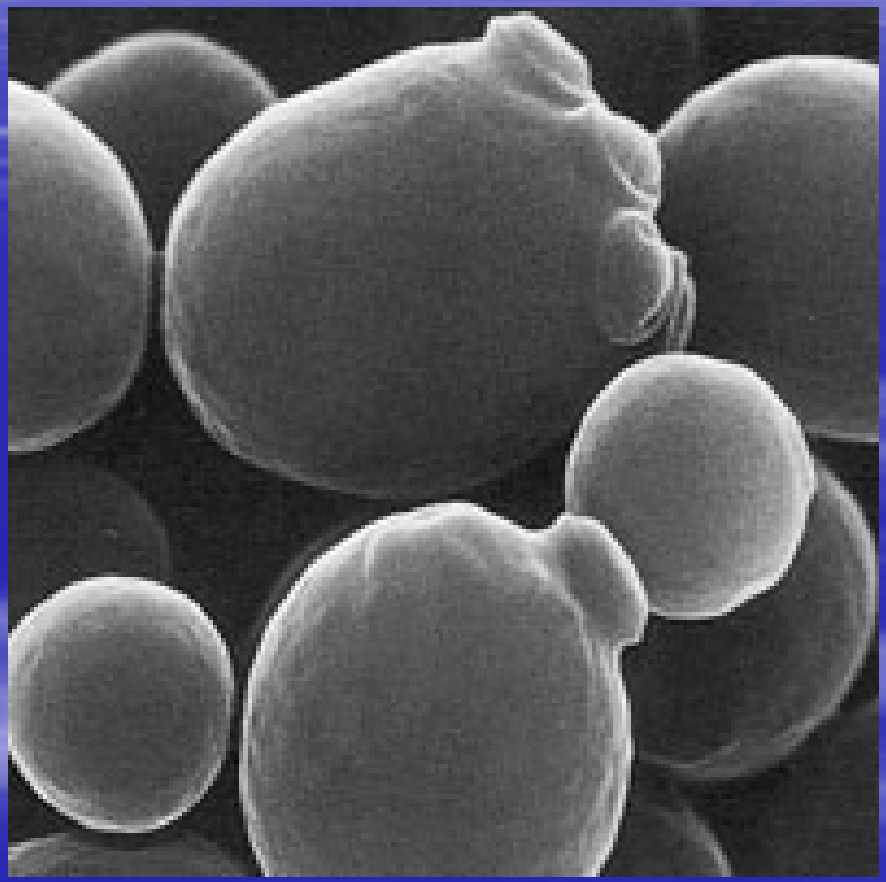
Basic Brewing Microbiology



BSI
Yeast Supplier & Microbiology Lab

www.brewingscience.com

Brewing Microbiology

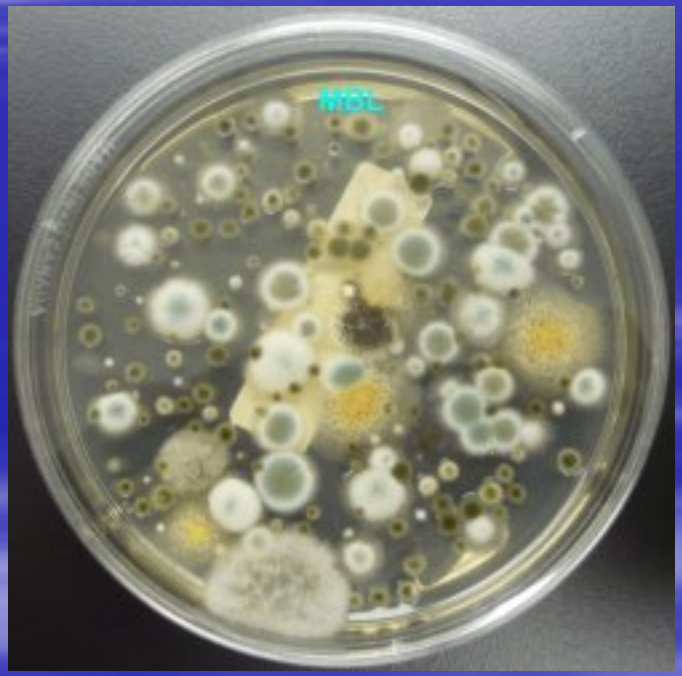
- Reduction or Elimination of Unwanted Beer Spoilage Organisms
- Detection of Beer Spoilage Organisms
- Optimizing Brewing Yeast Fermentations

Craft Breweries vs Traditional Breweries

- Most craft breweries are not designed by brewery engineers or brewing microbiologists
- Brewhouse and fermentation equipment are very often in the **SAME** room
- Traditional breweries were designed to separate the hot/cold side of brewing
- Malt dust contains many beer spoilers

Microbiology of the Brewery

The Brewery is a dirty place



Sources of Potential Beer Spoilers

- Water
- Air
- Malt dust
- Traffic into the brewery
- Sewers
- Fruit flies or other critters

Aseptic Technique

- What's practical?
- Room isolated from brewhouse to perform testing
- Aerial fallout must be minimized
- Testing wort/beer for contamination requires a clean space to work
- Clean clothes/lab coat reduces the chance of false positive results
- Hands must be sanitized with 70% isopropyl alcohol
- Lysol can be used on work surfaces

Aseptic Technique

- Vessels/bottles used to collect samples must be sterilized by heat
- Transfer pippetes must be bought pre-sterilized or must be sterilized by heat
- Sample valves must be sprayed with 70% isopropyl and flamed
- Samples valves must not have dried wort/beer from previous sampling

Review: Sanitized vs. Sterilized

- Brewers must **sanitize** tanks, hoses, etc. due to practicality

- Laboratory supplies can be either sterilized in an autoclave or pressure cooker or purchased sterile

Lab supplies

Autoclave/Pressure cooker

15# pressure for 15 minutes

Pipette: Aseptic liquid transfer

HEPA/UV Lab air Filter

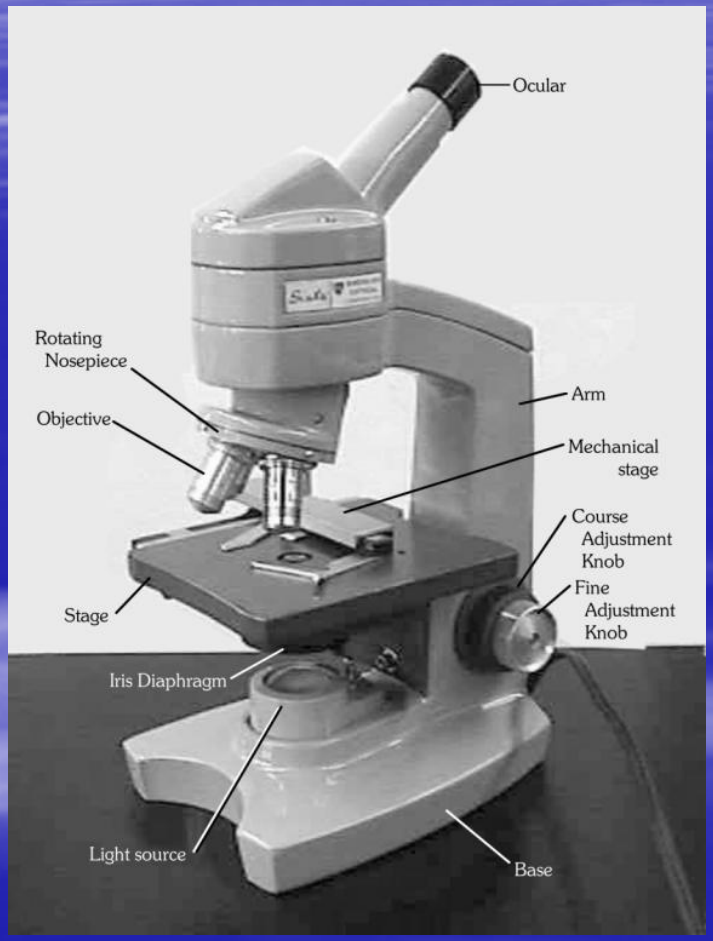
Beer is a relatively hostile environment

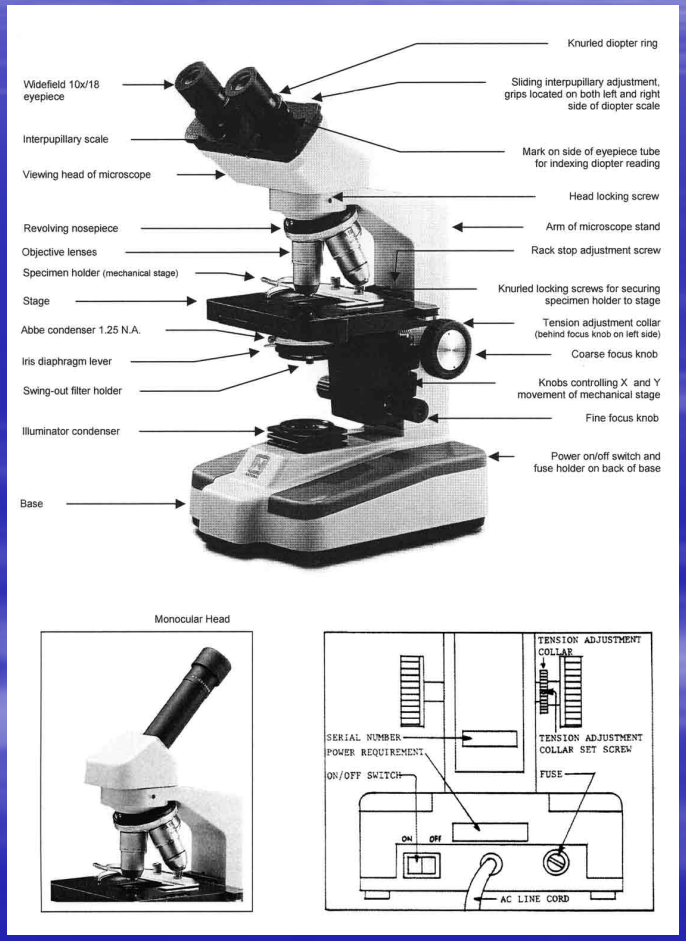
- Low pH
- Alcohol
- Anaerobic
- Cold stability
- Hop bittering compounds that inhibit most Gram + bacteria
- All beer has some microbial load

Quick and Dirty "Film Test" for Bottled Beer

- Bottle necks with a ring and beer/headspace interface
- Cloudiness
- Residue at the bottom of filtered beer
- Excessive foaming of opened/chilled beer
- Off-flavors or aromas

Microscopic Examination



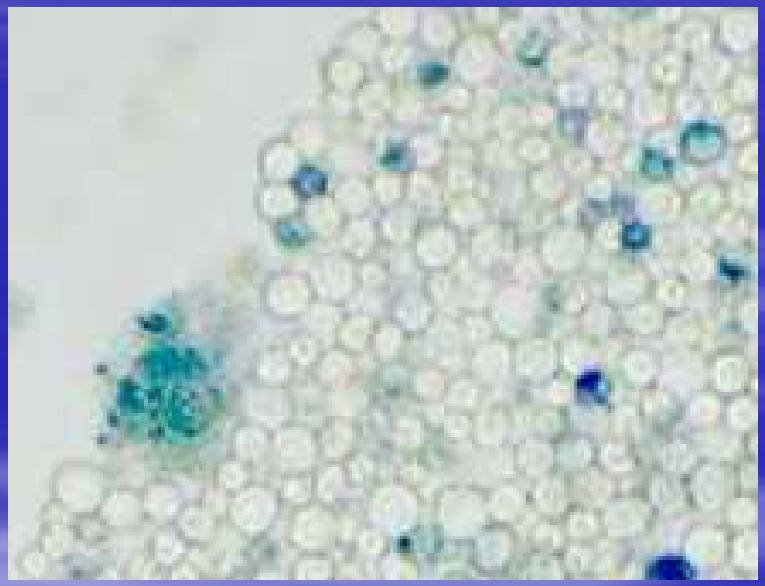


Microscopic Examination

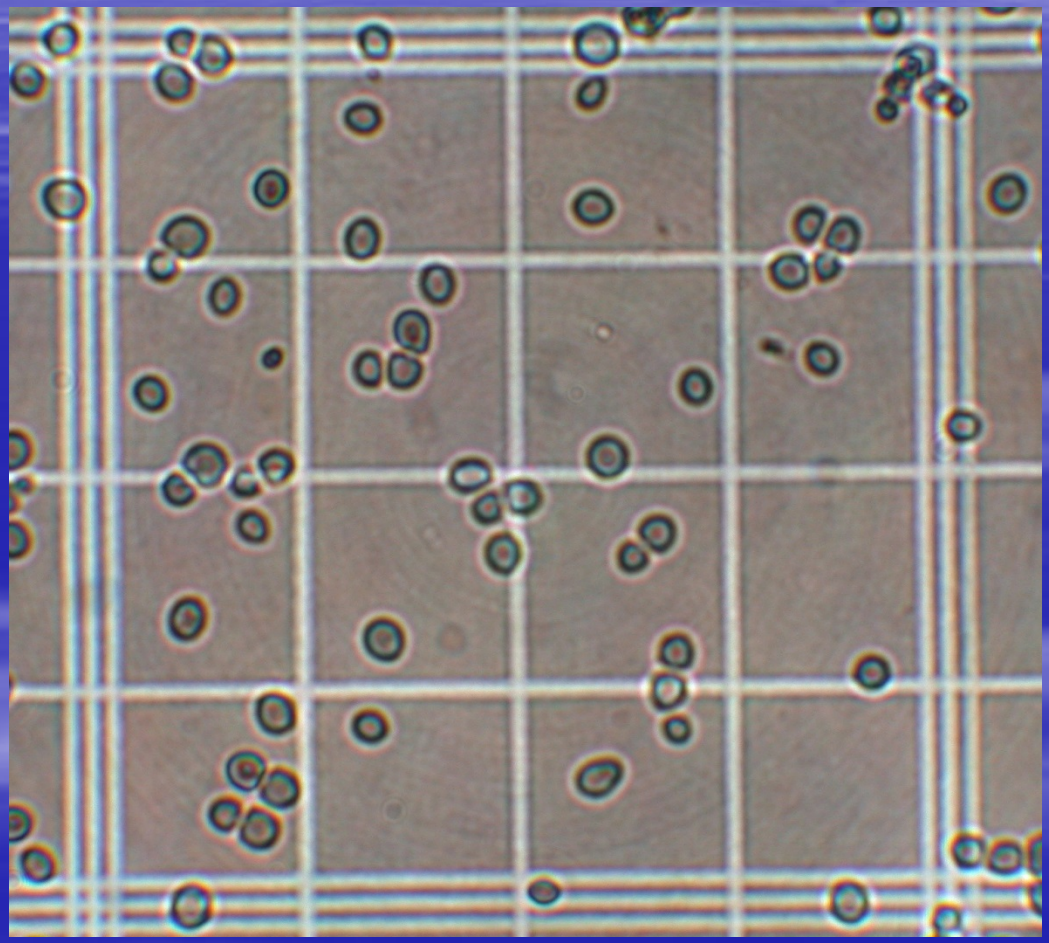
- Yeast: Use the 40x objective(400 total magnification)
  - % viability
  - Yeast concentration
- Bacteria: Use the 100x objective(1000 total magnification)
  - Use oil immersion lens
  - Whether cells are rods (bacilli) or round (cocci)
  - The approximate length or diameter in microns
  - The gram stain reaction
  - Microscopic examination of beer samples has limited use. Very limited sensitivity compared to selective media

Brewer's Yeast(400x)

Brewer's Yeast (dead cells are blue)

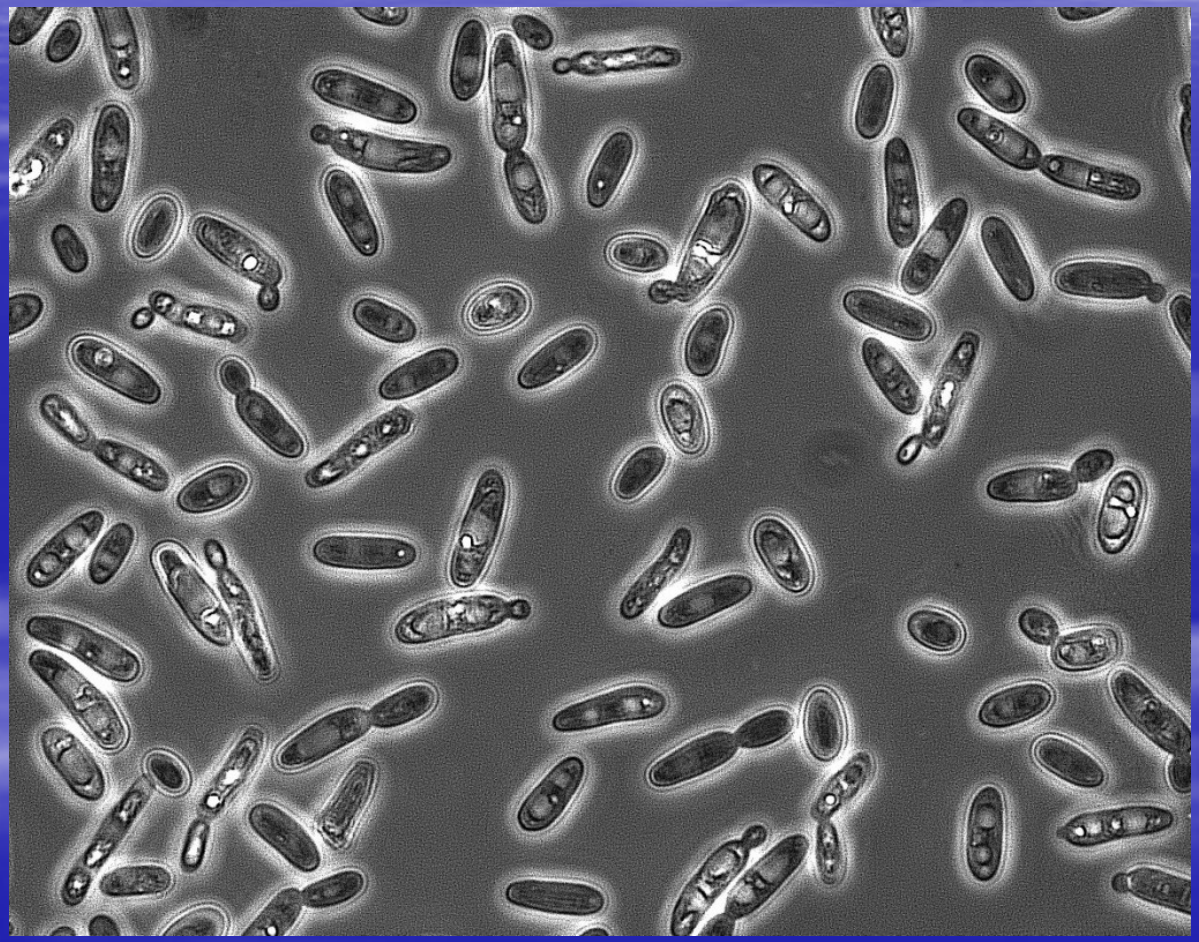


Brewer's Yeast

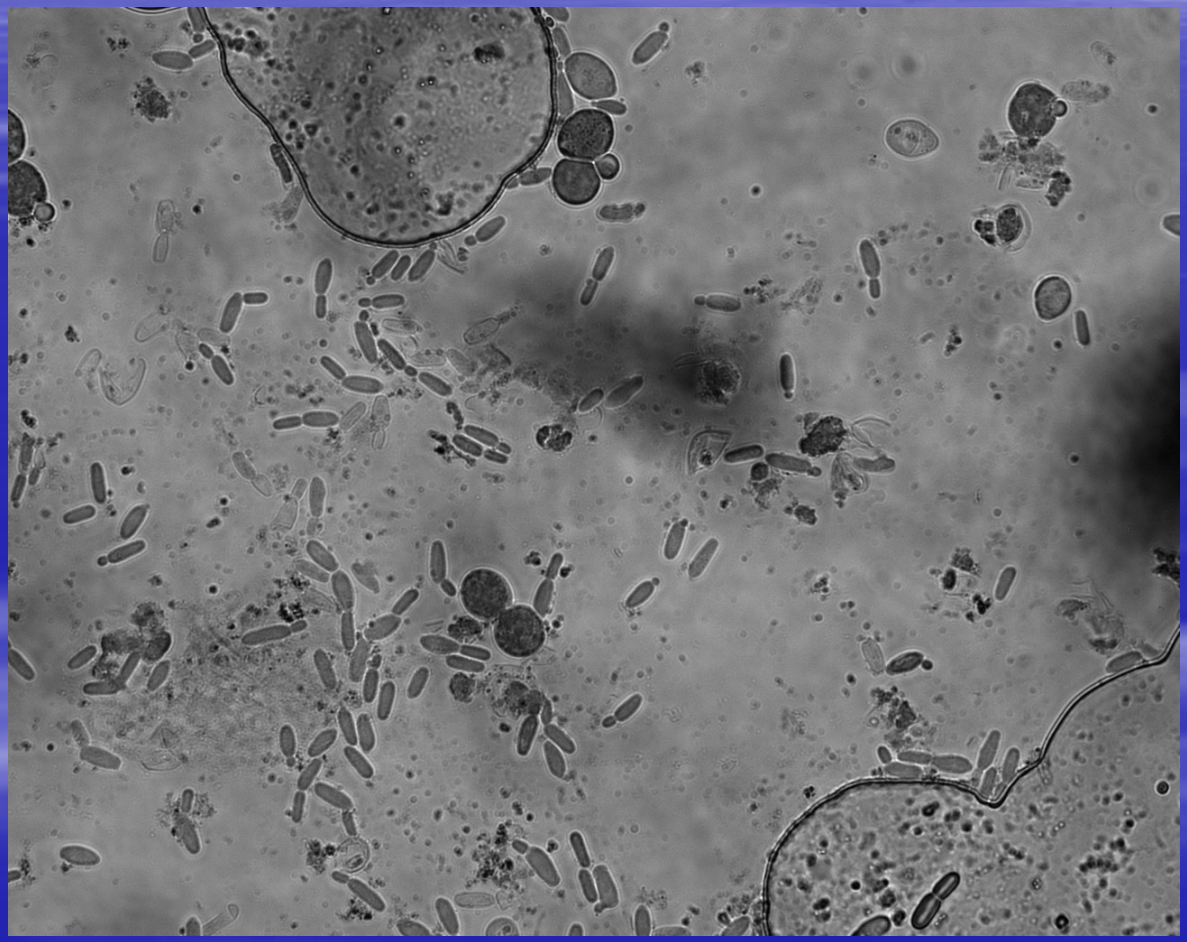


Brettanomyces yeast 400x

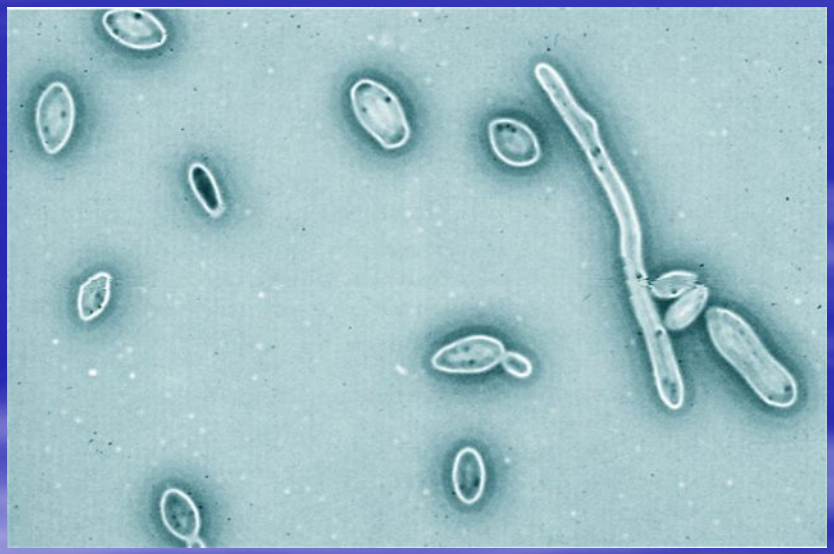
Wild Yeast



Wild Yeast 400x

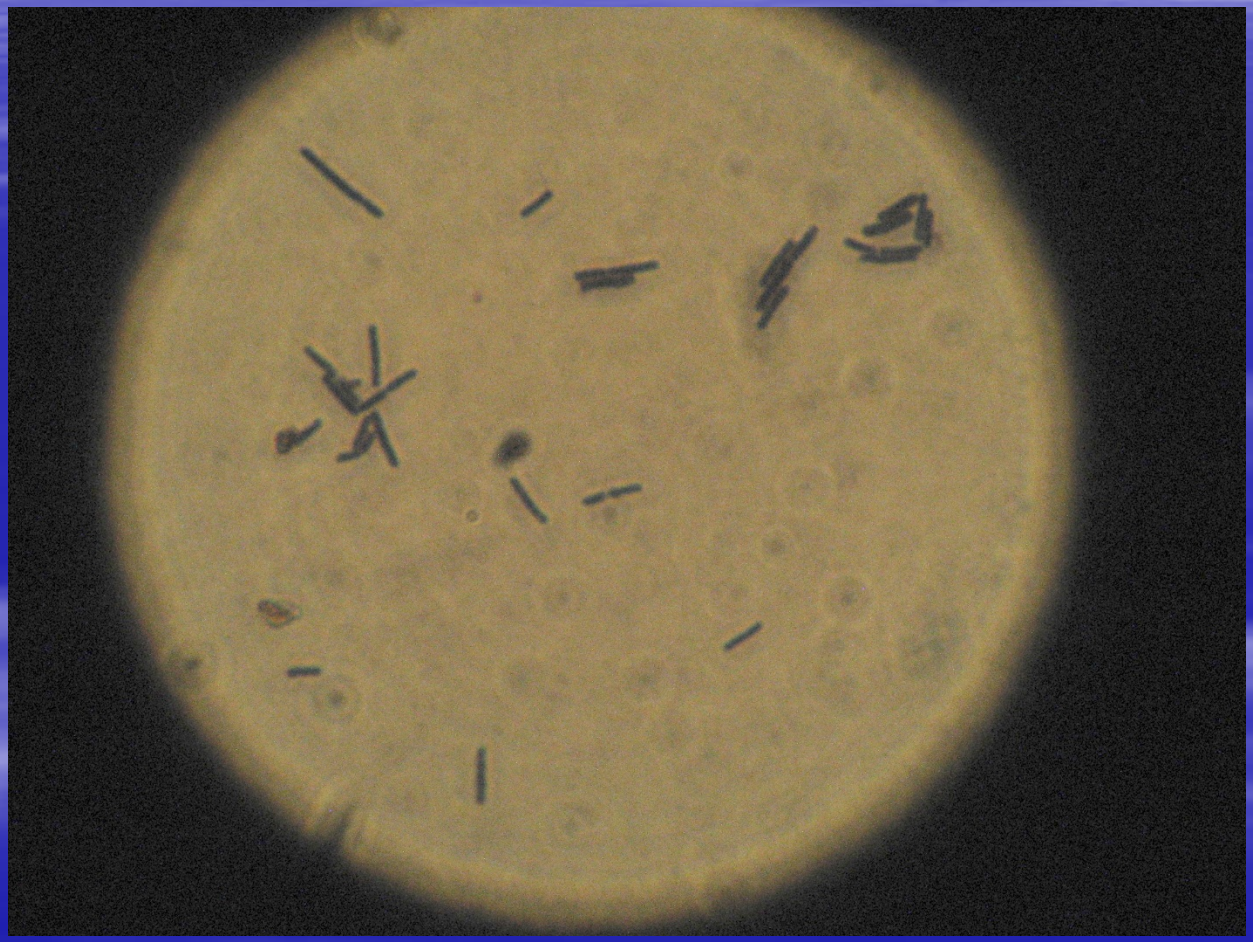


Brettanomyces 400x

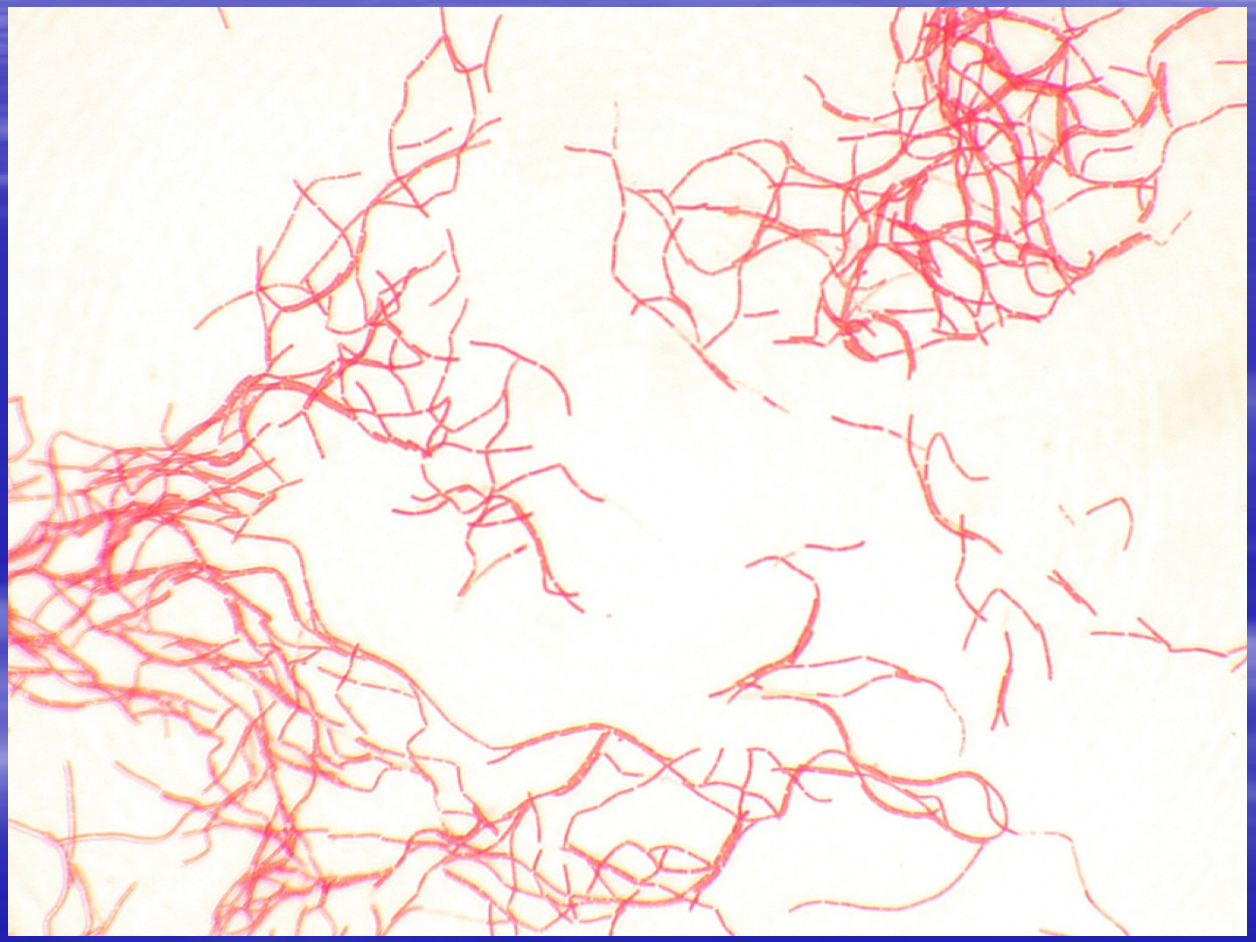


Bacteria: Gram + Rods 1000X

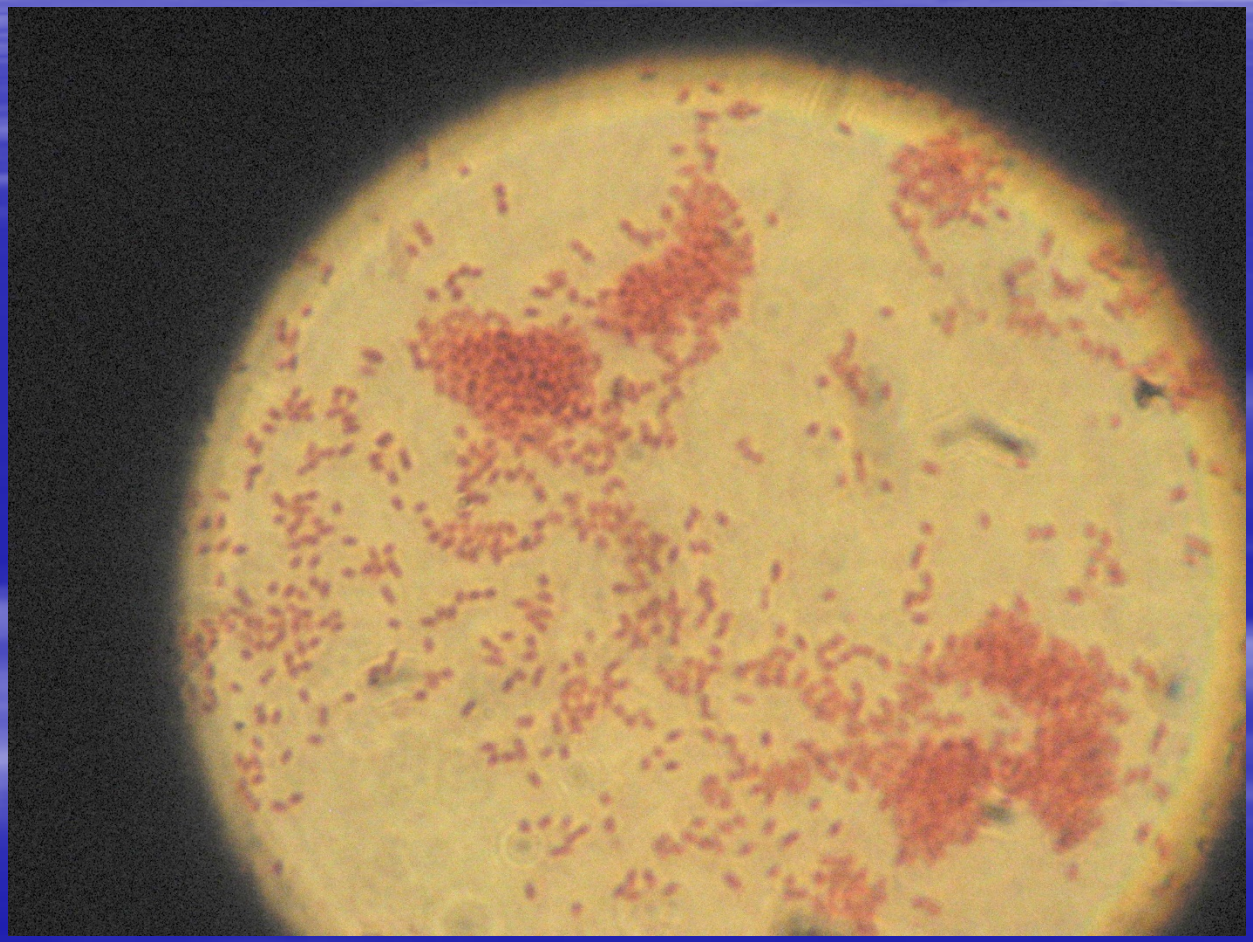
Gram + Rods
Lactobacillus
1000X



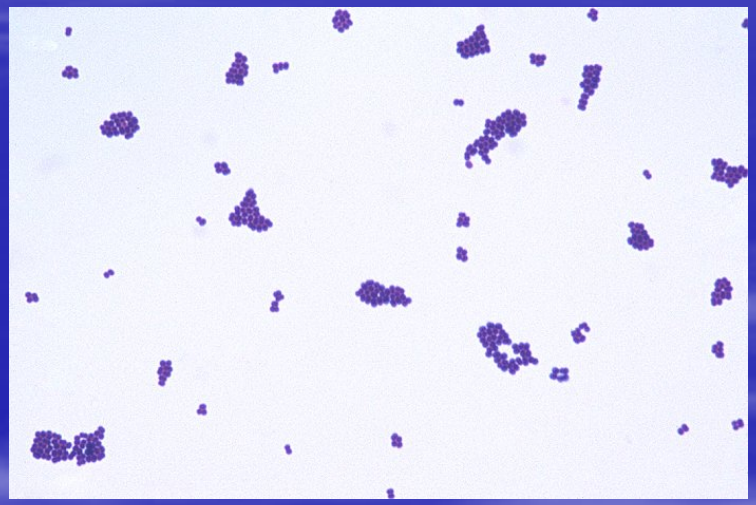
Gram – Rods(long) 1000X



Gram - Rods(short) 1000X



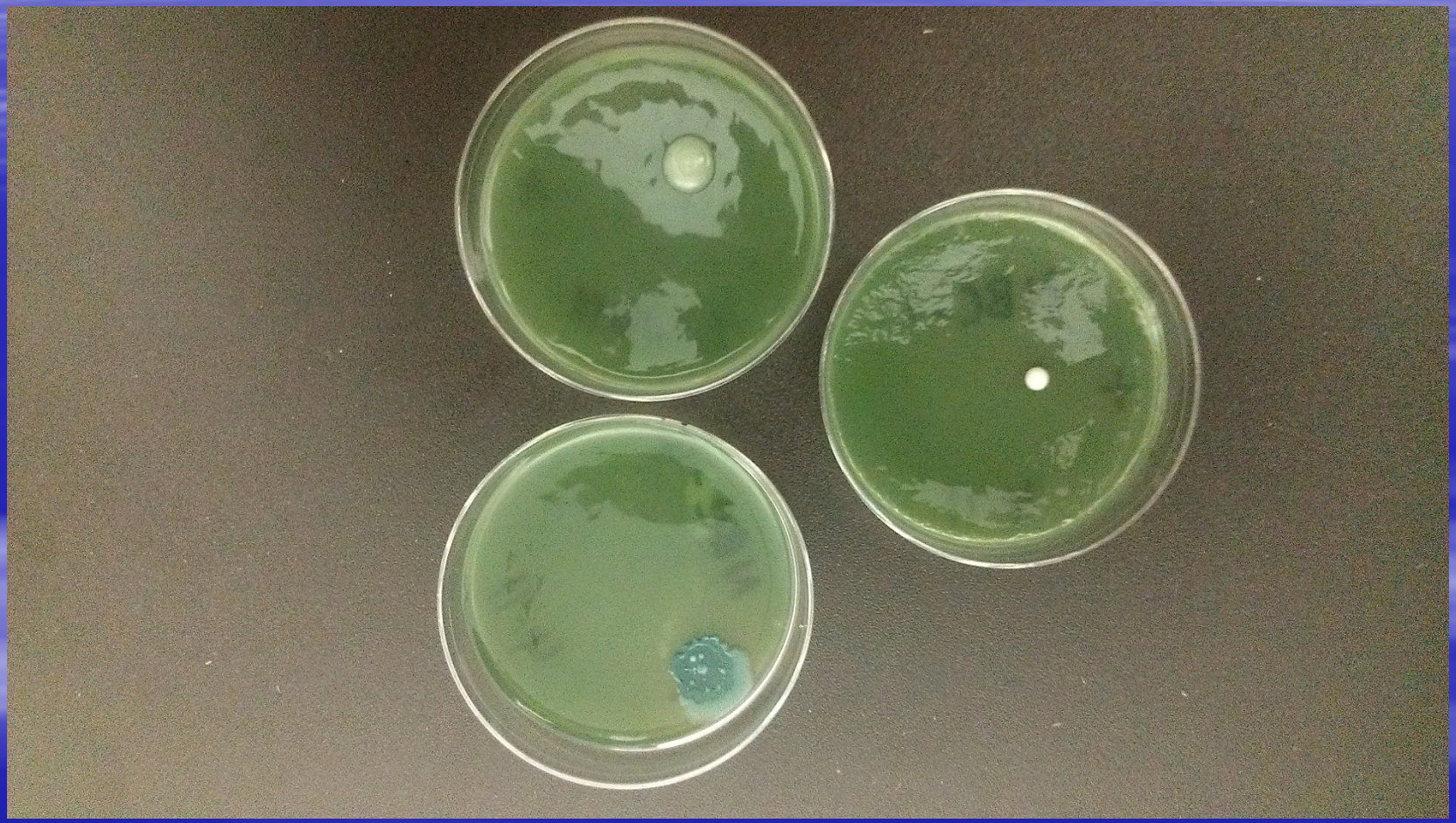
Gram + Cocci
Pediococcus
1000X



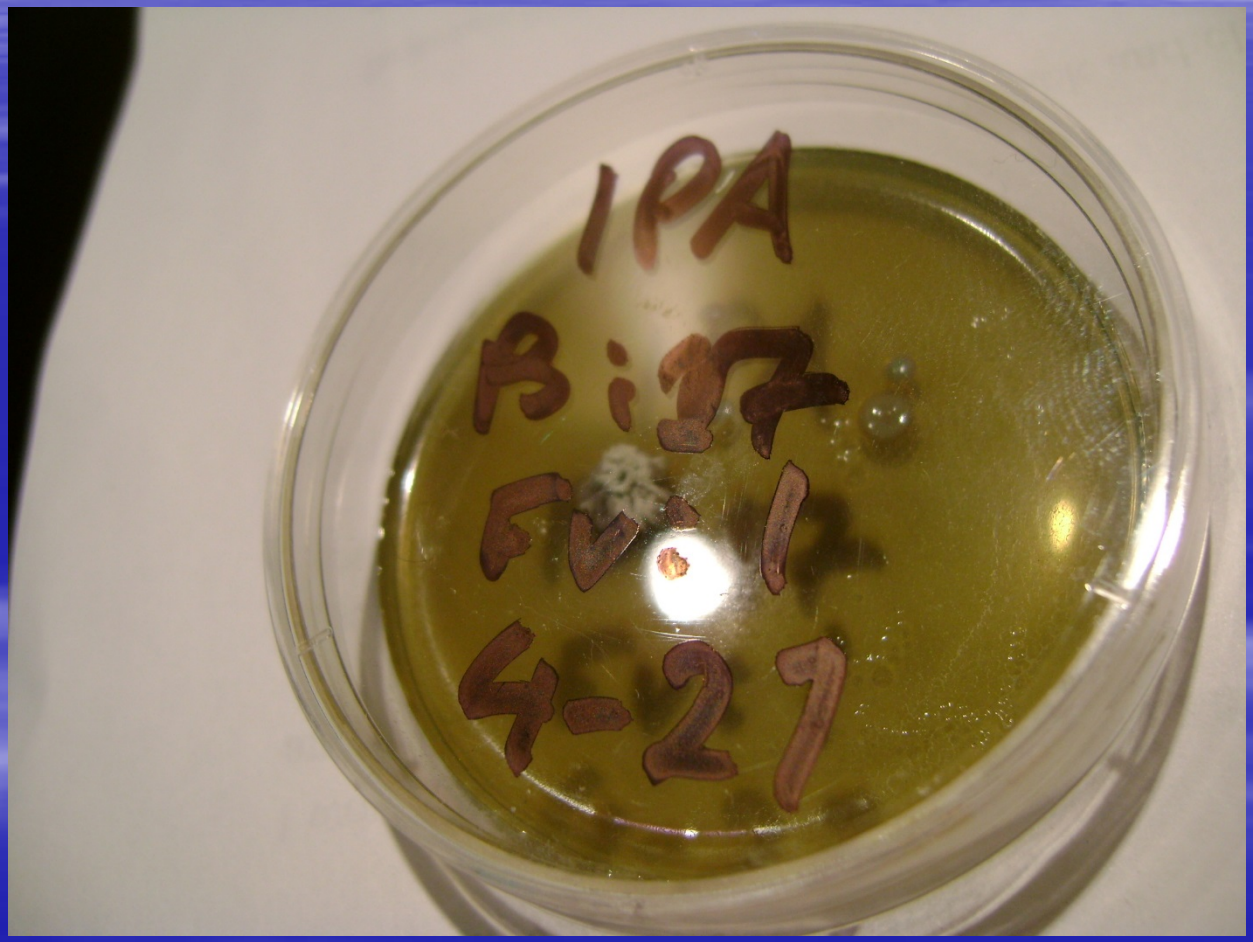
Detection of Spoilage Organisms using Selective Media

- LMDA(SDA)
  - 7 ppm cycloheximide inhibits brewing yeast but allows wild yeast to grow
  - Bromocresol green is used as a pH indicator for detecting acid producing beer spoilers
  - Solid media is enumerative, colony counts possible
  - All common beer spoilers grow on this media
  - Not completely selective, allows non-brewery bacteria to grow as well as mold/bacillus spores

Growth of Bacteria and Wild yeast on LMDA

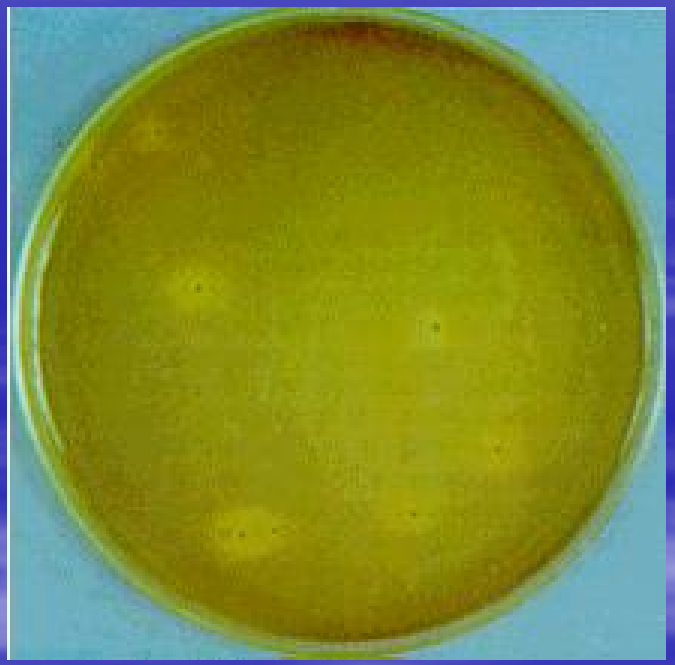


Enteric (wort spoiler)



Acetobacter

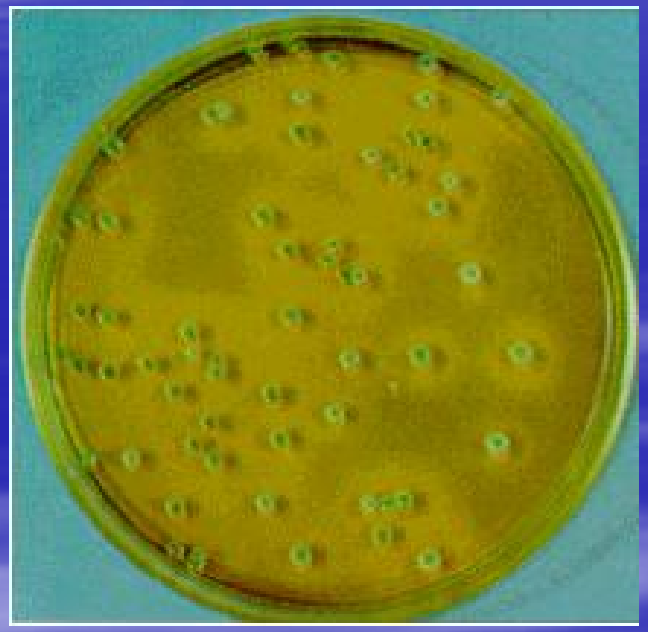
- **Acetic acid bacteria (*Acetobacter, Acidomonas*)**
- Gram-negative, strictly-aerobic rods common in plant material such as fruit and grain. Normally encountered in stored or fermenting wort and bottled beer. Produce acetic acid, which lowers pH and lends a vinegary flavor/odor. Recommended limit: 5 per 1-ml sample or per 100-ml yeast-free sample.
- colony: greenish-blue
- colony size: 1-2mm
- colony texture: smooth
- Changes media color or cloudiness?: YES
- Bubble formation when exposed to peroxide? YES
- Turns royal purple when exposed to oxidase reagent? NO *(Acetobacter)*, YES *(Acidomonas)*



Enteric (wort Spoiler)

- Enteric bacteria (*Citrobacter, Enterobacter, Hafnia, Klebsiella, Obesumbacterium)*
- *G*ram-negative, facultatively-anaerobic rods common in water, soil, and plant material. Normally encountered in stored and fermenting wort. Produce sulfur compounds, fusel alcohols, phenolics and acetaldehyde, which cause a variety of off-flavors/odors. Recommended limit: 8 per 1-ml sample or per 100-ml yeast-free sample.
- colony: greenish-blue, yellowish-green
- colony size: 2-5mm, may spread to cover entire plate
- colony texture: smooth, slimy
- Changes media color or cloudiness?: NO
- Bubble formation when exposed to peroxide? YES
- Turns royal purple when exposed to oxidase reagent? NO *(all except Obesum)*, YES *(Obesum)*

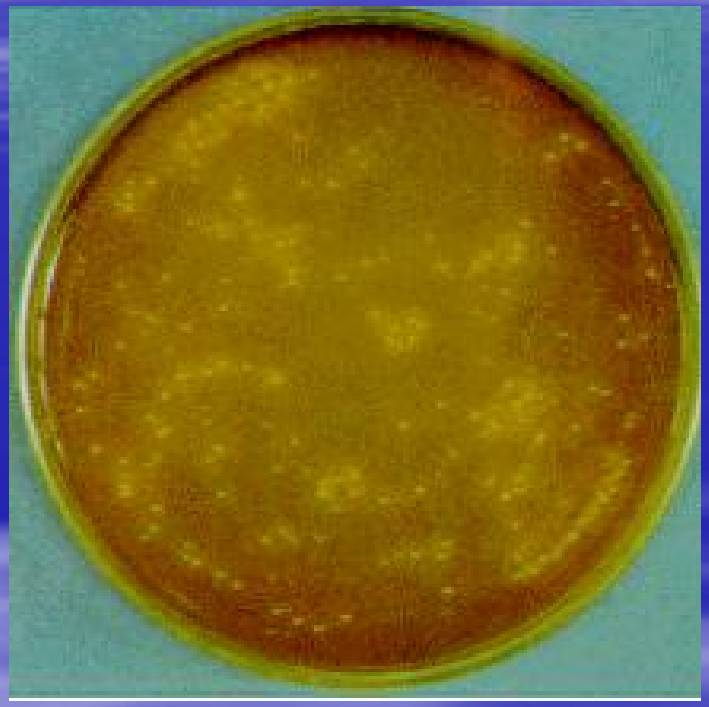
Lactobacillus



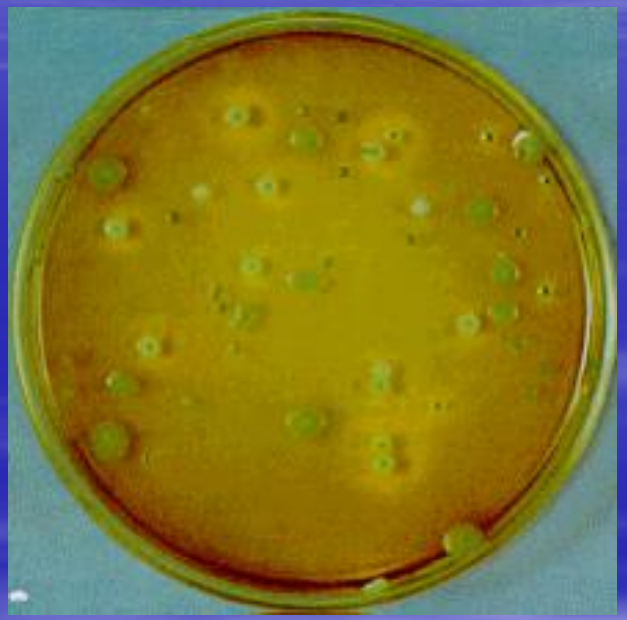
Lactic Acid Bacteria

- **lactic acid bacteria *(Lactobacillus, Pediococcus)***
- *Lactobacillus* (rod) and *Pediococcus* (coccus) are gram-positive, facultative anaerobes common in plant material such as fruit and grain. Encountered in all stages of brewing. Produce lactic acid, which lowers pH and lends a tart, sour flavor/odor. Recommended limit: 3 per 1-ml sample or per 100-ml yeast-free sample.
- colony: yellow-green *(Pedio)*, white with bluish center *(Lacto)*
- colony size: 1-3mm
- colony texture: smooth *(Pedio)*, rough or smooth *(Lacto)*
- Changes media color or cloudiness?: YES
- Bubble formation when exposed to peroxide? NO

Pediococcus



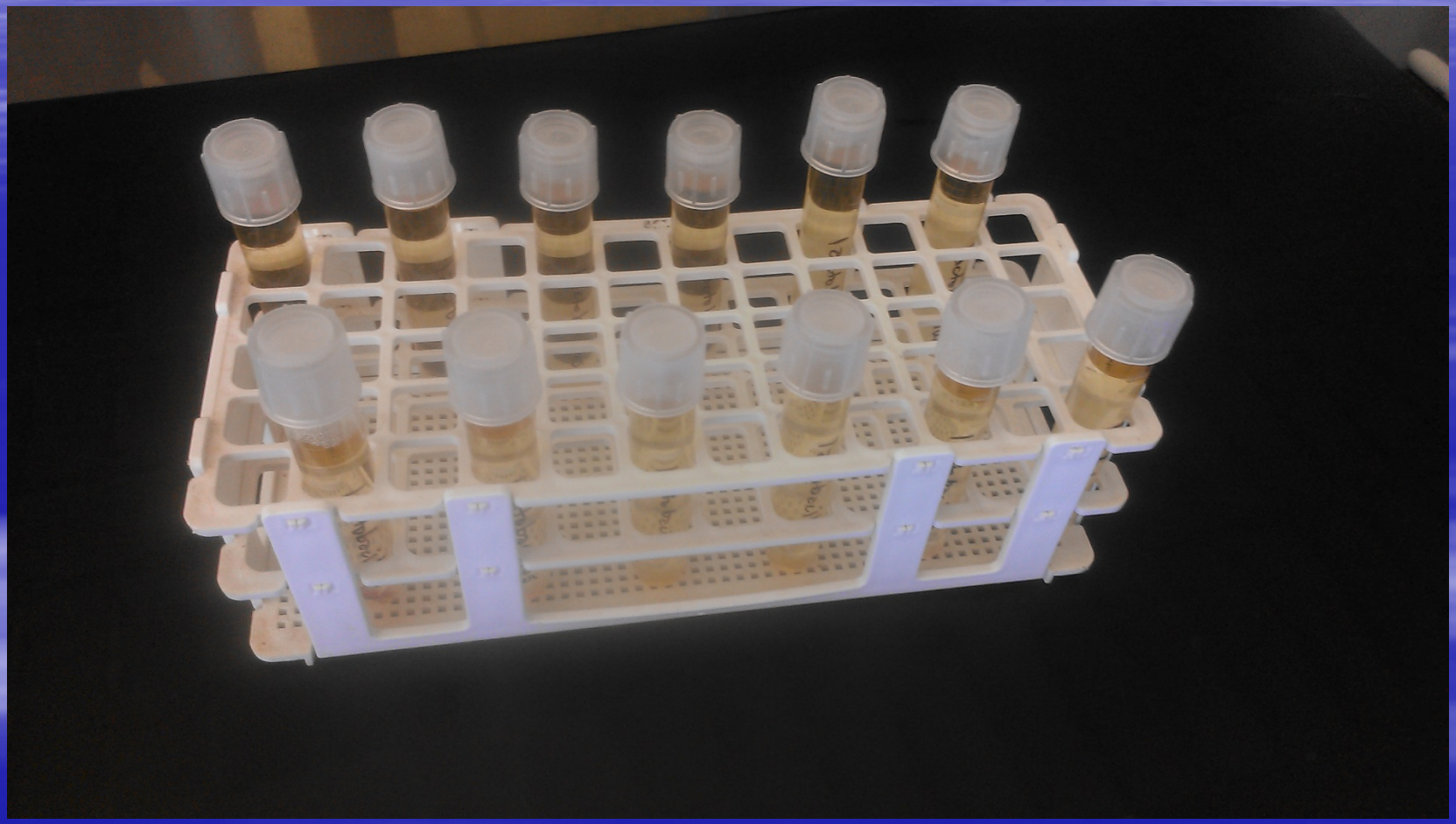
Bacteria Mixture



Other Brewing Testing Media

- HLP
  - Selective for Lactobacillus/Pediococcus
  - Easy to prepare
  - Autoclave/pressure cooker not needed
  - Anaerobic only
- Wild Yeast Media
  - LCSM vs LWYM
  - Detects non-Saccharomyces vs Saccharomyces
  - Limited shelf life
  - Commonly gives false positives
  - Difficult to produce small quantities

HLP



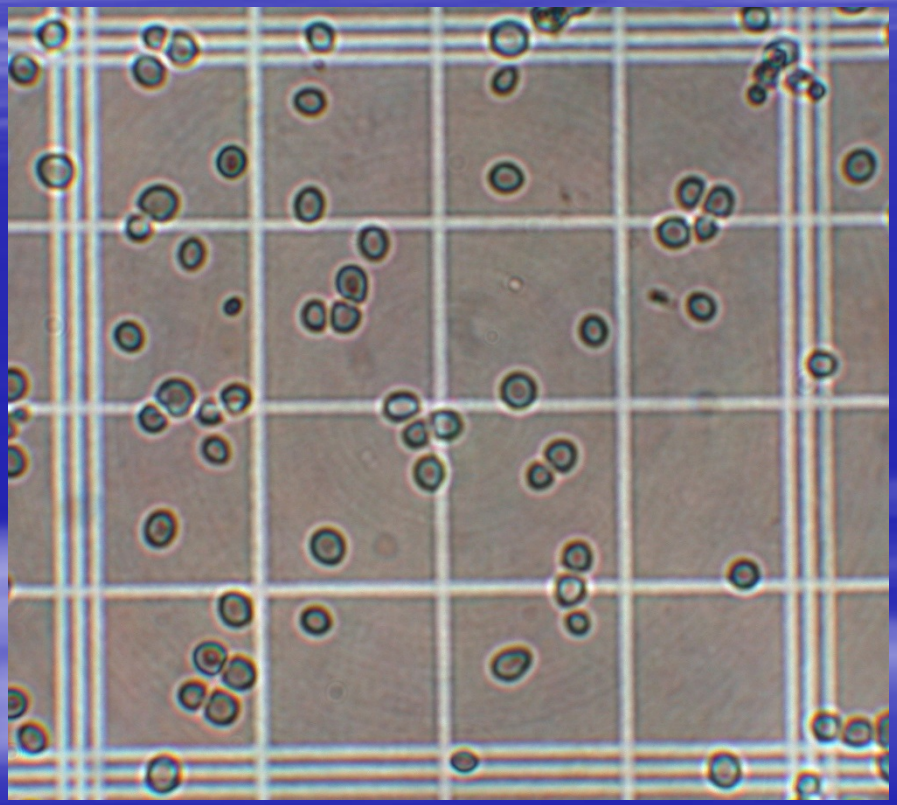
LWYM

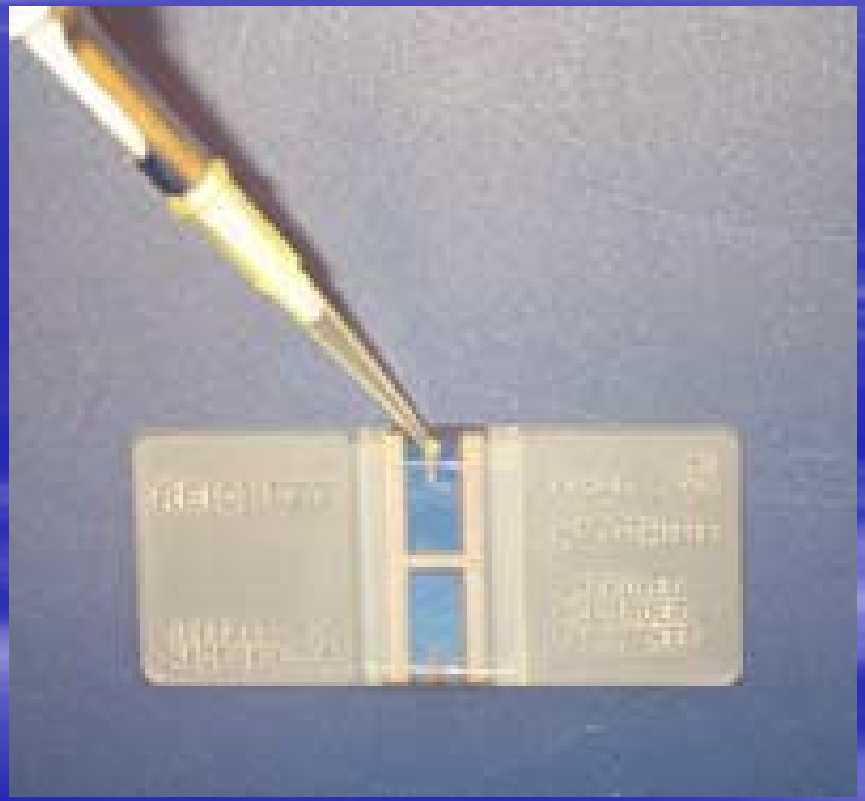
Brewing Yeast grown on non-selective Universal Beer Agar

Simple Tests for Identifying Bacteria

- Colony characteristics on LMDA
  - Odor (vinegar, lactic, sulphur?)
  - Acid Production?(media turns from green to yellow)
  - Colony color, size, texture and shape
- Gram stain (purple or pink?)
- Catalase reaction (positive or negative?)

Hemacytometer Yeast Cell Counts





Protocols

- Where and When to sample?
- Drawing samples
- Swabbing
- Wort Stability Test
- Plating Samples Directly
- Plating Samples Using Membrane Filtration

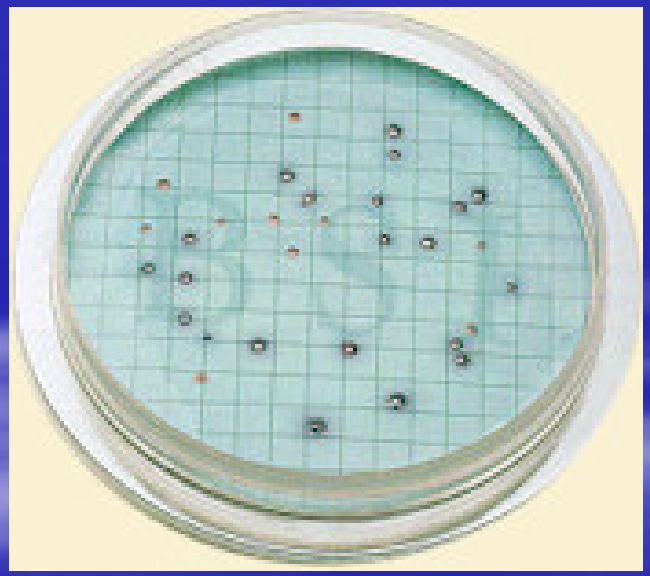
Wort Stability Test

Plating Beer Samples

- Yeasted or unfiltered samples: Direct Plating of **1 ml samples**

- Filtered Beer Samples: Membrane Filtration of **100ml** samples concentrated on membrane. Membrane is then aseptically transferred onto LMDA plate

Membrane Filtration Plating

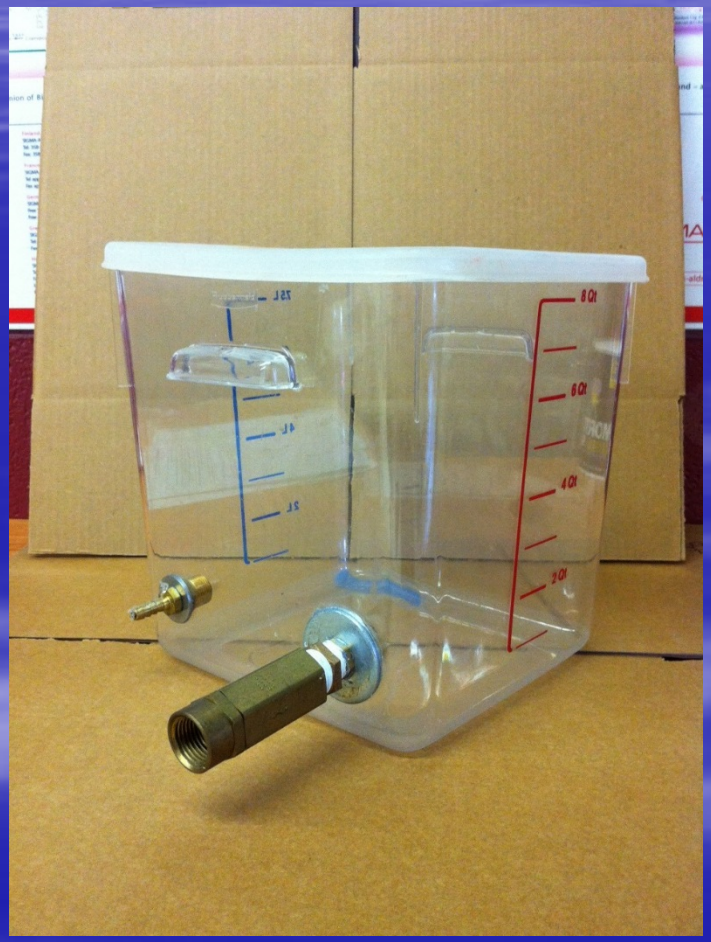


Plating Beer Samples

- After plating on selective LMDA, samples need to be incubated aerobically to isolate wild yeast, enterics and acetic acid bacteria

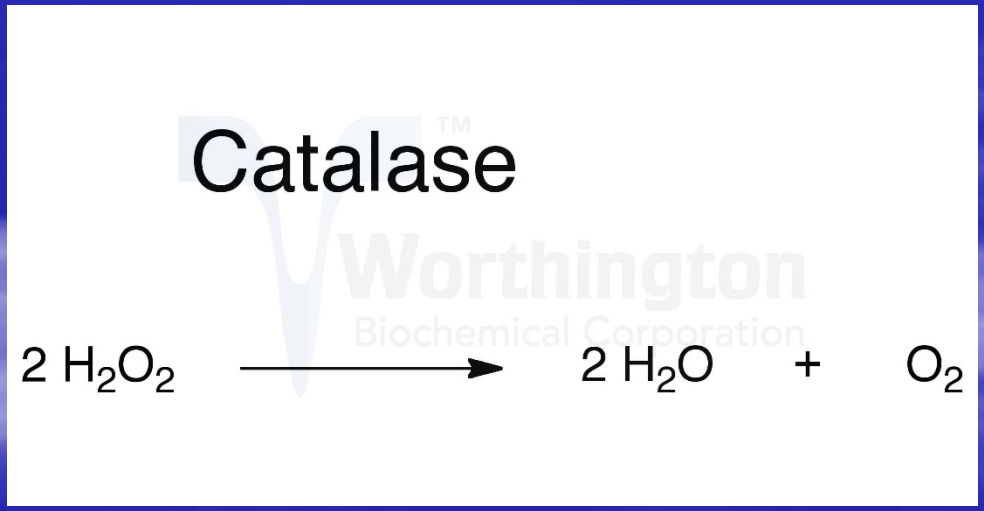
- Duplicate LMDA plates can be separately incubated anaerobically to isolate Lactobacillus and Pediococcus

Anaerobic Incubator

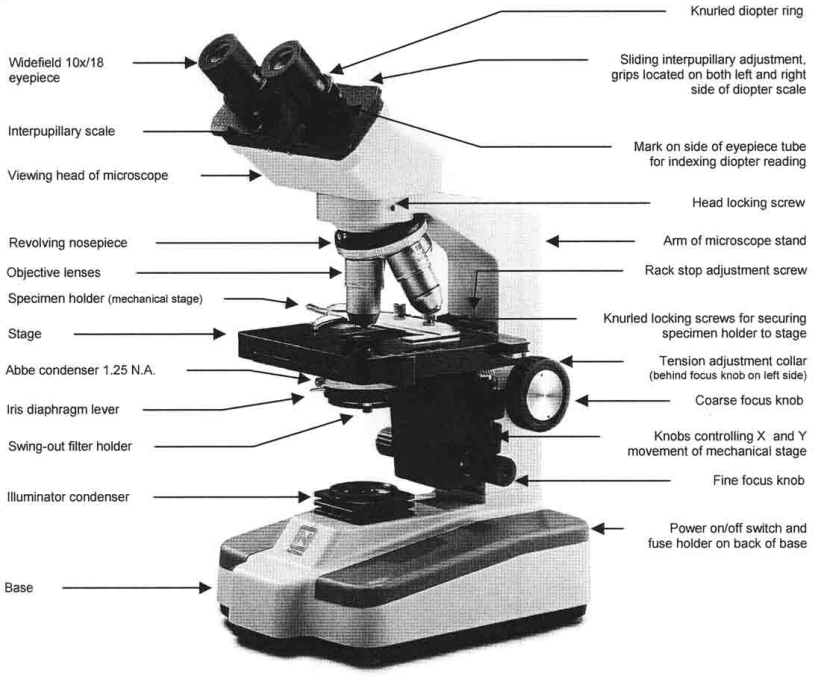


Catalase Test

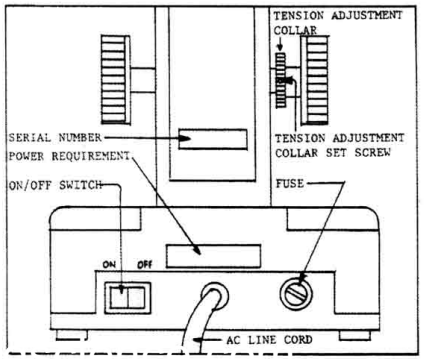
- Bacteria that are catalase positive will produce bubbles on a glass slide when hydrogen peroxide is added

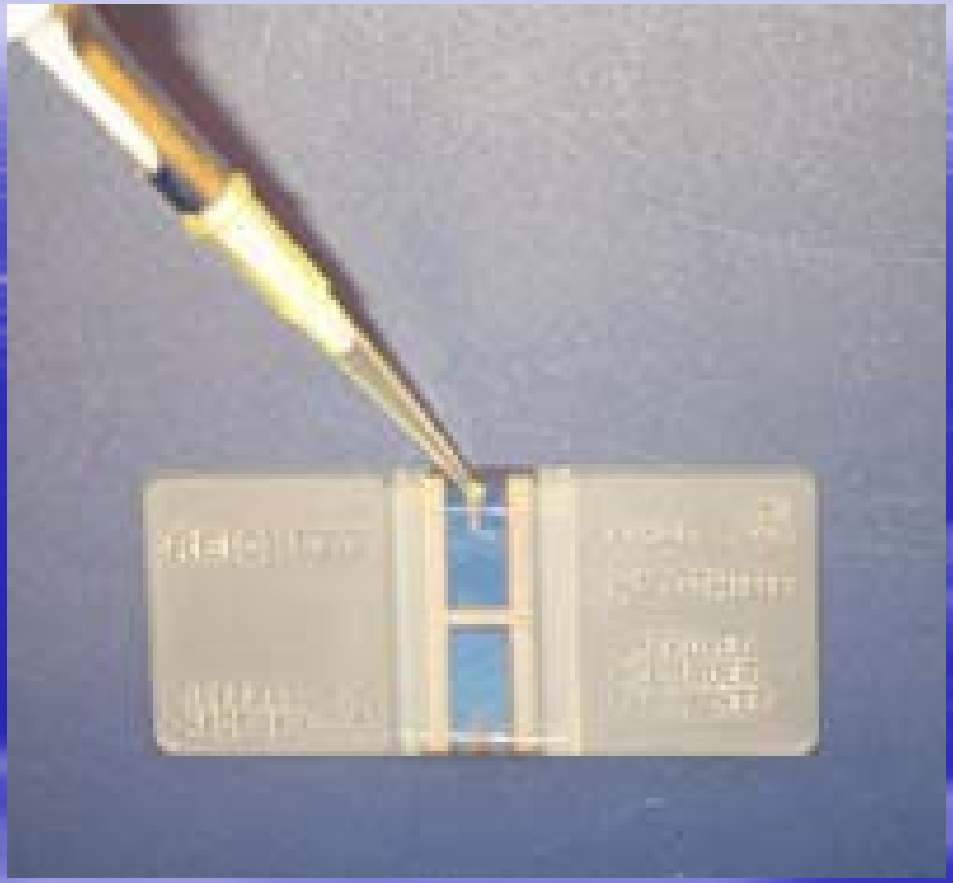


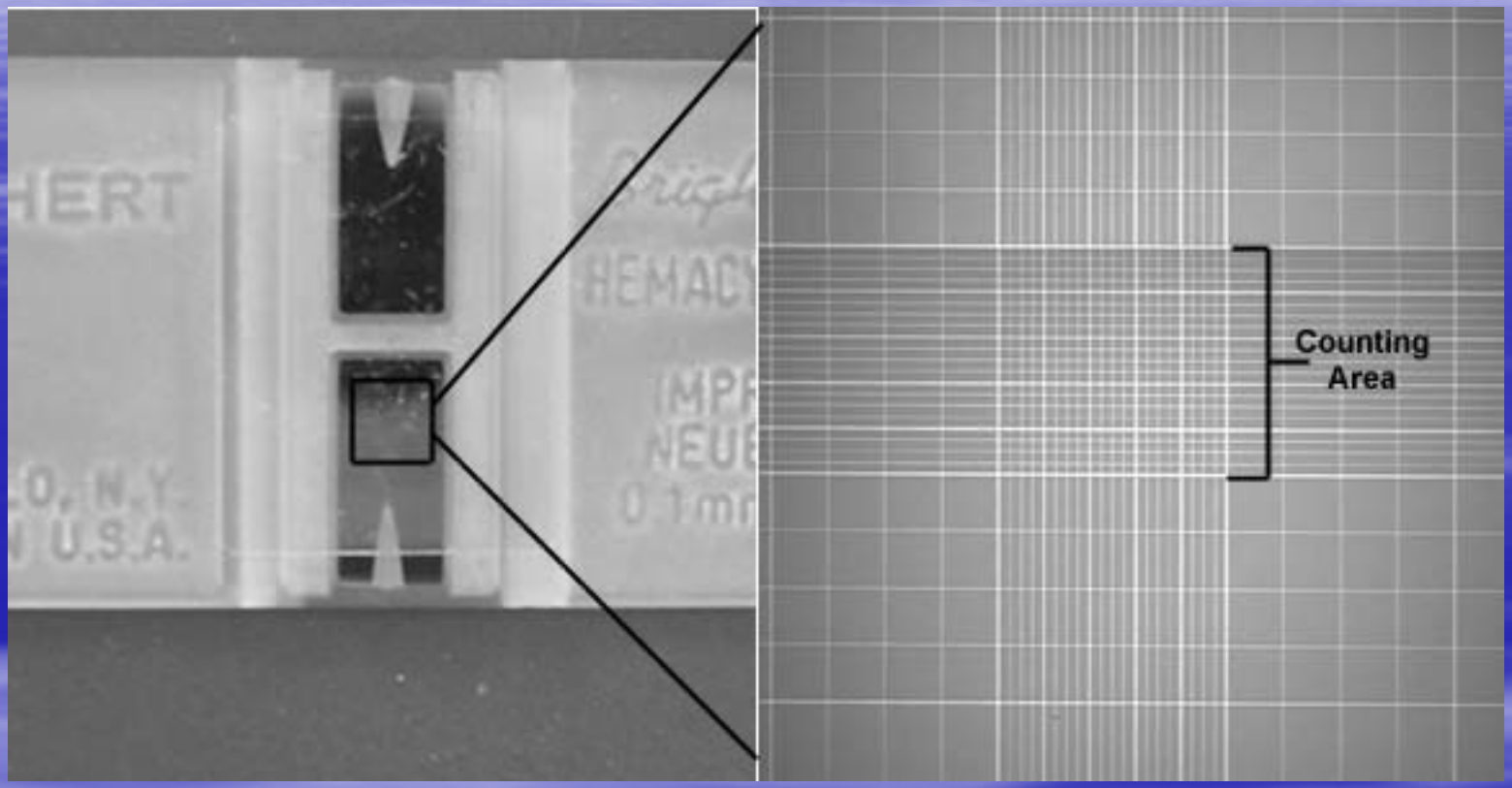
How to use a microscope



Monocular Head






Counting
Area

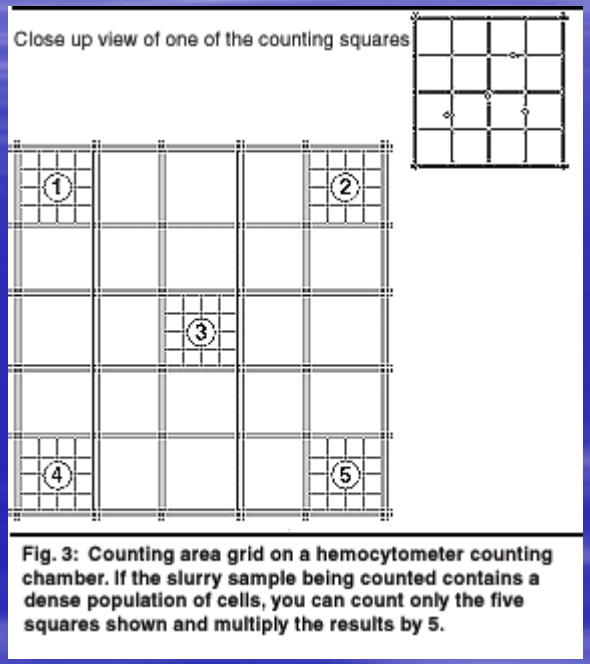


Fig. 3: Counting area grid on a hemocytometer counting chamber. If the slurry sample being counted contains a dense population of cells, you can count only the five squares shown and multiply the results by 5.

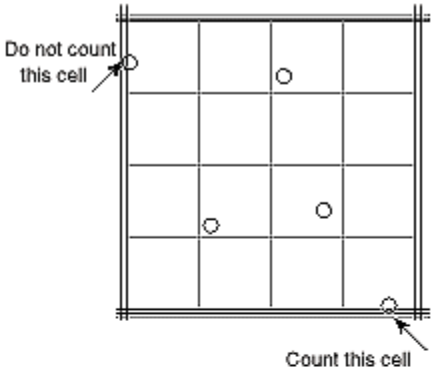


Fig. 4: Close up view of a counting square showing an example protocol for arriving at accurate estimates of total cell count.

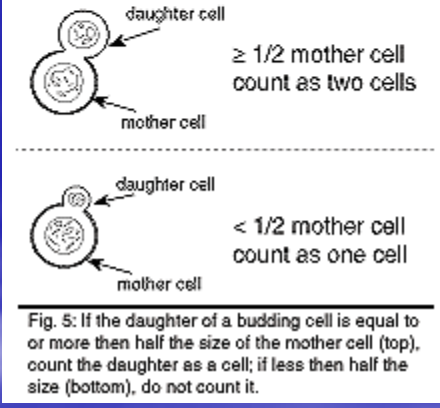


Fig. 5: If the daughter of a budding cell is equal to or more then half the size of the mother cell (top), count the daughter as a cell; if less then half the size (bottom), do not count it.