Lab Manual

NAME: _____

BYO Yeast Course 2021

WHITE LABS

PURE YEAST & FERMENTATION

GENERAL GUIDELINES FOR ASEPTIC TECHNIQUE

Success microbiology labs rely heavily on good aseptic technique. Performing microbiological testing on dirty work surfaces or with nonsterile supplies can lead to issues. Aseptic technique is designed to provide a barrier between the environment and your culture. Below are some guidelines on performing good aseptic technique to reduce the potential of contaminating your cultures.

Clean Work Area

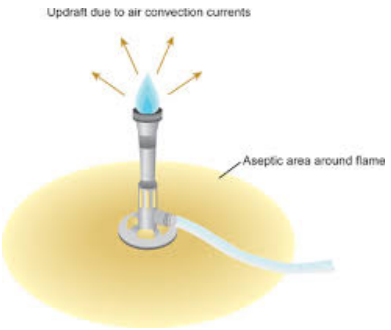
- Avoid air drafts and foot traffic
- Wash hands before touching sterile/sanitized equipment or containers
- Working surfaces should be washed with 70% isopropanol
- Be conscious of the work environment and equipment sterility/cleanliness

Good personal hygiene

- Tie back long hair
- Gloves should be worn and washed with 70% isopropanol
- Skin should be shielded with clothing or a lab coat

Handling

- Use sterilized glass or disposable plastic apparatuses when necessary
- Open and close caps on bottles, flasks, or tubes quickly to minimize air exposure
- Work with open bottles or flasks near a flame to avoid particles dropping into the apparatus



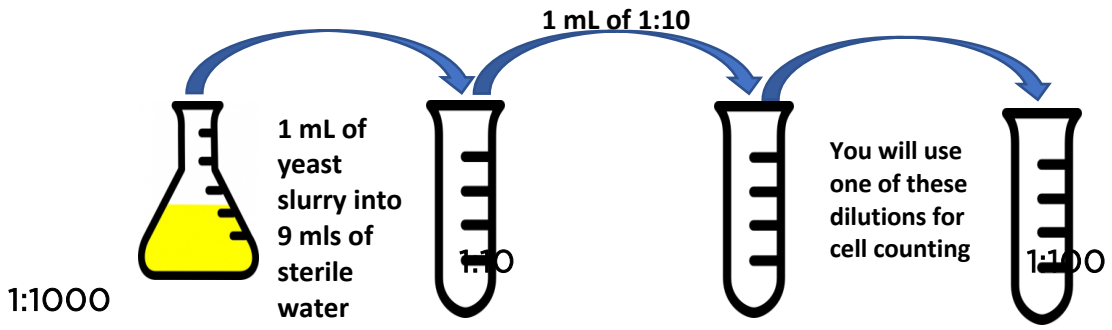
Serial Dilutions

MATERIALS

- Sterile water tubes (at least 2 will be needed for this dilution series)
- Transfer pipette
- Yeast slurry

METHOD

1. Set up test tubes in racks for dilutions with successive water tubes (depending on your desired dilution rate)
2. Label your tubes appropriately using a permanent marker.
3. Loosen the caps of the dilution tubes. Re-homogenize the yeast, carefully remove the lid, and pipette 1 mL of yeast and place into the 9mL water tube (for the primary dilution) using sterile transfer pipette. Pump the pipette to mix up the yeast and water. You have just made a 1:10 dilution
4. Using the same pipette, remove 1 mL of diluted yeast and place into the next water tube. This will make a 1:100 dilution.



HLP TESTING

MATERIALS

- Diluted yeast slurry or beer sample
- 15 mL conical tube
- Prepared HLP media (Microwaved)
- 70% Isopropanol
- Transfer pipette
- Bunsen Burner

METHOD

1. Wipe down working space with 70% isopropanol and turn on Bunsen burner
2. Label 15ml conical tubes with your sample name
3. Arrange tubes as close as possible next to flame to work inside the "aseptic bubble" generated by the flame.
4. Aseptically transfer 1ml of sample from to the sterile conical tube. Replace cap after dispensing sample.
5. Fill tube with approximately 14 ml of HLP media or until the media reaches the top of the tube. After securely closing the cap, invert tube multiple times to thoroughly mix sample.
6. After 5 days inspect plates. Negative tubes have no growth. Positive samples have white dots or tubes are opaque. (See included positive control)

STREAK PLATE

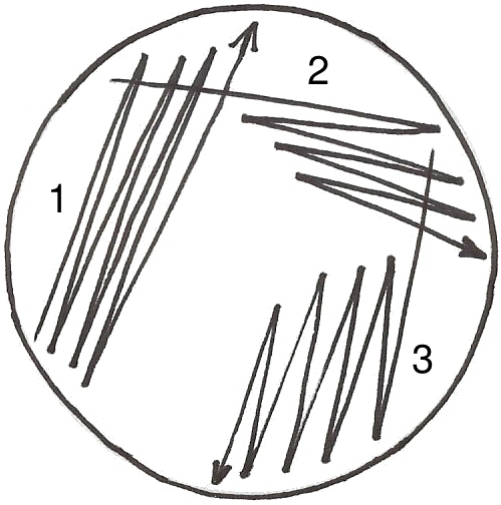
MATERIALS

- Agar plate with colony that you'd like to streak out (working plate)
- Sterile one time use inoculating loop(s)
- Pre-poured agar plate or slant (Generally YPD will be used for *Saccharomyces cerevisiae*)

METHOD

*Note: There are several acceptable methods that can be utilized to streak plates. One such method is described below.

1. Wipe down working space with 70% isopropanol and turn on Bunsen burner
2. Label YPD plate with your name, group number, and date
3. Arrange plates as close as possible next to flame to work inside the "aseptic bubble" generated by the flame.
4. Pick up 1 colony from working plate
5. Streak in a zigzag on 1/3 of the plate
6. Use new loop to streak through first zigzag
7. Use new loop to streak through second zigzag
8. Incubate at 30°C for 3-5 days



FORCED WORT

MATERIALS

- Wort (with out yeast
- Yeast slurry
- Sterile container- Whirlpak bag, sanitized flask or mason jar with foil
- Warm incubation space

METHOD

1. Sanitize sample valve
2. Aseptically transfer 50mL of wort to sterile container. Run wort out of the sample valve for at least 5 seconds before collecting a sample.
    a. Option to pipette sample from fermentor
3. Close container and incubate in warm area

Results:

Clear = 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

FORCED DIACETYL

MATERIALS

- Two glasses
- Aluminum Foil
- Hot Water Bath
- Thermometer

METHOD

Procedure:

1. Heat the water bath to 140 to 160 F

2. Collect beer into each glass and cover with aluminum foil

3. Place one glass in the hot water bath, while keeping the other at room temperature. Do not microwave!

4. After 10 to 20 min remove the beer from the hot bath, and cool to the same temperature as the other sample. Ice water bath is effective for cooling.

5. Remove the aluminum foil and smell each sample. If you smell the buttery character of diacetyl in either or both samples, you know your beer has the diacetyl precursor.

CELL COUNTING & VIABILITY

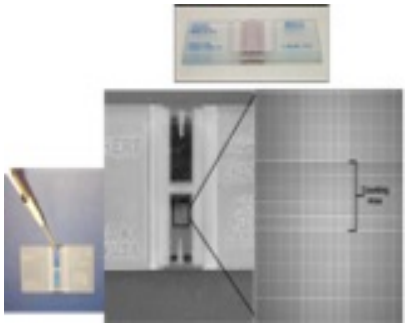
MATERIALS

- Methylene blue solution
- Hemocytometer
- Hand held counter
- Transfer pipets
- Kimwipes
- Gloves (optional)

You will need a microscope with 400X capability (40x lens + 10x eyepiece).

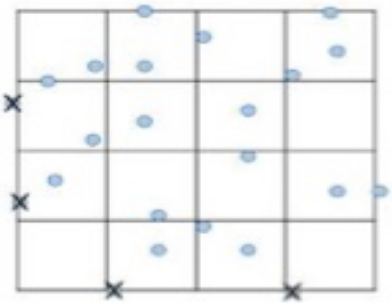
METHOD

1. Make certain hemocytometer is clean and dry before use. Hemocytometers can be easily cleaned with water and/or 70% EtOH. Chamber and cover slip may be scrubbed gently using a lint free towelette (kimwipe).
2. Dilute yeast sample to an appropriate concentration. Usually a 1:100 or 1:1000 works well for yeast slurry. Sample can be diluted with deionized/distilled water or with 0.5% H2SO4 if cells clump excessively. **Note your dilution factor.**
3. Invert sample several times to mix, taking care not to introduce bubbles.
4. Mix 1mL of your diluted yeast sample with 1mL of methylene blue solution and allow to incubate for 1 - 2 minutes.
5. Invert sample 1-2 times and take up sample by using a transfer pipette.
6. Fill chamber by placing 2 drops of sample onto center of counting grid.
7. Gently position cover slip so that glass covers both counting areas equally.
8. Carefully place hemocytometer on microscope stage. As you focus at each objective leading up to the 40X lens, note the distribution of yeast cells. If cells are well distributed, then you can use the short cell count method.



CONSIDERATIONS FOR WHEN CELL COUNTING

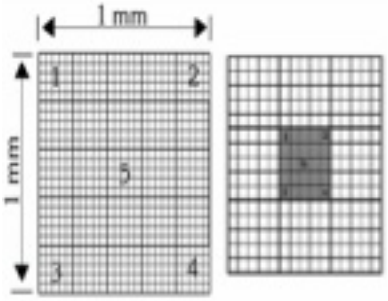
1. You will be counting squares within the 1mm^2 ruled area centrally located on the chamber (see images below, in next section)
2. It is helpful to establish a counting protocol for all cell counts and stick with it! Consistency is key!
   --For example, cells touching or lying on the top and right boundary lines are not counted, whereas cells touching or lying on the bottom or left boundary lines are counted (see image below).
3. Yeast cell buds emerging from mother cells are counted as a separate cell if the bud is at least one-half the size of the mother cell.
4. If performing viability counts, dead cells will stain dark blue. Non-viable cells do not have the metabolic capability to reduce the blue dye.
5. Do not count cells that are pale blue in color as dead. Some budding cells will also stain blue, do not count these cells as non-viable.
6. If you are performing a cell count and viability count simultaneously, it is best to count all cells on the hand-held counter and record noted dead cells on a written tally.



SHORT METHOD: FOR AN EVENLY DISTRIBUTED SAMPLE

1. You will be counting cells within the 5 numbered squares (see image below)
2. Estimate the total number of cells in grid by taking # of cells counted in 5 numbered squares and multiplying by 5
3. Your 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 = 1360000000 = 1.36×10^9 = 1.36 billion cells/mL

YEAST PITCHING CALCULATION

Example:

Parameter	Value	Unit
Batch size	5	Gallon
Strength of wort	1.056	S.G
Wanted re-pitching rate	1	Million cells / ml / degree Plato
Concentration of slurry	0.8	Billion cells / ml

Question: How much yeast slurry (volume) should the brewer add to achieve the wanted pitching rate?

First, let's calculate the total number of cells we want to add to the full batch of wort

Goal: We want 1 million cells / ml / degree Plato. With a 1.056 S.G wort, we need to convert to Plato. (Use chart) Therefore we want 13.8 million cells / ml or $13.8 * 10^6\ cells/ml$

We have 5 gal wort. 5 gal is 18927mls.

We have just calculated that we have 18927 ml wort. Therefore, our total goal of how many cells to add is:

$$18927ml * 13{,}800{,}000\ cells/ml = \mathbf{2.6 * 10^{11}}\ cells$$

Slurry concentration obtained by cell count is:

800 million cells / ml, or 8 *10^8 cells /ml

How much slurry to add?

Our goal was to add $\mathbf{2.6 * 10^{11}}\ cells$ cells to the fermentor and we have 0.8 million cells/ml in our slurry. To calculate this we must use the formula for pitching

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

So, now we have all we need:

$$Volume\ to\ pitch\ (ml)\ = \frac{\mathbf{2.6 * 10^{11}}\ cells}{8 * 10^{8} cells/ml}$$

$$Volume\ to\ pitch\ (ml) = 325ml$$

SPECIFIC GRAVITY TO PLATO CONVERSION FIGURE

Brix	Plato	SG
0.0	0.0000	1.0000
0.2	0.1970	1.0008
0.4	0.3970	1.0016
0.6	0.5970	1.0024
0.8	0.7970	1.0031
1.0	0.9970	1.0039
1.2	1.1970	1.0047
1.4	1.3971	1.0054
1.6	1.5971	1.0062
1.8	1.7971	1.0070
2.0	1.9972	1.0078
2.2	2.1972	1.0086
2.4	2.3973	1.0094
2.6	2.5973	1.0101
2.8	2.7974	1.0109
3.0	2.9975	1.0117
3.2	3.1975	1.0125
3.4	3.3976	1.0133
3.6	3.5977	1.0141
3.8	3.7977	1.0149
4.0	3.9978	1.0157
4.2	4.1979	1.0165
4.4	4.3980	1.0173
4.6	4.5981	1.0181
4.8	4.7982	1.0189
5.0	4.9983	1.0197
5.2	5.1984	1.0205
5.4	5.3985	1.0213
5.6	5.5986	1.0221
5.8	5.7987	1.0229
6.0	5.9988	1.0237
6.2	6.1989	1.0245
6.4	6.3990	1.0253
6.6	6.5991	1.0261
6.8	6.7992	1.0269
7.0	6.9994	1.0277
7.2	7.1995	1.0285
7.4	7.3996	1.0294
7.6	7.5997	1.0302
7.8	7.7998	1.0310
8.0	7.9999	1.0318
8.2	8.2000	1.0326
8.4	8.4002	1.0334
8.6	8.6003	1.0343
8.8	8.8004	1.0351
9.0	9.0005	1.0359
9.2	9.2006	1.0367
9.4	9.4007	1.0376
9.6	9.6009	1.0384
9.8	9.801	1.0392
10.0	10.0011	1.0400
10.2	10.2012	1.0409
10.4	10.4013	1.0417
10.6	10.6014	1.0425
10.8	10.8015	1.0434
11.0	11.0016	1.0442
11.2	11.2017	1.0450
11.4	11.4018	1.0459
11.6	11.6019	1.0467
11.8	11.8020	1.0475
12.0	12.0021	1.0484
12.2	12.2022	1.0492
12.4	12.4023	1.0501
12.6	12.6024	1.0509
12.8	12.8025	1.0518
13.0	13.0026	1.0526
13.2	13.2027	1.0534

Brix	Plato	SG
13.4	13.4027	1.0543
13.6	13.6028	1.0551
13.8	13.8029	1.0560
14.0	14.0030	1.0568
14.2	14.2030	1.0577
14.4	14.4031	1.0586
14.6	14.6031	1.0594
14.8	14.8032	1.0603
15.0	15.0032	1.0611
15.2	15.2033	1.0620
15.4	15.4033	1.0628
15.6	15.6033	1.0637
15.8	15.8034	1.0646
16.0	16.0034	1.0654
16.2	16.2034	1.0663
16.4	16.4034	1.0672
16.6	16.6034	1.0680
16.8	16.8034	1.0689
17.0	17.0034	1.0698
17.2	17.2034	1.0706
17.4	17.4034	1.0715
17.6	17.6034	1.0724
17.8	17.8034	1.0733
18.0	18.0033	1.0741
18.2	18.2033	1.0750
18.4	18.4033	1.0759
18.6	18.6032	1.0768
18.8	18.8032	1.0777
19.0	19.0031	1.0785
19.2	19.2030	1.0794
19.4	19.4030	1.0803
19.6	19.6029	1.0812
19.8	19.8028	1.0821
20.0	20.0027	1.0830
20.2	20.2026	1.0839
20.4	20.4025	1.0848
20.6	20.6024	1.0857
20.8	20.8023	1.0866
21.0	21.0021	1.0875
21.2	21.2020	1.0884
21.4	21.4018	1.0892
21.6	21.6017	1.0901
21.8	21.8015	1.0911
22.0	22.0014	1.0920
22.2	22.2012	1.0929
22.4	22.4010	1.0938
22.6	22.6008	1.0947
22.8	22.8006	1.0956
23.0	23.0004	1.0965
23.2	23.2002	1.0974
23.4	23.4000	1.0983
23.6	23.5997	1.0992
23.8	23.7995	1.1001
24.0	23.9992	1.1011
24.2	24.1990	1.1020
24.4	24.3987	1.1029
24.6	24.5984	1.1038
24.8	24.7982	1.1047
25.0	24.9979	1.1057
25.2	25.1976	1.1066
25.4	25.3972	1.1075
25.6	25.5969	1.1084
25.8	25.7966	1.1094
26.0	25.9963	1.1103
26.2	26.1959	1.1112
26.4	26.3956	1.1122
26.6	26.5952	1.1131

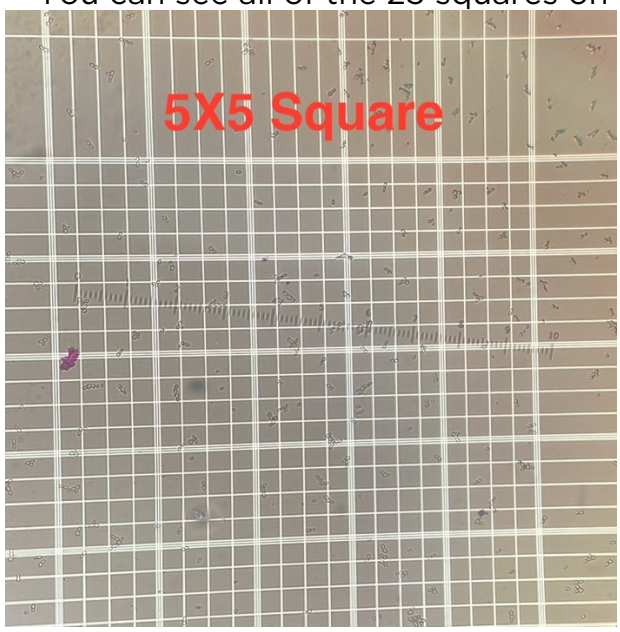
Brix	Plato	SG
26.8	26.7948	1.1140
27.0	26.9944	1.1150
27.2	27.1940	1.1159
27.4	27.3936	1.1168
27.6	27.5932	1.1178
27.8	27.7928	1.1187
28.0	27.9924	1.1197
28.2	28.1919	1.1206
28.4	28.3915	1.1216
28.6	28.5910	1.1225
28.8	28.7905	1.1235
29.0	28.9901	1.1244
29.2	29.1896	1.1254
29.4	29.3891	1.1263
29.6	29.5886	1.1273
29.8	29.7880	1.1282
30.0	29.9875	1.1292
30.2	30.1870	1.1302
30.4	30.3864	1.1311
30.6	30.5859	1.1321
30.8	30.7853	1.1330
31.0	30.9847	1.1340
31.2	31.1841	1.1350
31.4	31.3835	1.1359
31.6	31.5829	1.1369
31.8	31.7823	1.1379
32.0	31.9817	1.1389
32.2	32.1810	1.1398
32.4	32.3804	1.1408
32.6	32.5797	1.1418
32.8	32.7791	1.1428
33.0	32.9784	1.1437
33.2	33.1777	1.1447
33.4	33.3770	1.1457
33.6	33.5763	1.1467
33.8	33.7756	1.1477
34.0	33.9749	1.1487
34.2	34.1741	1.1497
34.4	34.3734	1.1507
34.6	34.5727	1.1516
34.8	34.7719	1.1526
35.0	34.9711	1.1536
35.2	35.1703	1.1546
35.4	35.3695	1.1556
35.6	35.5687	1.1566
35.8	35.7679	1.1576
36.0	35.9671	1.1586
36.2	36.1663	1.1596
36.4	36.3655	1.1606
36.6	36.5646	1.1617
36.8	36.7638	1.1627
37.0	36.9629	1.1637
37.2	37.1620	1.1647
37.4	37.3612	1.1657
37.6	37.5603	1.1667
37.8	37.7594	1.1677
38.0	37.9585	1.1688
38.2	38.1576	1.1698
38.4	38.3566	1.1708
38.6	38.5557	1.1718
38.8	38.7548	1.1728
39.0	38.9538	1.1739
39.2	39.1529	1.1749
39.4	39.3519	1.1759
39.6	39.5509	1.1770
39.8	39.7500	1.1780
40.0	39.9490	1.1790

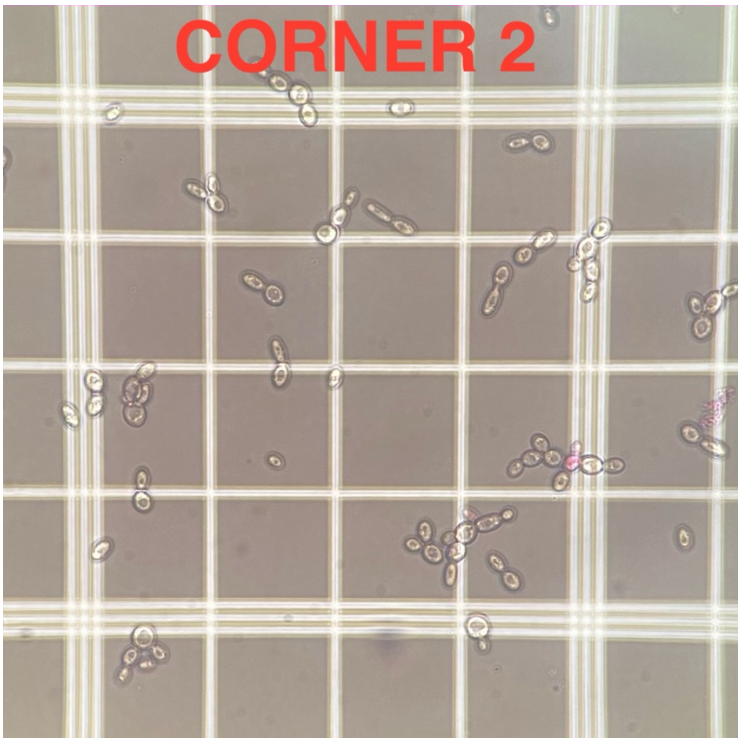
EXERCISES

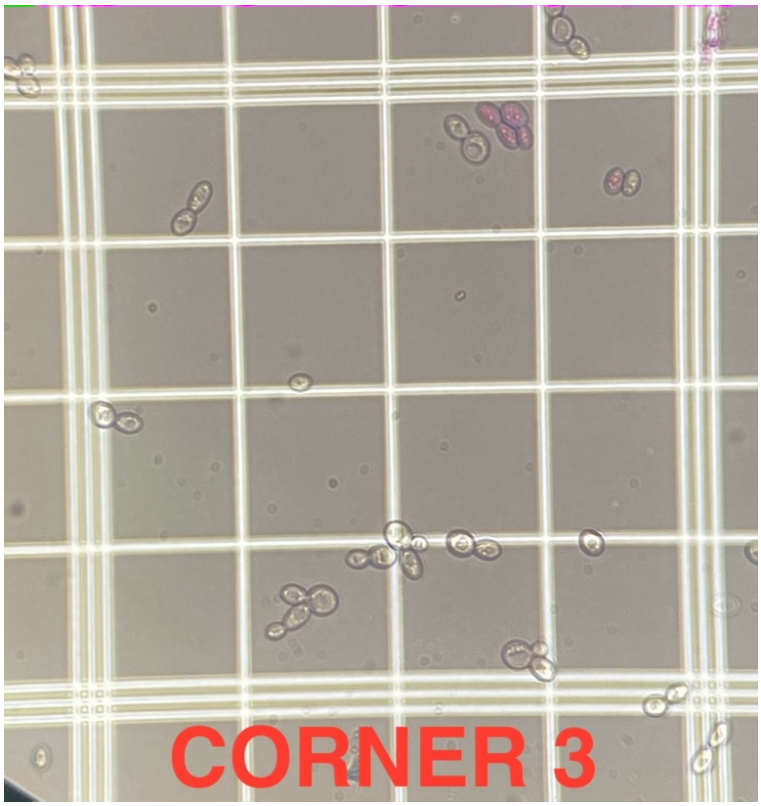
CELL COUNTING EXERCISE

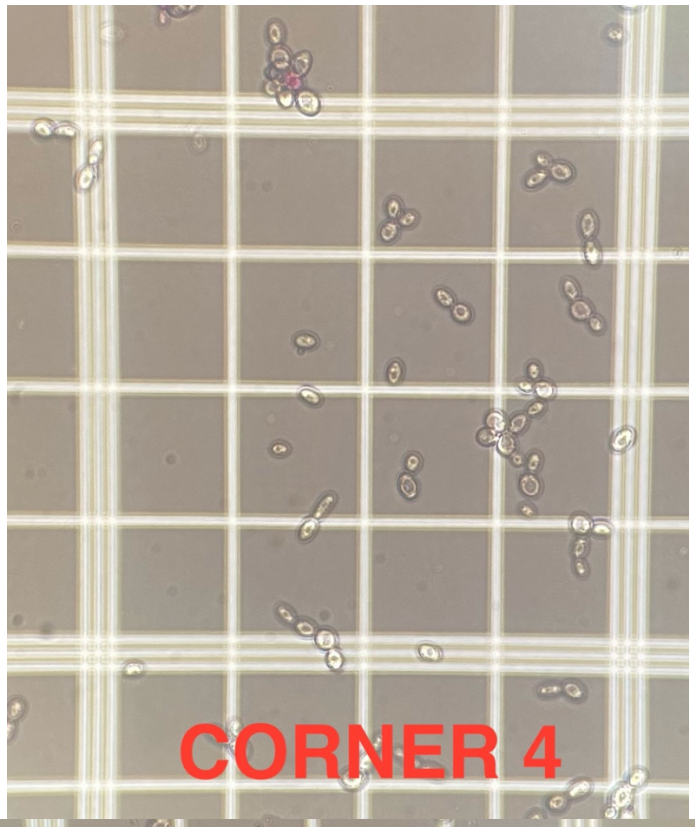
Counting the amount of cells dead and alive in each photo or microscope view.

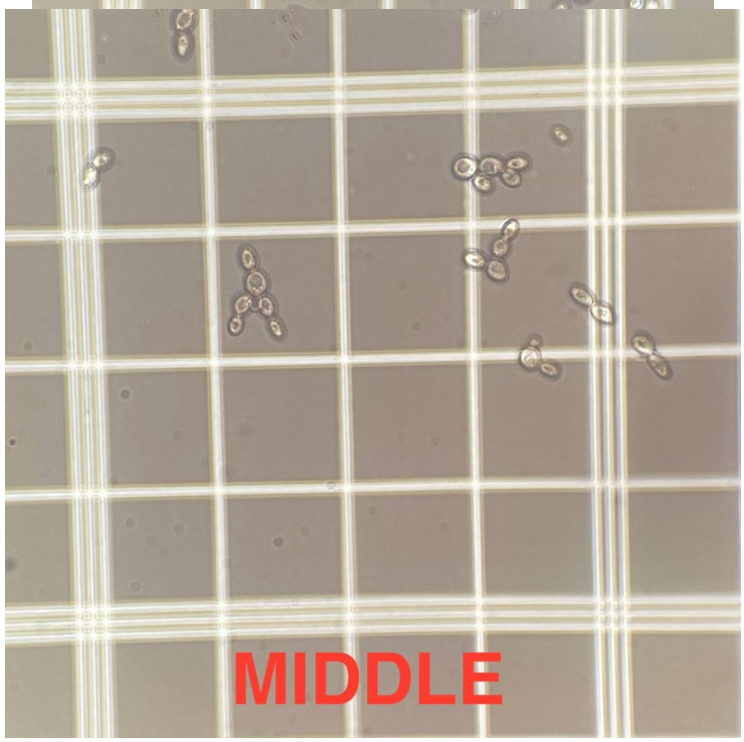
This view is at 100X – You can see all of the 25 squares on the hemocytometer




CORNER 2


CORNER 3


CORNER 4


MIDDLE

Based on the following parameters, calculate the cell count of the slurry.

Dilution Factor 1: 200

Yeast cells/mL = Total number of cells in grids (reminder to multiple by 5 for the average) x dilution factor x 10^4 (or 10,000, this number is a constant.)

PITCHING RATE EXERCISE

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

Question: How much yeast slurry (volume) should the brewer add to achieve the wanted pitching rate? Reminder of the equation is below

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