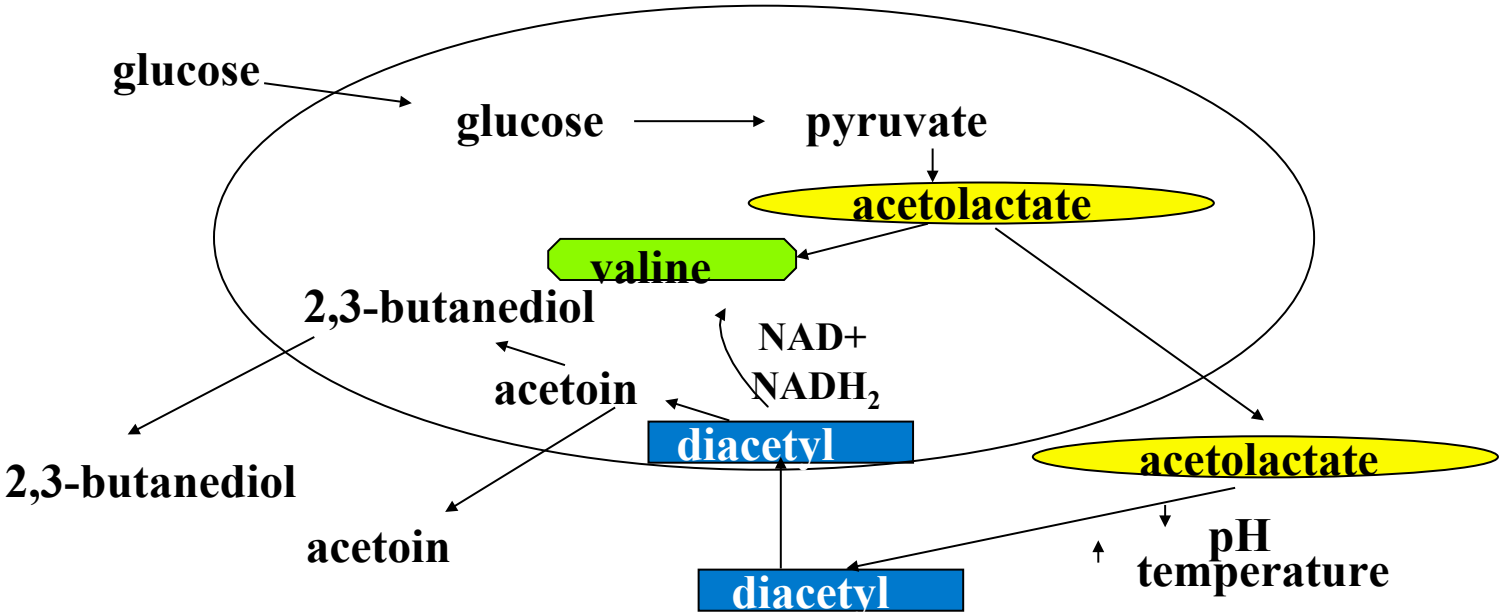
- Clear wort = Beer is clean
- Cloudy wort or wort with bubbles = contamination

Duration	Result
1 day	Very dirty, clean heat exchanger and hoses. Beer will need to be dumped.
2–3 days	Major contamination. Need to clean problem, beer most likely will be affected. Do not collect yeast for re-use from this batch.
3–6 days	Mild contamination build up, clean problem. Beer may or may not be affected.
7 or more	Very clean, keep up the good work

BREAK

- 5 min

Forced Diacetyl Testing



Forced Diacetyl Testing

Forcing conversion of precursor to diacetyl with heat and oxygen

Sample 1→ water bath (140-160°F)

Sample 2→ Room temperature

10-20 min

- Cool
- Smell

Forced Diacetyl Testing

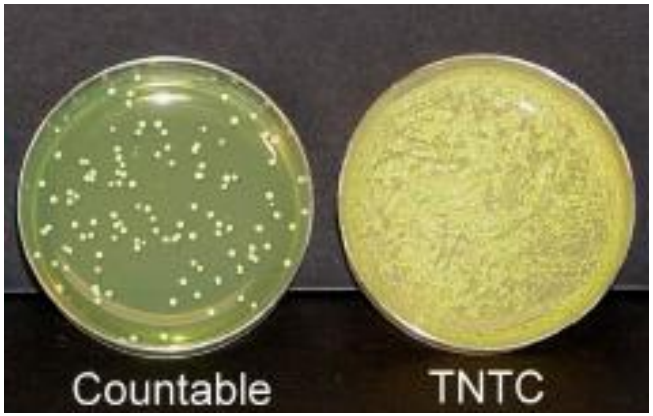
Room Temp Beer	Heated Beer	Conclusion
Negative	Negative	No precursor present, beer is ready to go
Negative	Positive	Precursor present, beer needs more time on yeast
Positive	Positive	Beer is loaded with precursor or possibly contaminated

Dilutions

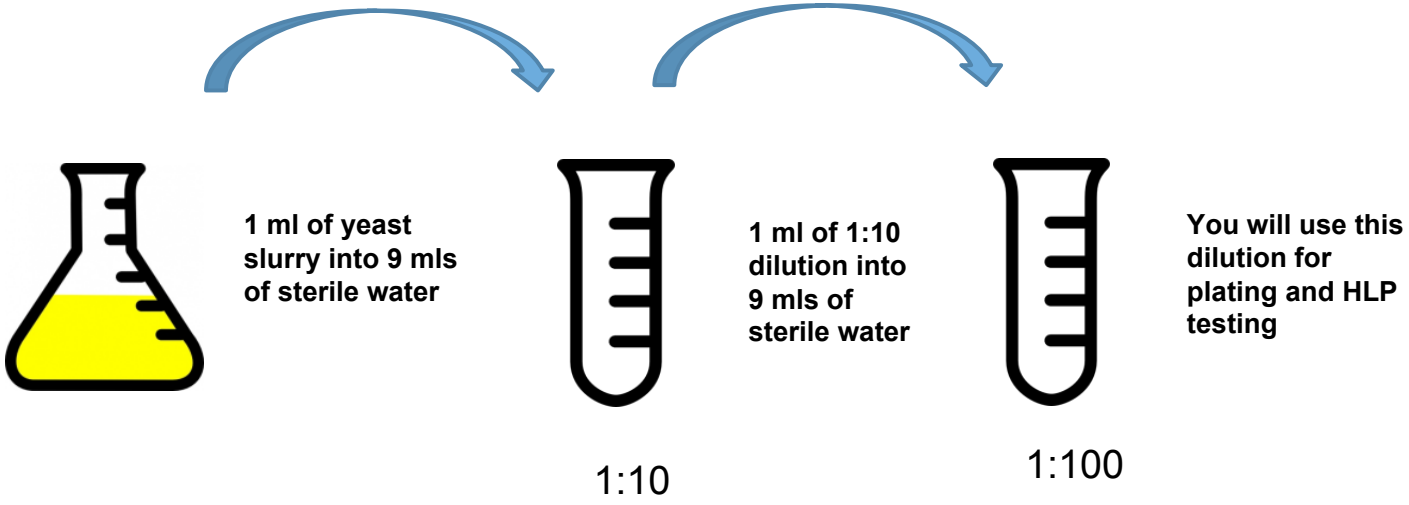
Why do we dilute samples?

Yeast Counting: Need to be able to physically count the yeast

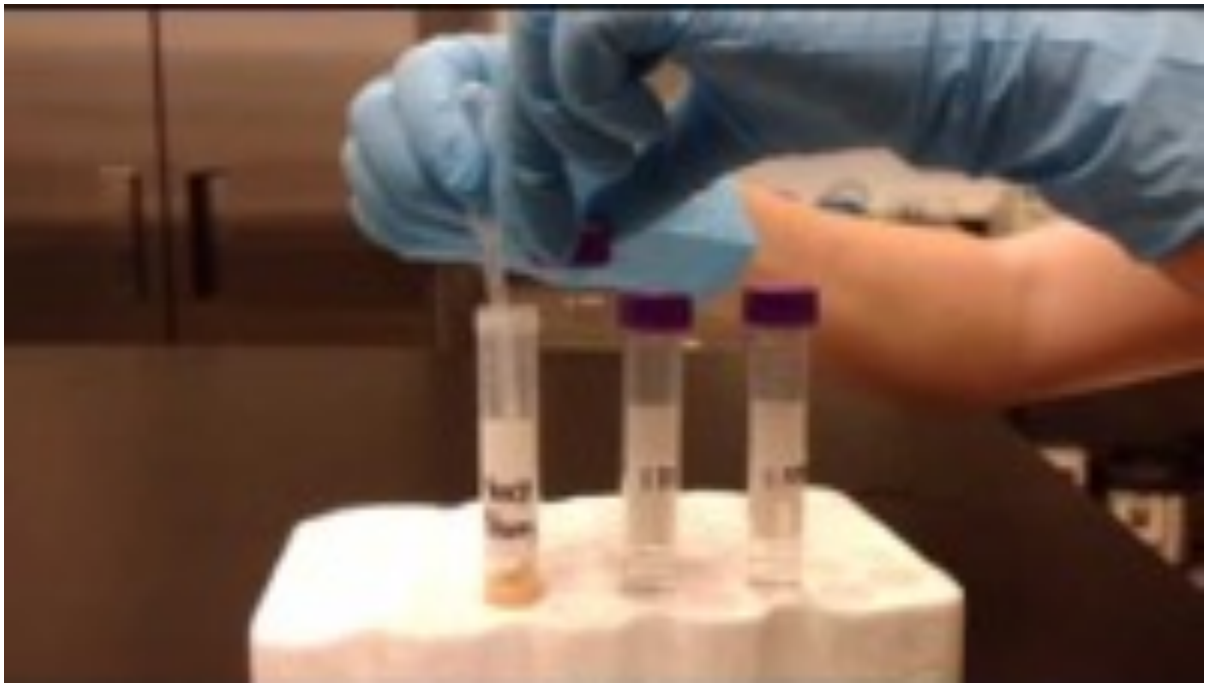
Microbiological: Reduce the chances of overcrowding a plate



Dilutions


1 ml of yeast slurry into 9 mls of sterile water
1 ml of 1:10 dilution into 9 mls of sterile water
You will use this dilution for plating and HLP testing
1:10
1:100

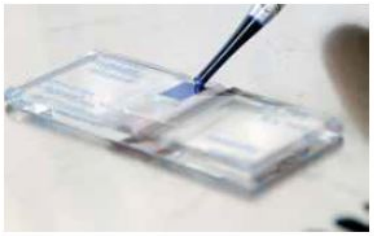
Dilution Video

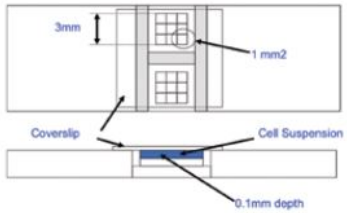


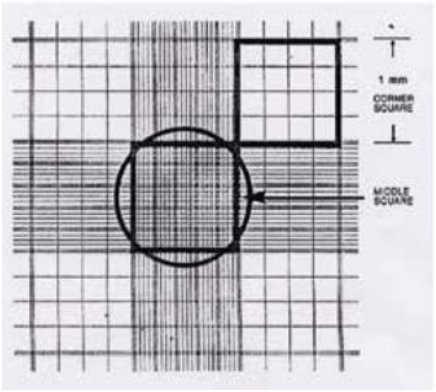
Dilution Lab

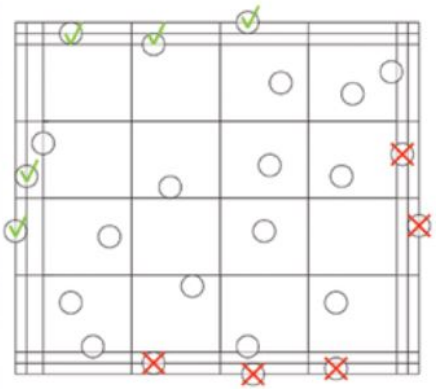
Cell Counting

Why do we cell count?









- Knowing the concentration of cells in a slurry prior to pitching is a prerequisite for calculating the correct amount of yeast to pitch
- The yeast pitching rate has a great impact of the performance, yeast derived flavor compounds, as well as the longevity of a yeast culture
- *Bonus effect*: Visual evaluation of the yeast culture helps the brewer understand the state of the culture

Cell Counts/Yeast Morphology

- Knowing the concentration of cells in a slurry prior to pitching is a prerequisite for calculating the correct amount of yeast to pitch
- The yeast pitching rate has a great impact of the performance, yeast derived flavor compounds, as well as the longevity of a yeast culture
- *Bonus effect*: Visual evaluation of the yeast culture helps the brewer understand the state of the culture

Yeast Viability and Vitality

Definition of “viability”

Capacity of a cell to exhibit life functions

DEAD OR ALIVE

Definition of “vitality”

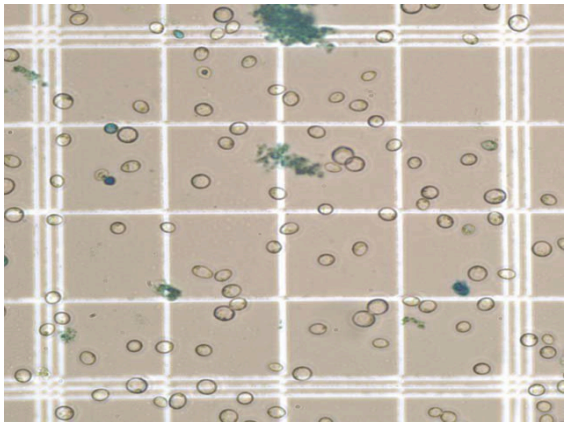
Yeast metabolic fitness or potential to endure stress and still perform

METABOLIC FITNESS

Viability Dyes

0.01% Alkaline Methylene Violet

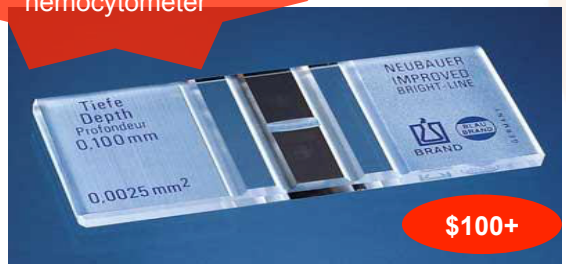
0.01% Citrate Methylene Blue



Specifications to consider when purchasing a microscope:

- Magnification – you'll need at least 1000X magnification to see bacteria
- Having an adjustable stage is helpful
- Monocular vs. Binocular

Alert! Only buy a **bright-line** hemocytometer



$100+

HEMOCYTOMETER

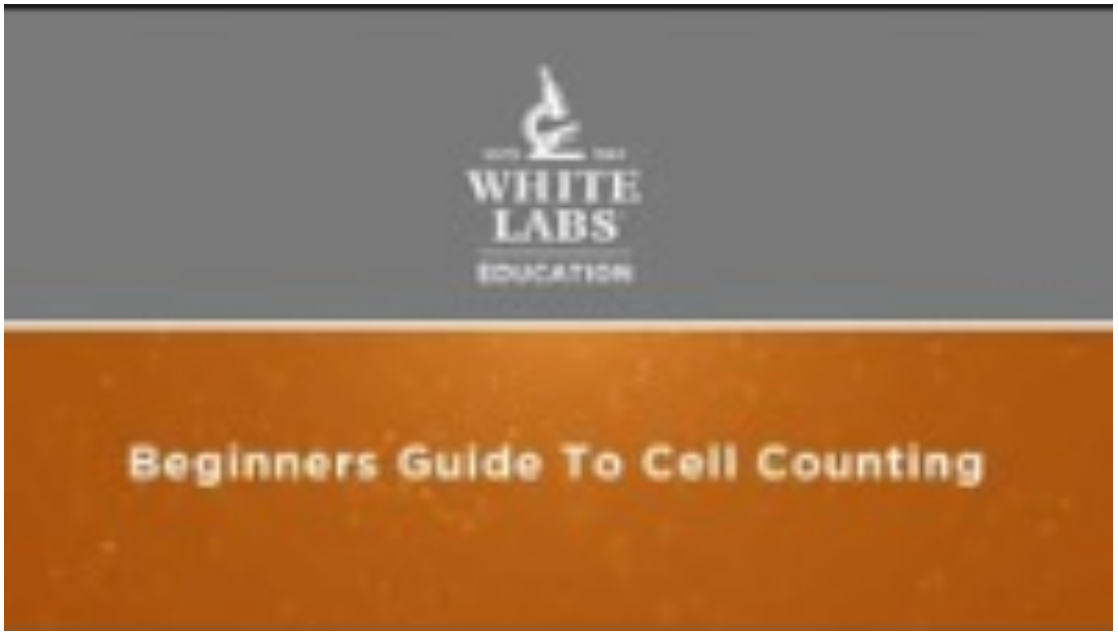
$9+

TALLY COUNTER

LIGHT MICROSCOPE

$150-$500

Cell Counting Video



Cell Counting Example

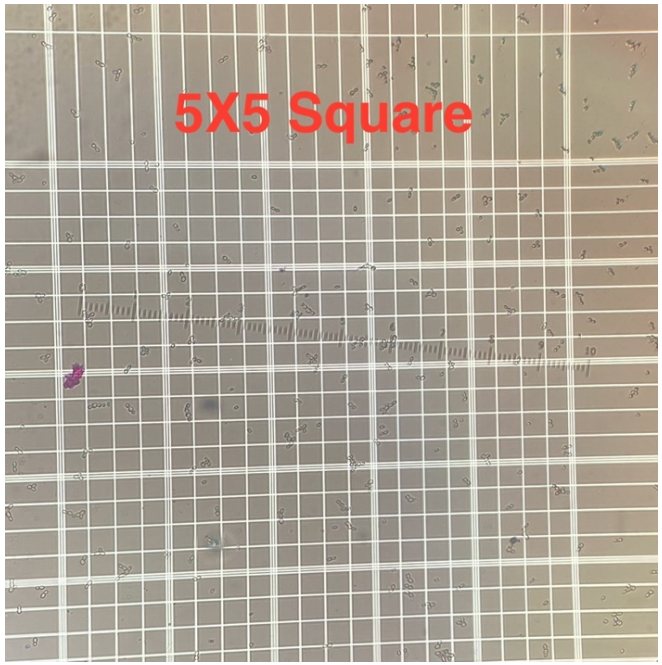
Yeast count equation is:

Yeast cells/mL = Total number of cells in grid x dilution factor x 10^4 (or 10,000, this number is a constant.)

Example: Let's say you counted 136 cells in the 5 numbered squares, using a 1:200 dilution.

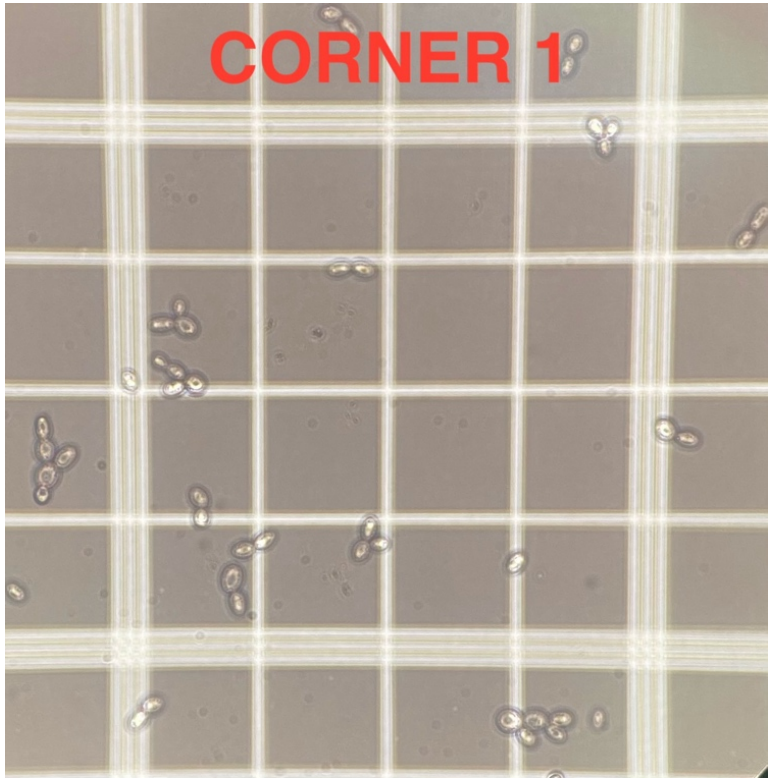
Your cell count would be: 136 x 5 x 200 x 10,000 = 1,360,000,000 = 1.36×10^9 = 1.36 billion cells/mL

Cell Counting Lab



Cell Counting Lab

- Count the cells in the provided photos
- Calculate the cells per ml



Cell Counting Lab

Calculations

Corner 1= 23 Cells

Corner 2= 49 Cells

Corner 3= 27 Cells. 5 dead

Corner 4= 40 Cells

Corner 5= 25 Cells

5 X 164= 820

820 X 200 (dilution factor) X 10^4 =

1.64 x 10^9 cells/ml

1.64 billion cells/ml

ADVANCING FERMENTATION. CULTIVATING COMMUNITY.

Calculating Pitching Rates

Calculating Pitching Rate

Parameter	Value	Unit
Batch size	5	Gallon
Strength of wort	1.050	S.G
Wanted re-pitching rate	1	Million cells / ml / degree Plato
Concentration of slurry	Use concentration from cell counting exercise	Billion cells / ml

Need to determine needed amount of cells for batch

Calculating Pitching Rates

1) Convert batch size to mls 5gal = 18927 ml

2) Convert S.G to Plato (Use chart) 1.050 = 12 P

1 mil cells/ ml / P

1 million X 18927 X 12 = 2.27 x 10^11 cells needed for the 5 gal batch

How much do I add?

$$Volume\ of\ slurry\ to\ pitch\ (ml) = \frac{\text{total cells wanted (cells)}}{\text{concentrations of slurry (cell s/m l)}}$$

2.27 x 10^11 cells. =. 138 ml of the yeast slurry

1.64 x 10^9 cells/ml

Pitching Rates

How do I put this together?

When collecting yeast or making a yeast starter you can count the cells collected and calculate how much yeast you need to add

Lab Equipment

Check out EBay, Amazon, used lab equipment

Sous vide for a water bath

Pressure cooker for sterilization

Portable Flame- No hard gas lines needed

Phone adapter for microscope

Entire lab made from Ikea kitchen

DIY Incubator

Q&A