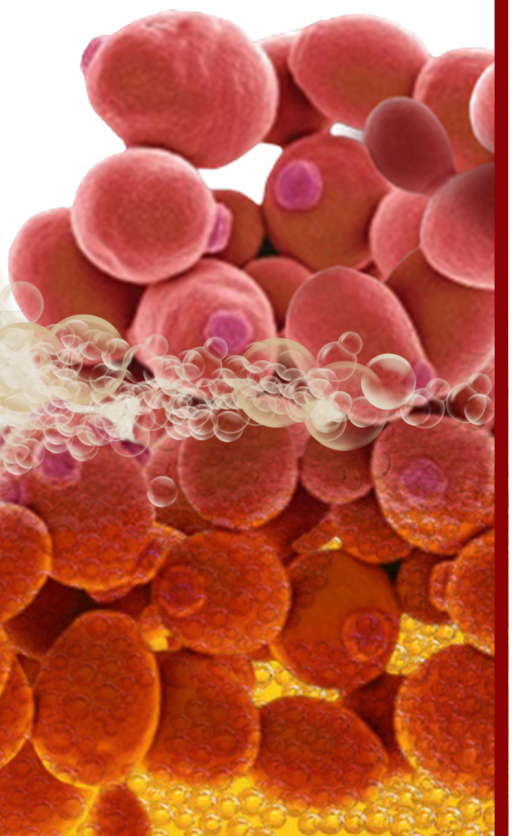
BREWING
WITHOUT THE BLINDFOLD™

BSI

Yeast Supplier & Microbiology Lab



BREWER'S LABORATORY HANDBOOK

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"Brewing without the blindfold" means keeping an eye on your microbes, be they brewing yeast or the myriad contaminants that plague breweries. This handbook helps you do just that, and respects the fact that you want to spend time in the brewery, not in the lab!

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ASEPTIC TECHNIQUE

The ability to transfer cultures without contamination is imperative to good microbiology. Keep in mind:

- ☐ Hands must be washed and work areas must be cleaned with a 5% solution of disinfectant, or sanitizer.
- ☐ Sterile vessels and liquids must be used (subject to sterilization at 15 psi pressure for 15 minutes in a pressure cooker, or autoclave).
- ☐ Sterile transfer tools must be used (dip in 70% ethanol or rubbing alcohol and flame).
- ☐ Aerial fall-out and drafts must be minimized.
- ☐ Use laminar flow hood if available.

For More Information See Protocols:

- ¤ Pouring Plates
- ¤ Drawing Samples
- ¤ Swabbing
- ¤ Plating Samples Directly
- ¤ Plating Samples Using Filtration

MICROSCOPIC EXAMINATION

For YEAST, use the 40x lens using a hemacytometer and methylene blue and make note of:

- ☐ Percent viability

For BACTERIA, use the 100x oil immersion lens and make note of:

- ☐ Whether cells are rods (bacilli) or round (cocci).
- ☐ The approximate length or diameter in microns.
- ☐ The gram reaction.

For RAPID microbial determination:

- ☐ Use 40x lens and wet mount method.

Microscopic examination of samples has limited use. The microscope is not very helpful for detecting even moderate levels of contamination, whether it be in yeast, wort or beer. If enough bacteria or wild yeast is present to be seen while surveying only a few fields, contamination levels are very high. Also keep in mind that different yeast strains can have very similar appearances under the microscope, so it can be difficult to tell a wild strain from a brewing strain, or to tell one brewing strain from another. Petite mutants are so-named because of their small colony size. They cannot be differentiated from normal cells under the microscope.

DETECTING MIXED STRAINS OF BREWING YEAST

If you use more than one strain of yeast in the brewery, you may wish to determine whether or not they are becoming intermingled. By plating the slurry for single colonies on wort agar and incubating for 5-7 days, you will be able to tell one from another after careful examination of colony shapes, sizes and colors. Any differences in these characteristics are solid indications of different strains.

For More Information See Protocols:

- ¤ Pouring Plates
- ¤ Streaking for Single Colonies
- ¤ Strain Purification

DETECTING BACTERIA and WILD YEAST with SELECTIVE MEDIA

If you know what type of contaminant you're encountering, you are better equipped to determine how serious a problem it presents and how to get rid of it. On a non-selective medium, such as wort agar, yeast colonies are relatively large, opaque, whitish and waxy-looking, while bacteria colonies are generally smaller, more translucent and slick-looking. Knowing this is fine, but a selective medium is needed for more specific identification. We recommend the following selective media for the basic groups of brewing contaminants:

- ☐ Brewing Yeast Bacteria - LMDA/SDA
- ☐ Wild Yeast - LCSM and LWYM

Each of these media are easy-to-use and allow for enumeration (counting the number of contaminants in the sample tested) and differentiation (knowing one type of organism on the plate from another), which is more than can be said for most testing media.

For More Information See Protocols:

- ¤ Pouring Plates
- ¤ Plating Samples Directly
- ¤ Plating Samples Using Filtration
- ¤ Streaking for Single Colonies
- ¤ Strain Purification

QUICK and DIRTY "FILM TEST" FOR BOTTLED BEER

Bottled products can be checked for gross contamination without much difficulty.

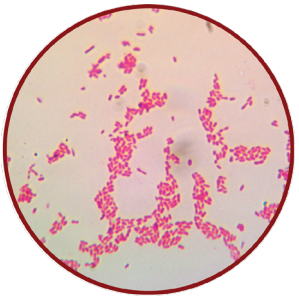
The following are undesirable:

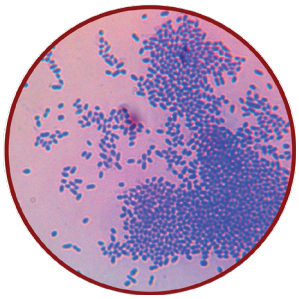
- ☐ Bottle necks with rings at the beer/headspace interface.
- ☐ Cloudiness in any part of the body.
- ☐ Residue at the bottom of filtered beer.
- ☐ Excessive foaming of opened, chilled beer.

Odor and taste are indicators as well. However, what is most useful is catching contamination well before it reaches taste, odor and visibility thresholds.

SIMPLE TESTS FOR IDENTIFYING BACTERIA

Make the following observations of any bacteria colonies before disturbing the colony with stain or other tests:





- ☐ Odor (vinegary, rotten, sulfury, fruity)
- ☐ Acid Production (halo surrounding colony with color change and/or clearing of medium)
- ☐ Colony Color, Size, Texture, Shape

The gram stain takes advantage of certain differences in cell membrane properties of bacteria. All bacteria are divided into two categories: gram-positive (takes on crystal violet stain and turns blue) and gram-negative (takes on safranin stain and turns pink). Yeasts are gram-positive. Armed with all the above information, check Table of Brewing Bacteria for bacteria's identification.

For More Information See Protocols:

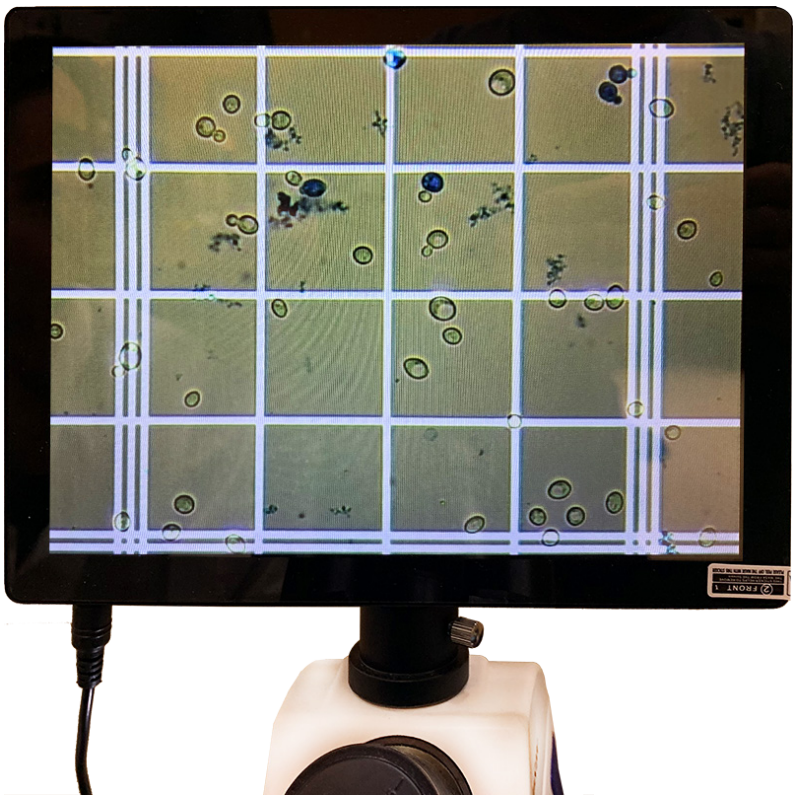
- ¤ Gram Staining and Protocol.
- ¤ Catalase & Oxidase Tests.

CELL COUNTS and DETERMINATION of PROPER PITCHING RATE

A hemacytometer is essential for accurate cell counts (determination of number of yeast cells/ml in a slurry). The number of cells counted within a chosen grid is converted into cells/ml. This number is compared to the number of cells needed for healthy fermentation.

For More Information See Protocols:

- ¤ Cell Counting



SAMPLE	FREQUENCY	SAMPLE SIZE	COMMON CONTAMINANTS	TOLERANCE
Water Supply	Once per Week	100ml, Filtered	Enteric, Molds	<10 cfu
Wort	Every Brew	1.0ml	Enteric, Acetic & Lactic, Wild Yeast	<10 cfu; 0 cfu wild yeast
Pitching Yeast	Every Crop	1.0ml (Dilution required)	Enteric, Acetic & Lactic, Wild Yeast	<10 cfu; 0 cfu wild yeast
Fermenting Beer, days 1-2	Every Tank	1.0ml	Enteric, Acetic & Lactic, Wild Yeast	<10 cfu; 0 cfu wild yeast
Fermenting Beer, days 3-5	Every Tank	1.0ml	Acetic & Lactic, Wild Yeast	<10 cfu; 0 cfu wild yeast
Storage Tank	3 Times per Week	1.0ml	Acetic & Lactic	<10 cfu
Finishing Tank	3 Times per Week	1.0ml	Lactic	<10 cfu
Bottling Tank	Once per Month	100ml, Filtered	Lactic	<10 cfu
Bottled Beer	Every Batch	100ml, Filtered	Acetic & Lactic	<10 cfu
CIP'd Surfaces	Every CIP	Swab	-	0 cfu
Note: CFU = colony-forming units, or the number of colonies growing on the test plate.				

PROTOCOL: DRAWING SAMPLES

Equipment

- ☐ 70% ethanol or rubbing alcohol
- ☐ Sterile sample tube

Procedure

- ¤ Wash and dry hands thoroughly.
- ¤ Sterilize intervening "outside" surfaces, such as valves, with 70% ethanol and flame.
- ¤ Do not uncap tube until immediately before sampling.
- ¤ Do not touch inside of tube or cap to any surface.
- ¤ If possible, allow sample to flow or crumble directly into tube without using utensils.
- ¤ Do not allow sample to overflow onto outside of tube.
- ¤ Recap tube as soon as possible.

PROTOCOL: SWABBING

Equipment

- ☐ Sterile swab tube

Procedure

- ¤ Wash and dry hands thoroughly.
- ¤ Unscrew swab/cap from tube without touching inside of tube or cap to any surface.
- ¤ Firmly swab area to be tested, twisting swab to expose entire tip to area in question.
- ¤ Immediately place exposed swab back into tube, tighten cap and label.

Notes:

Exposed swabs can be tested by streaking across the surface of plated media, or by adding 10ml sterile wort to swab tube and allowing to grow in a warm area (~86ºF/30ºC) for 3 - 5 days.

For More Information See Protocols:

- ¤ Plating Samples Directly
- ¤ Wort Stability Test

PROTOCOL: PLATING SAMPLES DIRECTLY

Plated media are generally used to test the purity of liquid samples such as wort, yeast slurry and bottled beer. It is important that the plates do not become contaminated with anything other than the sample you want tested, so store your plates refrigerated and upside-down in a clean and sanitized plastic box until you are ready to use them.

Equipment

- ☐ Starsan or a 5% disinfectant solution
- ☐ Media plates
- ☐ Sterile pipettes
- ☐ Plastic box

Procedure

- ¤ Choose an area that is enclosed or away from drafts and messy or dusty operations.
- ¤ Wipe down counter top or table with Starsan or a 5% Lysol or bleach solution.
- ¤ Wash and dry hands thoroughly.
- ¤ Inspect each new plate for contamination; label underside with date, sample name, sample site and size of sample plated.
  - » *Liquid sample*: place sample (based on sample size Protocol page 3). on media using a sterile pipette, gently spread over entire surface without gouging media and immediately replace plate lid.
  - » *Swab sample*: streak swab tip over entire media, twisting swab to expose all of the tip to the media surface and immediately replace plate lid.
- ¤ Place plates in box upside-down; loosely fit box lid and allow to grow in a warm area (~86ºF/30ºC) for 3 - 5 days. Use incubator to store plates if available.
- ¤ Do not wrap plates or seal plates in any way.

PROTOCOL: POURING PLATES

Equipment

- ☐ 5% disinfectant, sanitizer, Isopropyl alcohol
- ☐ Sterile petri dishes
- ☐ Sterile prepared media
- ☐ Permanent marker

Procedure

- ¤ Prepare media according to instructions.
- ¤ Wash and dry hands thoroughly.
- ¤ Clean work area with a 5% disinfecting solution such as Lysol or bleach.
- ¤ Lay out, label and date underside of plates and turn each back over with the covers up.
- ¤ Allow media to cool until it is comfortably-warm to the touch (about 50ºC or 122ºF).
- ¤ Swirl media occasionally to keep any insoluble components of media suspended.
- ¤ Pour 15ml (7ml for small plates) directly into each plate (about 5mm thick), lifting covers just enough to allow for pouring.
- ¤ Do not disturb plates until media has solidified.
- ¤ Invert and store plates refrigerated in a clean plastic bag.

Notes:

Pouring media while too hot will result in condensate inside the plate, which increases the chances of contamination. You can check plates for contamination by examining them after 48 hours of incubation in a warm area (~86ºF/30ºC). Wort agar (instead of commercial nutrient agar) = 30ml house wort, 70ml water, 2g agar; sterilize for 15 minutes at 15psi.

PROTOCOL: WORT STABILITY TEST

Equipment

- ☐ 70% ethanol or rubbing alcohol
- ☐ Sterile sample tube

Procedure

- ¤ Wash and dry hands thoroughly.
- ¤ Sterilize intervening “outside” surfaces, such as valves, with 70% ethanol and flame.
- ¤ Do not uncap sample tube until immediately before sampling.
- ¤ Do not touch inside of tube or cap to any surface.
- ¤ If possible, allow cooled, aerated wort to flow directly into tube without using utensils; fill tube about halfway.
- ¤ Do not allow sample to overflow onto outside of tube; recap loosely.
- ¤ Allow to grow in a warm area (~86ºF/30ºC) for 3 - 5 days.

Notes:

This test determines whether or not brewing organisms are present without allowing for their identification. Clear, bubble-free wort after 3 days of incubation at room temperature indicates the sample contains no viable brewing organisms. Cloudiness, bubbles or gas escaping when lid is loosened indicates that live organisms are present, which means either your sanitizing regime is inadequate or sterile materials and surfaces are being exposed and contaminated.

PROTOCOL: PLATING SAMPLES USING FILTRATION

If the entire sample is too large to be plated directly (>1.0ml), sample filtration is necessary. The purpose of filtration is to trap any organisms present onto a membrane filter by drawing the sample through it. The filter is then laid directly onto plated media.

Equipment

- ☐ Starsan or a 5% disinfectant
- ☐ 70% alcohol
- ☐ Gas burner
- ☐ Vacuum pump
- ☐ Sterile filtration apparatus
- ☐ Sterile 0.45 µm filters
- ☐ Tweezers
- ☐ Media plates

Procedure

- ¤ Choose an area that is enclosed or away from drafts and messy or dusty operations.
- ¤ Wipe down counter top or table with Starsan or a 5% disinfectant.
- ¤ Wash and dry hands thoroughly.
- ¤ Inspect each new plate for contamination; label underside with date, sample name and sample site.
- ¤ Dip tweezers in alcohol and light with flame. Allow flame to extinguish on its own.
- ¤ Use tweezers to place sterile filter into filtration apparatus; reassemble apparatus.
- ¤ Flame mouth of sample vessel and add 100ml to the top receptacle.
- ¤ Cap top receptacle and attach vacuum to bottom receptacle. Draw entire sample through filter.
- ¤ Sterilize tweezers as above; remove top receptacle and retrieve filter[1].
- ¤ Place filter face-up on agar, making sure membrane's entire underside is in contact with media.
- ¤ To filter another sample, liberally spray alcohol into apparatus and draw through with vacuum; load with sterile filter.
- ¤ Wipe down a plastic box with Starsan or a 5% disinfectant.
- ¤ Place plates in box upside-down; loosely fit box lid and allow to grow in a warm area (~86ºF/30ºC) for 3 days.
- ¤ Do not wrap plates or seal plates in any way.

Notes:

[1]If another sample is to be filtered, disassemble apparatus and liberally spray down interior of top receptacle with alcohol; empty using vacuum suction. Sterilize tweezers, put fresh filter in place and repeat filtration procedure.

PROTOCOL: CATALASE & OXIDASE TESTS

Equipment

- ☐ 3% hydrogen peroxide
- ☐ Glass slide
- ☐ Oxidase "dry slides"

Procedure: Catalase Test

Drop a little hydrogen peroxide onto the colony in question, or onto bacteria smeared on a clean glass slide.
A positive result is indicated by the formation of bubbles.

Procedure: Oxidase Test

Smear a little of the colony in question onto oxidase "dry slide."
A positive result is indicated by the bacteria turning a very dark "royal" purple color within 2 minutes.

PROTOCOL: GRAM STAINING

Equipment

- ☐ Microscope with 100x objective
- ☐ Immersion oil
- ☐ Glass slide
- ☐ Gram stain kit

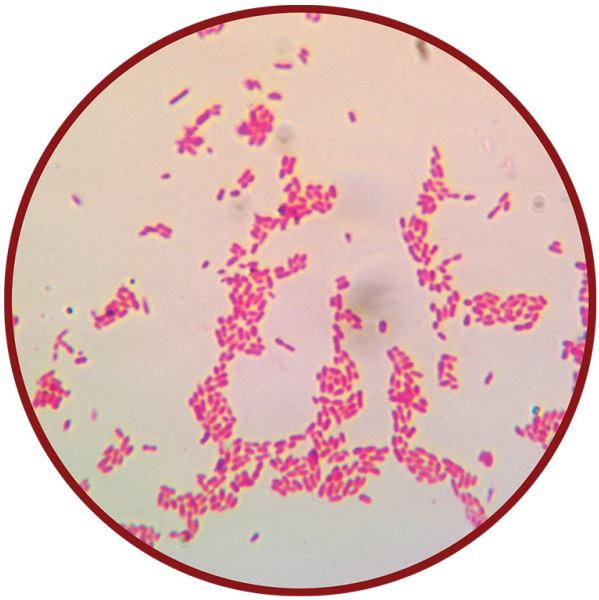
Procedure:

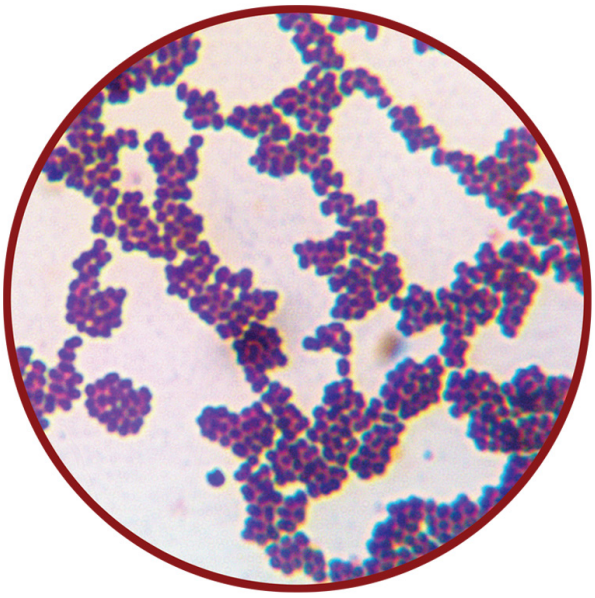
- ¤ Place a drop of water on a clean glass slide. Barely touch a flamed and cooled inoculating loop to the colony[1]
- ¤ "Wash" loop in drop of water and evenly smear the suspension around in a pea-sized area; allow to air-dry.
- ¤ Hold glass slide about 3 inches above flame and hold for 4 seconds to heat fix organisms to slide; allow slide to cool.
- ¤ Cover smear with crystal violet stain and leave on for 1 minute.
- ¤ Rinse and flood slide with iodine mordant; leave on for 3 minutes then drain.
- ¤ Slowly drip decolorizing rinse onto smear while holding slide vertically; stop as soon as color ceasing flowing from smear[2]
- ¤ Cover smear with safranin counterstain, leave on for 1 minute, then rinse gently with water and allow to air dry.
- ¤ Examine under microscope using oil-immersion lens. Gram$^+$ is blue or purple; gram $^-$ is red or pink.

Notes:

[1]Adding too much bacteria to the water droplet will lead to too dense a smear, making good staining almost impossible. The proper amount of bacteria will be a barely visible dot of material on the loop.

[2]The decolorizing rinse is the most critical step. If you continually get gram$^-$ or variable results for known gram$^+$ bacteria, you are over-rinsing. Gram$^+$ results for gram$^-$ bacteria can result from too dense a smear.





PROTOCOL: STREAK-PLATING FOR SINGLE COLONIES

Equipment

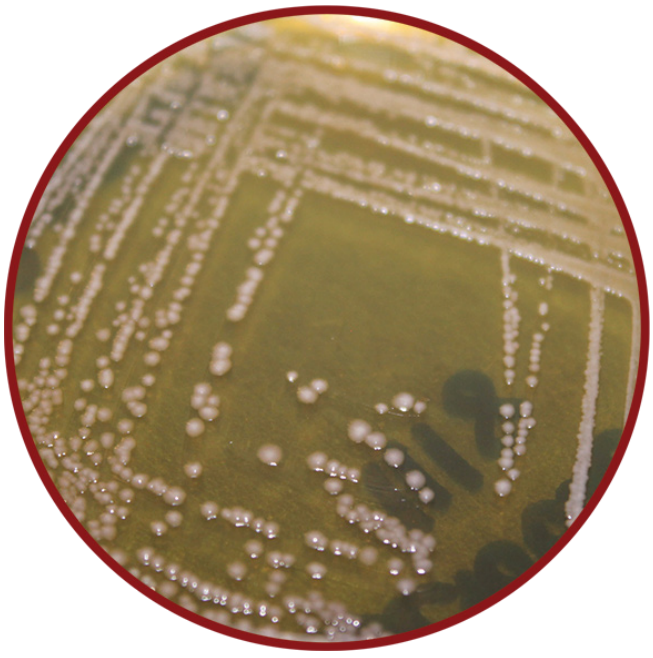
- ☐ Wort agar[1] plate
- ☐ Yeast culture
- ☐ Loop
- ☐ Flame

Procedure:

- ¤ The inoculation loop is first sterilized by passing it through a flame. When the loop is cool, barely touch the edge of the inoculating loop to a yeast colony or suspension of yeast.
- ¤ The inoculation loop is then dragged across the surface of the wort agar back and forth in a zigzag motion until approximately 30% of the plate has been covered.
- ¤ The loop then is re-sterilized and the plate is turned 90 degrees.
- ¤ Starting in the previously streaked section, the loop is dragged through it two to three times continuing the zigzag pattern.
- ¤ The procedure is then repeated once more being cautious to not touch the previously streaked sectors.
- ¤ Each time the loop gathers fewer and fewer yeast cells until it gathers just single yeast cells that can grow into a colony.
- ¤ The plate should show the heaviest growth in the first section.
- ¤ The second section will have less growth and a few isolated colonies, while the final section will have the least amount of growth and many isolated colonies.

Notes

[1]Wort agar = 30ml house wort, 70ml water, 2g agar; sterilize for 15 minutes at 15psi.



PROTOCOL: YEAST HARVEST & STORAGE

Equipment

- ☐ 70% ethanol or rubbing alcohol
- ☐ Sanitizer
- ☐ Storage container
- ☐ Cold water
- ☐ Sterile Wort

Procedure:

- ¤ Sterilize valves with 70% ethanol and flame, and sanitize any hoses, utensils and storage containers to be used.
- ¤ Catch the middle layer of yeast in a storage container as follows, discarding top and bottom layers:
- ¤ CONICAL vessels require draining the bottom trub-filled layer until good color and consistency is seen.
- ¤ ROUND vessels require manual harvesting with a paddle (layers mix if allowed to drain through the valve).
- ¤ Acidify about 100ml of water to ~pH 3 using a food-grade acid.
- ¤ For every gallon of yeast to be treated, add 2ml sodium chlorite[1] to the acidified water.
- ¤ When concoction turns a very pale yellow (at about 1 - 2 minutes) add to the yeast slurry, mixing well.
- ¤ Allow to sit for a minimum of 30 minutes (longer reaction time ensures effectiveness).

- ¤ Use yeast as needed or add an equal volume of sterile wort and store at 34°F/1°C with pressure relief for up to 2 weeks.
- ¤ If stored, test for viability, cell count and contamination before pitching.

Notes:

It is best to harvest yeast immediately after final gravity has been reached, before diacetyl rest. Even if it is kept very cold, yeast should not be stored in the cone or under beer. Finished beer is a nutrient-poor waste product to yeast, and forcing it to reside there will cause the viability to drop precipitously (over 50% within 48 hours), no matter how cold it is kept!

[1]DioxyChlor™ from BIRKO at (800) 525-0476, or Star-Xene™ from Five Star at (800) 782-7019.

PROTOCOL: CELL COUNTING

Materials

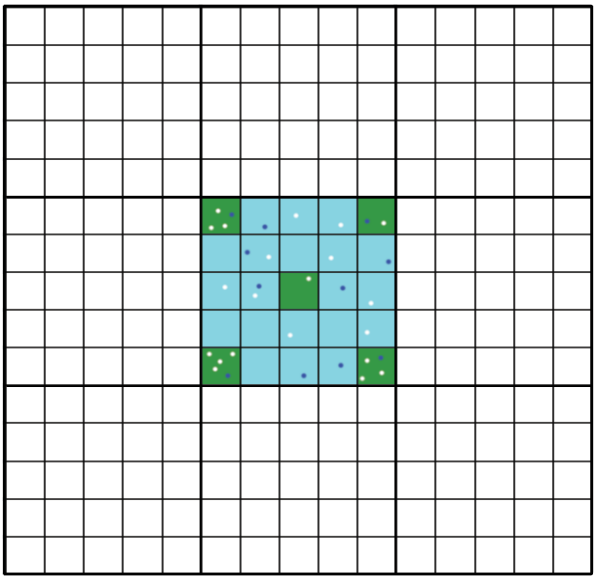
- ☐ Microscope with 40x objective
- ☐ 0.01% Methylene Blue Solution
- ☐ Hemacytometer and its Clean, Dry Cover Slip
- ☐ Vortex
- ☐ Handheld Counter
- ☐ Pipets

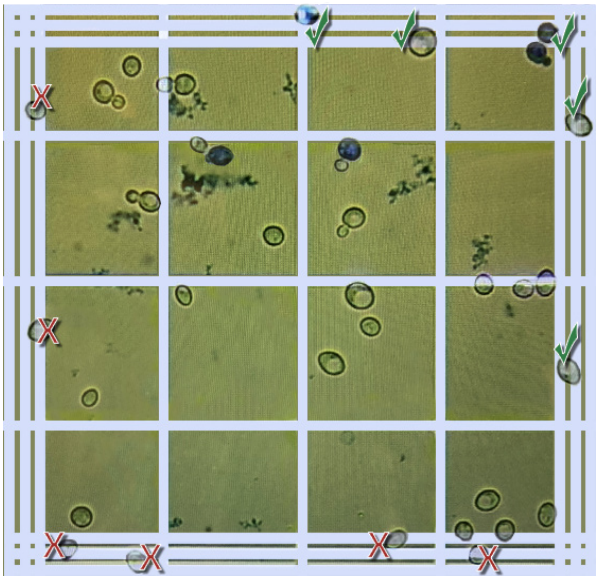
Staining

- ¤ Obtain a homogenized yeast cell suspension, keeping track of any dilutions[1] made.
- ¤ Vortex the diluted yeast sample and mix it in a ratio of 1:1 with 0.01% Methlyene Blue solution. For example, mix 1ml of dilute yeast with 1ml of 0.01% Methylene Blue solution.
- ¤ Mix the sample until the color is uniform; allow it to sit for 1 - 2 minutes.
- ¤ Pipet cell sample into one of the hemocytometer chamber and let it fill by capillary action.
- ¤ Carefully place hemocytometer on microscope stage and view under 40x objective.
- ¤ For viability count; dark blue cells are non-viable dead cells while clear are viable cells.

Counting:

- ¤ Locate the central square with 25 smaller squares which are gridded in a 4x4 pattern.
- ¤ Count the total number of yeast cells in 5 of the squares using pattern shown at the page bottom. Record dead cells separately for the viability count.
- ¤ Count cells touching or resting on the top and right middle lines. Don't count cells touching or resting on the bottom or left middle lines.
- ¤ Yeast cells that are budded are counted as one cell if the bud is less than ½ the size of the mother cell.





PROTOCOL: CELL COUNTING

Calculating Pitch Rate:

HOW MUCH YEAST DO YOU HAVE?

Yeast Slurry Density: ____cells counted in 5 squares x 5* x 10,000 x ____dilution factor[1] = ____million cells/ml

** Multiply by 5 to get total number of squares in the grid.*

Slurry Volume: _____gal x 3800ml/gal = _____ml

Total Viable Cells Available: _____million cells/ml x _____ml x viability/100 = _____Cells Available

Calculating Viability:

$$\text{Viability \%} = \frac{\text{Live yeast cells}}{\text{Live + dead yeast cells}} \times 100$$

How Much Wort Do You Have?:

Pitch Rate: _____°P x 1 million cells/ml = _____million cells/ml

Wort Volume: _____bbl x 31gal/bbl x 3800ml/gal = _____ml

Total Cells Required: _____million cells/ml x _____ml = _____Cells Required

Notes:

[1]Confused about making dilutions and calculating dilution factors? Consider a 1:10 dilution. The number on the left side of the colon is the volume (in ml, let's say) you begin with; the number on the right is the volume you end up with. A dense slurry may require a 1:100 dilution, namely, the addition of 1ml yeast to 99ml water, in order to render it countable. There is another way to get a 1:100 dilution.

You may add 1ml yeast to 9ml water, mix, then remove 1ml of that suspension and add it to 9ml of fresh water. In other words, two 1:10 dilutions made in series are equivalent to a single 1:100 dilution. Similarly, three 1:10 dilutions made in series are equivalent to a single 1:1000 dilution. The dilution factor is the product of the numbers on right side of the colon. For example, a 1:10 dilution followed by a 1:4 dilution is equal to a 1:40 dilution and has a dilution factor of 40.

Notes:

Note: if yeast cells are clumped you may use a declumping agent for dilution such as sulfuric acid and EDTA.

Or count 25 squares for a more accurate representation.

BACTERIA TYPES OVERVIEW

ACETIC ACID BACTERIA

Acetobacter, Acidomonas

- From plant material
- Found in wort and sometimes bottled beer
- Contributes vinegary or cidery flavor

ENTERIC BACTERIA

Citrobacter, Enterobacter, Hafnia, Klebsiella, Obesumbacterium

- From water, soil, plant material
- Found in wort, early stages of fermentation
- Enterobacter and Citrobacter killed at pH 4.4 and 2% alcohol, others are not
- Contribute fusel alcohols, sulfur compounds, phenolics, and diacetyl

LACTIC ACID BACTERIA

Lactobacillus, Pediococcus

- From grain dust
- Found throughout brewing process
- Contributes tart or sour flavor, diacetyl

NON-BREWING BACTERIA

Staphylococcus, Micrococcus, and Streptococcus

- From skin cells, hair cells, dust, air, malt, saliva
- Not considered brewing bacterias
- May survive in beer, but does not produce off flavors

OTHER BACTERIA

Megasphaera, Pectinatus, Zymomonas

- Occur rarely
- Sources vary
- Found in areas with little oxygen, such as CO_2 recovery systems
- Zymomonas tolerates up to 10% alcohol
- Contributes sulfur compounds, acetaldehyde

SUMMARY 1

BACTERIA IDENTIFICATION CHART

GENUS	GRAM	ACID	CATALASE	OXIDASE
Acetic Acid Bacteria *(smell sharp and vinegary)*				
Acetobacter	-	+	+	-
Acidomonas	-	+	+	+
Enteric Bacteria *(smell rotten, sulfury, or like a bad cold)*				
Citrobacter	-	-	+	-
Enterobacter	-	-	+	-
Hafnia	-	-	+	-
Klebsiella	-	-	+	-
Obesumbacterium	-	-	+	+
Lactic Acid Bacteria *(smell sharp and fruity)*				
Lactobacillus	+	+	-	-
Pediococcus	+	+	-	-
Other Bacteria				
Megasphaera	-	+	+	
Pectinatus	-	-		
Zymomonas	-	-	+	-
Non-Brewing Bacteria				
Staphylococcus	+	+	+	-
Streptococcus	+	-	-	-
Micrococcus	+	-	+	-

SUMMARY 2

Wort Aeration:

Aeration rates vary with wort volume, wort gravity and knockout time. In general, 8-10ppm of dissolved oxygen is desired for normal gravity worts. If aerating in-line with pure oxygen for the duration of a 1-hour knockout, this translates into 0.14LPM for every 10bbl of wort:

- **10ppm = 10mg/L = 11,780mg/10bbl**
- **1.4mg O_2= 1ml O_2**
- **11,780mg O_2 = 8,414ml O_2 = 8.4L O_2**
- **8.4L of O_2 delivered over 1 hour = 0.14L delivered over 1 minute**

Sanitizing Time vs Temperature:

The entire system must be kept at:

- **160°F (71°C) for 45 minutes**
- **170°F (77°C) for 30 minutes**
- **180°F (82°C) for 25 minutes**

Conversions

- **l = liter**
- **hl = hectoliter**
- **bbl = beer barrel**
- **1 gallon = 3.8L**
- **1hl = 100L**
- **1bbl = 118L**
- **1bbl = 31 gallons**
- **°C = 5/9 (°F - 32)**
- **°F = (9/5 °C) + 32**
- **° Plato = % sugar = ¼ (last two digits of specific gravity reading)**
- **5° Plato = 5% sugar = 1.020 sg**

OE = original extract, ° Plato **AE** - apparent extract , ° Plato **RE** = real extract, ° Plato

- % alcohol by weight = 0.42 (OE - AE)
- % alcohol by weight = 0.52 (OE - RE)
- % alcohol by weight = 2.22 (RE - AE)
- % alcohol by volume = (% alcohol by weight x specific gravity of beer) / 0.791

GLOSSARY

Aerobe: an organism which needs oxygen to survive (obligate aerobes will die without air).

Anaerobe: an organism which does not need air to survive (obligate anaerobes will die if exposed to air, while facultative anaerobes can grow with or without air).

Aseptic: against or without infection.

Contaminant: a relative term referring to any organism which lends undesirable characteristics; what is "bad" in one kind of brew may be intentionally cultured in another.

Inoculate: to introduce an organism to a sterile environment, intentionally or not.

Morphology: shape or appearance.

Petite mutant: a yeast with deficient respiratory abilities, resulting in slow growth and small (petite) colony size.

Strains: subspecies or "races" of *Saccharomyces cerevisiae*; all brewing yeasts are strains of this species.

Viability: percent of live cells in a population; not to be confused with vitality, which refers to metabolic activity or vigor of live cells.

Wild yeast: undesirable, undomesticated, or non-brewing yeast.

BSI
Yeast Supplier & Microbiology Lab

LAB SUPPLIES

ITEM	SIZE/QUANTITY	COMPANY
Wort Stability Test Jar	500ml	Your Science Hub
2 liter PP bottles	2000ml	Your Science Hub
Hemacytometer		Your Science Hub
Gram Stain Kit	each	Your Science Hub
Cotton Tip Applicators	box of 1000	Your Science Hub
Inoculating Loop	each	Your Science Hub
Sterile Transfer Pipettes	box of 500	Your Science Hub
Methylene Blue (1%)	each	Your Science Hub
Filter Apparatus	250ml	Fisher
Filter Apparatus	500ml	Fisher
Filter Apparatus	1000ml	Fisher
Filters/Filter App (0.45um)	box of 100	VWR
Hand Vacuum Pump	each	Your Science Hub
Sanitizer Spray Bottle	each	Your Science Hub
Forceps	each	Your Science Hub
Propane Torch	each	Hardware Store
Glass Slides	box	Your Science Hub
Immersion Oil	each	Your Science Hub
PP Samples Bottles	500ml	Your Science Hub
Anaerobic GasPak	box	Bacterius Ltd
New Monocular Microscope	each	Your Science Hub
New Binocular Microscope	each	Your Science Hub
Universal Beer Agar		Your Science Hub
70% Isopropyl Alcohol	each	Drugstore
3% Hydrogen Peroxide	each	Drugstore
Tupperware Box for Agar Plate Storage		
Tupperware Box for Incubator		

Fisher Scientific 1-800-766-7000
Your Science Hub 1-800-223-3517
VWR Labshop 1-888-897-5463
Bacterius Limited 1-832-474-4110*
**(additional charges for telephone orders may apply) sales@bacteria.us.com*