INTRODUCTION TO BASIC MICRO

YEAST, STORAGE & CONTAMINATION DETECTION

OVERVIEW

- Pitching Rates
- Cell Density
- Preparing Samples for Counting
- Beginning Growths
- Agar Media Types for Yeast Storage
- Contamination Detection

WHAT IS A PITCHING RATE?

- The number of yeast cells provided to a specified volume of wort
- Typically represented as:

Million Cells / mL / °Plato

- This often varies depending on the beer being made

HOW MANY CELLS ARE NEEDED FOR?

- 50L batch of 1.040 Session IPA?
- 20L batch of 1.050 Munich Helles?
- 10L batch of 1.095 Belgian Dark Strong?

PITCHING RATES ARE STYLE / BREWER DEPENDENT

- Lower pitching rates will often result in more *flavour* production (Esters, Higher Alcohols, etc.)
- Higher pitching rates are needed for stressful yeast environments or where ester production may not be desired (Lagers, High Gravity beers, etc.)
- Pitching rates are an approximation, being slightly off isn't going to end the world

CELLS FOR 50L 1.040 SESSION IPA AT 1M CELLS / ML / °PLATO?

- 1.040 S.G –> 10°Plato
- Therefore need 10 Million Cells / mL (10×10^6 cells/mL)
- 50L x 1000 mL per L = 50,000mL (5×10^4 mL)
- (5×10^4 ~~mL~~) x (10×10^6 cells / ~~mL~~) = 50×10^{10} cells

500 Billion Cells!

CELLS FOR 20L 1.050 MUNICH HELLES AT 1.5M CELLS / ML / °PLATO?

- 1.050 S.G –> 12.5°Plato
- Therefore need 18 Million Cells / mL (18×10^6 cells/mL)
- 20L x 1000 mL per L = 20,000mL (2×10^4 mL)
- (2×10^4 ~~mL~~) x (18×10^6 cells / ~~mL~~) = 36×10^{10} cells

360 Billion Cells!

CELLS FOR 10L 1.095 BELGIAN DS AT 2M CELLS / ML / °PLATO?

You have the answer for them I hope

EXERCISE FOR THE READER

CELL DENSITY

- The number of yeast cells present per unit of measure
- Common units:
  - cells / g (i.e. Dry Yeast)
  - cells / mL (i.e. Liquid Yeast / Slurries)

HOW TO DETERMINE DENSITY

+

I thought this was the bible LOL

DILUTIONS

- Slurry on it’s own is too dense and impossible to count
  - Yeast clumping, stacking, too many to count without massive amounts of error
- Diluting makes counting more manageable though will introduce slight error from minor imperfections of equipment

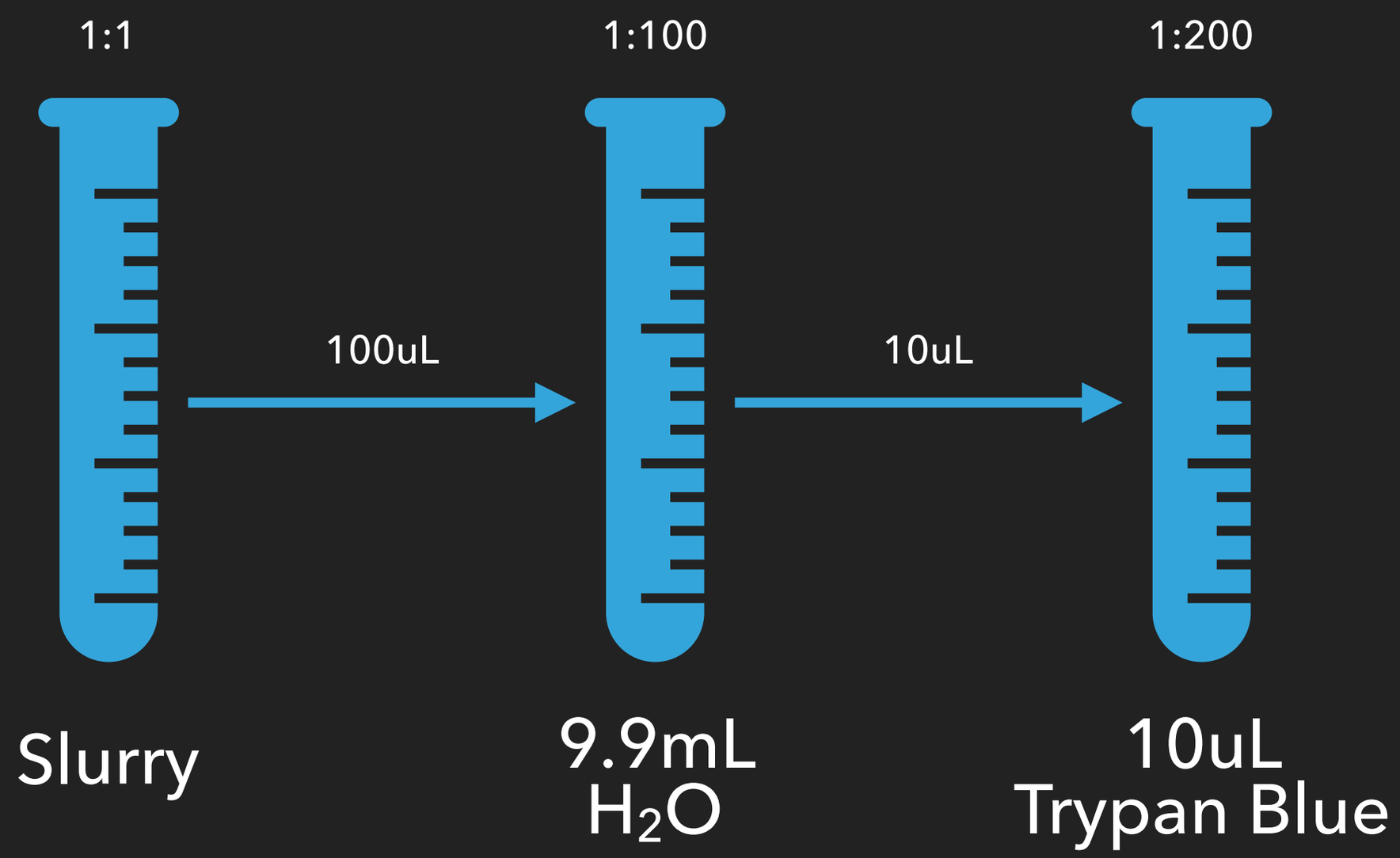
USEFUL DILUTION RATIOS

- 1:200 for Thick slurries (commercial pitches, tank bottoms, yeast cakes, decanted starters, etc)
- 1:20 for Thin slurries (starters, fermenting beer, etc)

HOW TO CALCULATE A DILUTION RATIO

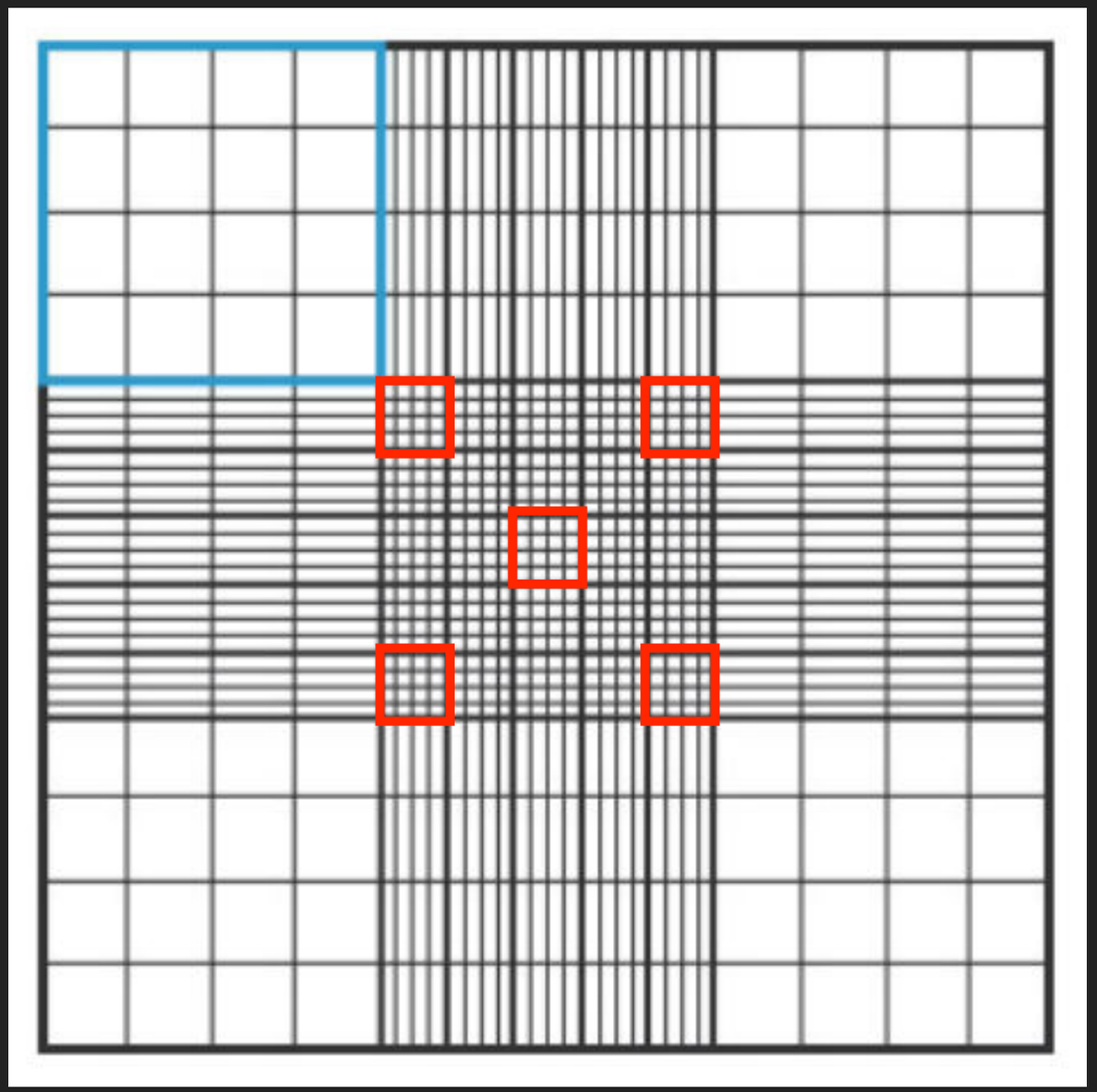
Should remind the audience that is this is to check viability, and you can perform cell counts using only the 9.9mL H2O

- Dilution Ratio = Slurry Volume / (Slurry Volume + Solvent)

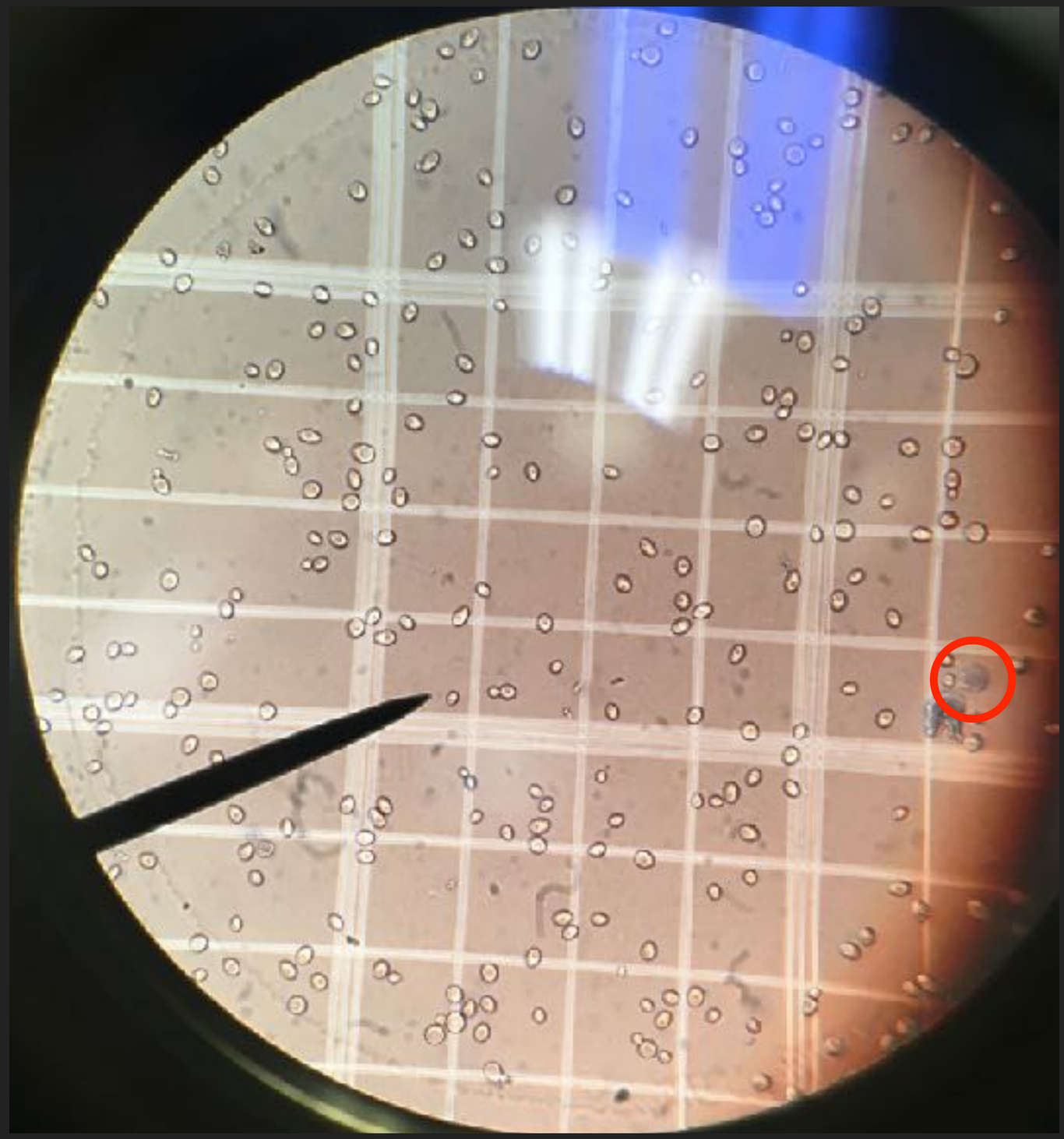


LOADING HAEMOCYTOMETER

COUNTING REGIONS



YEAST COUNTING



YEAST DENSITY & VIABILITY CALCULATIONS

- Average Cells = ∑(cellsInGrid) / 5
- ~Total Cells = Average Cells x 25
- Cells / mL = ~(TotCells/0.1u~~L within counting area*~~) x (1000 uL/mL) = ~TotCells/mL
- Cells / mL of Slurry = Cells / mL x Dilution Factor
- Viability = Live Cells / (Live Cells + Total Dead Cells)

* I believe… this makes the math work (and is required to hit the 10,000 seen in micro literature)

EXAMPLE

Total Alive: 215

Total Dead: 21

HOW MANY CELLS / ML AT 1:200 DILUTION?

- Tot Cells = 215 x 5 = 1075 cells
- Cells / mL = 1075 cells / 0.1 ~~uL~~ x 1000 mL / ~~uL~~
  = 10,750,000 cells / mL
  = 10.75×10^6 cells / mL
- Cells / mL of Slurry = $(10.75 \times 10^6) \times (2 \times 10^2)$
  = 21.5×10^8 cells / mL
  = 2.15×10^9 cells / mL
- Viability = 215 / (215 + 21) = 91.1%

HOW MUCH YEAST?

- 2.15 x 10^9 cells / mL
- Session IPA needs 500 Billion cells
  - mL Slurry = (500 x 10^9 cells) ÷ (2.15 x 10^9 cells / mL)
    = (500 ÷ 2.15) mL
    = 232.6 mL
- Munich Helles needs 360 Billion cells
  - mL Slurry = (360 x 10^9 cells) ÷ (2.15 x 10^9 cells / mL)
    = (360 ÷ 21.5) mL
    = 167.4 mL

GROWING UP YEAST

- Pull colony from plate and inoculate into small volume of sterile wort (25 - 50mL)
- After sufficient growth (visually turbid, flocculating out, ~24hrs max, etc) step into larger volume (500 - 1000mL)
- Repeat into larger volumes as necessary
- Can use previous growths as seeds for more yeast (i.e. inoculate into 1000mL, next day use some to inoculate another 1000mL)

INOCULATION RATES ARE CRITICAL!

- Too little yeast, poor growth and slow attenuation
- Too much yeast, fast attenuation of wort with very little growth
- 10-to-1 ratio is a safe bet for most strains

What would be ideal? Are there any checks you can do to make it ideal?

STARTERS WITH ESCARPMENT HOMEBREW PITCHES

- Approx 200 billion cells / container
- 10M cells / mL for 1.040 wort
- ~0.7M cells / mL for 1.060 wort
- Fresh? Starter maybe not needed
- Starter anyway? Use 1/3 - 1/2 yeast to ensure good growth

NO MICROSCOPE. HOW MANY CELLS IN STARTER?

- ¯_(ツ)_/¯
- Can base it on turbidity. More dense slurry will be more turbid.
- From plate –> 50mL –> 1L
  - ~200 Billion cells
- Not sure? Do second 1L starter and pitch both

DECREASING LAG TIMES

- Pitch fresh yeast
- Pitch more yeast
- Pitch active yeast at krausen – don’t crash starter
- Oxygenate wort prior to pitching
- Using a combination of these strategies can help

STARTERS FOR HIGH GRAVITY BEERS

- Stepping up increases risks of contamination
- Can end up using lots of raw materials for growths
- Resulting liquid from starter is garbage (starter *beer* tastes **terrible**)

- Have a “yeast farm” beer
  - 20L of 10 - 12.5 °Plato wort
  - Low hopping rate to reduce undesired material in slurry
  - Preferably pale wort – no/little colour impact in next beer

MAINTAINING A YEAST BANK

- Differential vs Non-Differential Media
- Maintaining culture purity
- Reducing genetic drift

DIFFERENTIAL VS NON-DIFFERENTIAL MEDIA

- Differential: WLN
  - Different strains take up the dye differently
  - Contamination detection can be very obvious
  - Somewhat expensive, typically requires accounts with lab suppliers
- Non-Differential: YPD
  - Fine for growing up yeast
  - Must rely on colony morphology to determine if plates are contaminated
  - Very cheap to make - easy to get ingredients online or from grocery stores

MAINTAINING PURITY

- Ensure plates aren’t contaminated
- Use aseptic techniques when working with colonies
- Sterile media / equipment only – Autoclave / pressure cook glassware, media, etc.

REDUCING GENETIC DRIFT

- Store cultures in as cold a temperature as possible
  - Warmer temps, more yeast activity, more mutation
- Sub-zero temperatures require special media preparation
  - Solution consisting of some kind of growth media (i.e YPD) & Glycerol
  - Home refrigeration isn’t perfect - self-thawing means temperature isn’t consistent
  - Can circumvent with an ice pack “locker” for cultures

REDUCING GENETIC DRIFT

- -80°C freezers are best – Access through yeast lab (may have cost or strings attached

DETECTING CONTAMINATIONS

- Stock plates – inspect colonies for differing morphologies, colours (differential media), mould, etc.
- Slurries – streak out onto differential media, look underneath scope – need to know your strains
  - i.e. Cali contaminated with French Saison is very easy to see – French Saison has very small cells
- Lacto Cultures – Plate onto media, look for yeast cells under scope, pressure buildup in container

WHAT DOES A CONTAMINATION LOOK LIKE?

Really good!!!

QUESTIONS?