



Gusmer Enterprises, Inc.*

Microbial Monitoring

Gusmer Enterprises
April 2013



Outline

- Intro: Why should I monitor for microbes?
- Micro Monitoring in the Lab
 - Monitoring the Product: Wine
 - Tools to monitor the wine
 - Troubleshooting
 - Plating demo
- Micro Monitoring in the Winery
 - Monitoring the Winery: Process Sanitation
 - Tools to monitor the winery
 - Best practices
 - Common contamination points and troubleshooting
- Advances in Rapid Micro



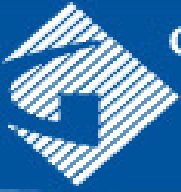
Why Should I Monitor for Microbes?

- Ensures no contamination and spoilage of finished product
 - If contamination, determine what
- Find contamination points
- Alert to failed equipment or sanitation
- Identify contaminated product from good product



Why Should I Monitor for Microbes?

- Common Spoilage Organisms
 - **LAB** (*Lactobacillus, Oenococcus, Pediococcus*)
 - **AAB** (*Acetobacter*)
 - **Spoilage Yeast** (*Brettanomyces, Saccharomyces, Zygosaccharomyces*)
- Lead to the formation of:
 - Off flavors and aromas
 - **4EP/4EG**
 - **VA** (volatile acidity)
 - **Ethyl acetate**
 - **Re-fermentation in bottle**
 - **Haze in bottle**



Monitoring the Product: Wine

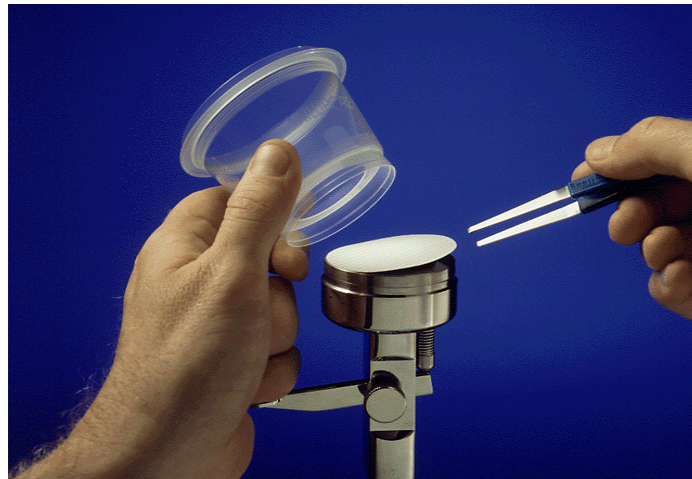
- Test the wine during aging
- Test wine samples post-bottling (BWS)
- Tools to test the wine:
 - Vacuum, manifold, funnel, membrane, media
 - Incubator, microscope

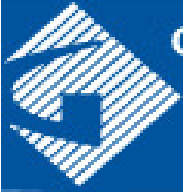




Manifolds

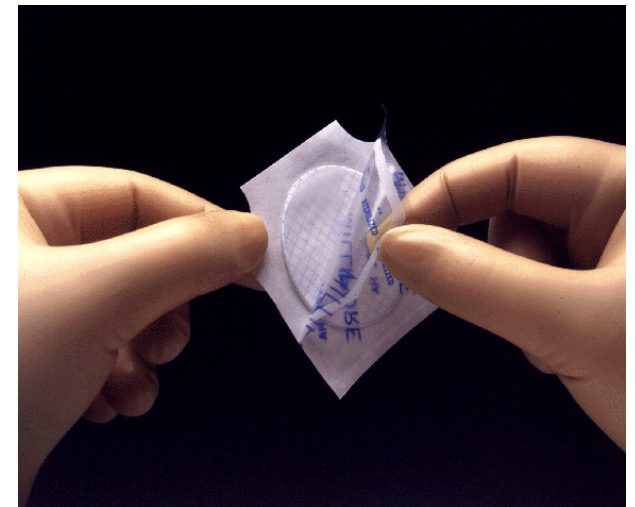
- Three main types
 - Glassware
 - Traditional (stoppered)
 - Flat top (Microfil)





Membranes

- 0.45 μm MCE (mixed cellulose ester)
- Should always use sterile membranes for plating
- Organisms remain on the membrane





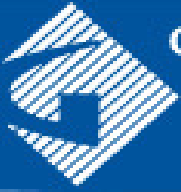
Media

- Dehydrated
 - Low cost, no refrigeration of unmade media
 - Agar compatible
- Selective Liquid Ampoules
 - Pre-sterilized, single doses
 - Often antibiotics or inhibitors for greater selectivity



- WL Wallerstein Nutrient
- Yeast and Mold
- Brettanomyces
- MRS





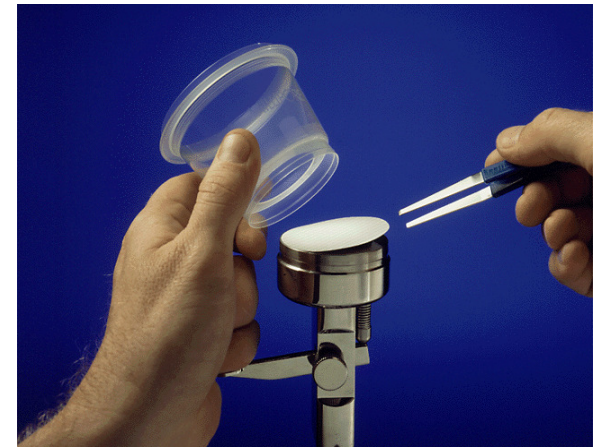
Re-useable vs. Disposable



- Must be sterilized in autoclave or water bath
- Higher initial purchase cost
- Lower ongoing materials cost
- More time consuming

VS

- Pre-sterilized
- Low initial purchase cost
- Higher ongoing materials cost
- Saves significant operator time
- Some available with preloaded membranes





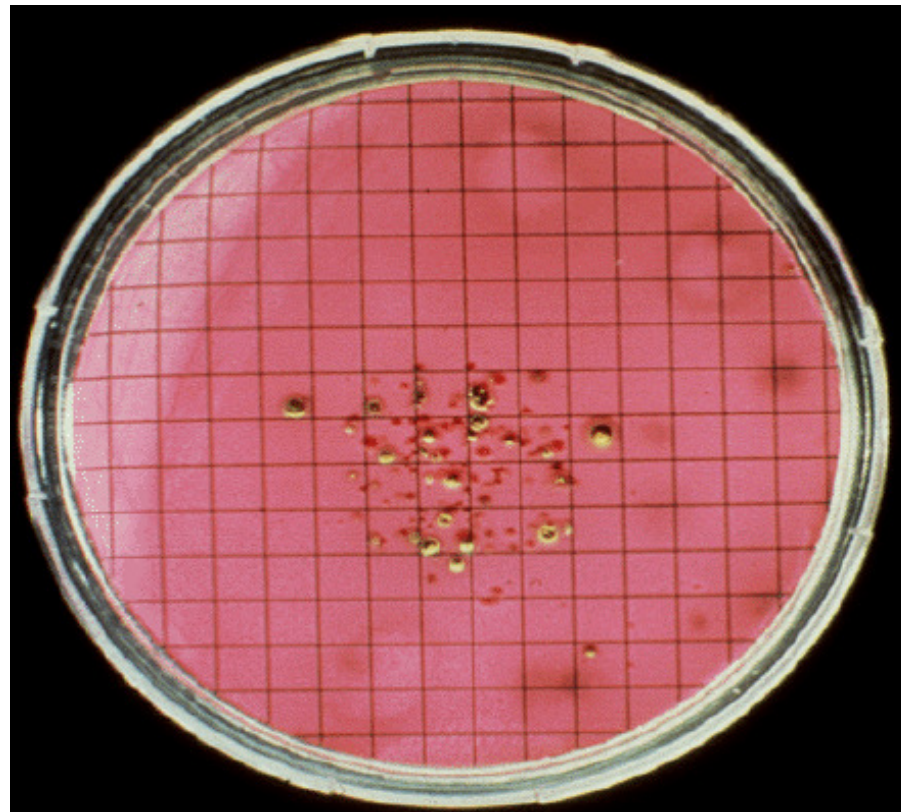
Troubleshooting

- **Unsterilized equipment** (funnels, forceps)
 - Re-useable funnels must be sterilized in water bath or autoclave
 - Forceps should be flamed
- **Contaminated media**
 - If using agar make sure it is fresh
 - Sterile ampoules eliminate this concern
- **Non-sterile membrane disks**
- **Manifold**
 - Stoppers, fritted disks and holders/funnels



Bad Micro-Tests

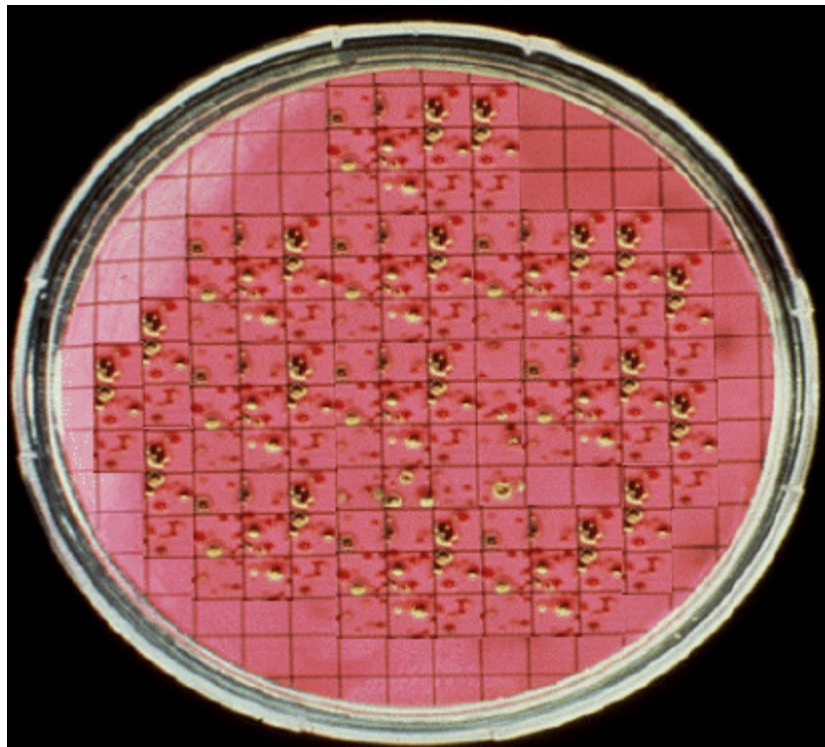
- Improper Sample Distribution
 - Contamination, pulling vacuum with media





Bad Micro-Tests

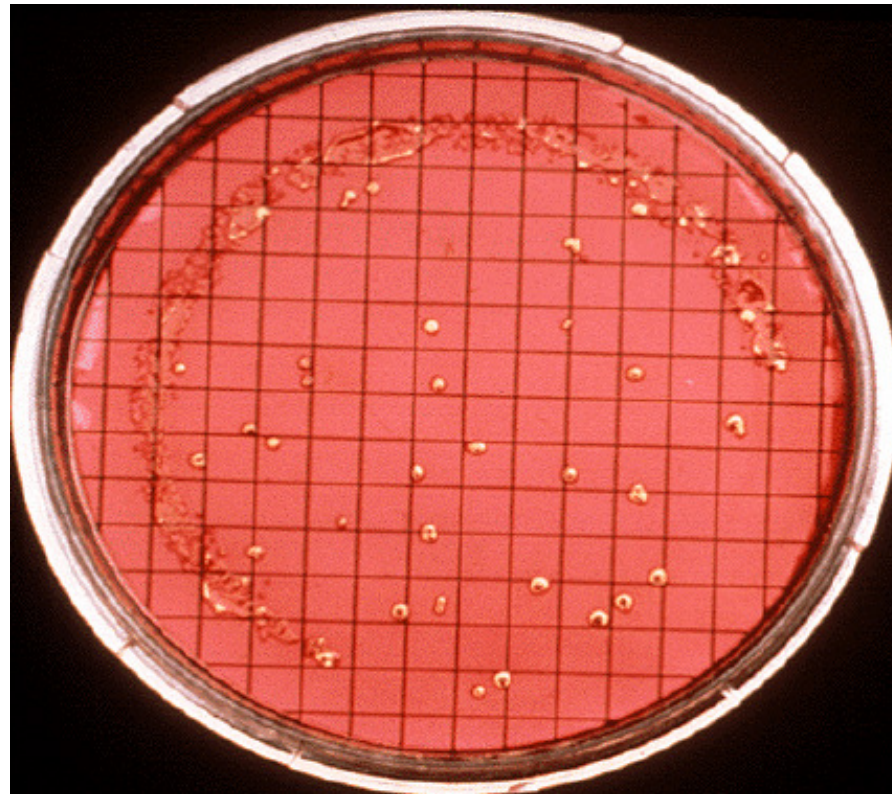
- Too Numerous to Count (TNTC)
 - Check sample size, media type/amount, contaminated media

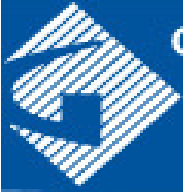




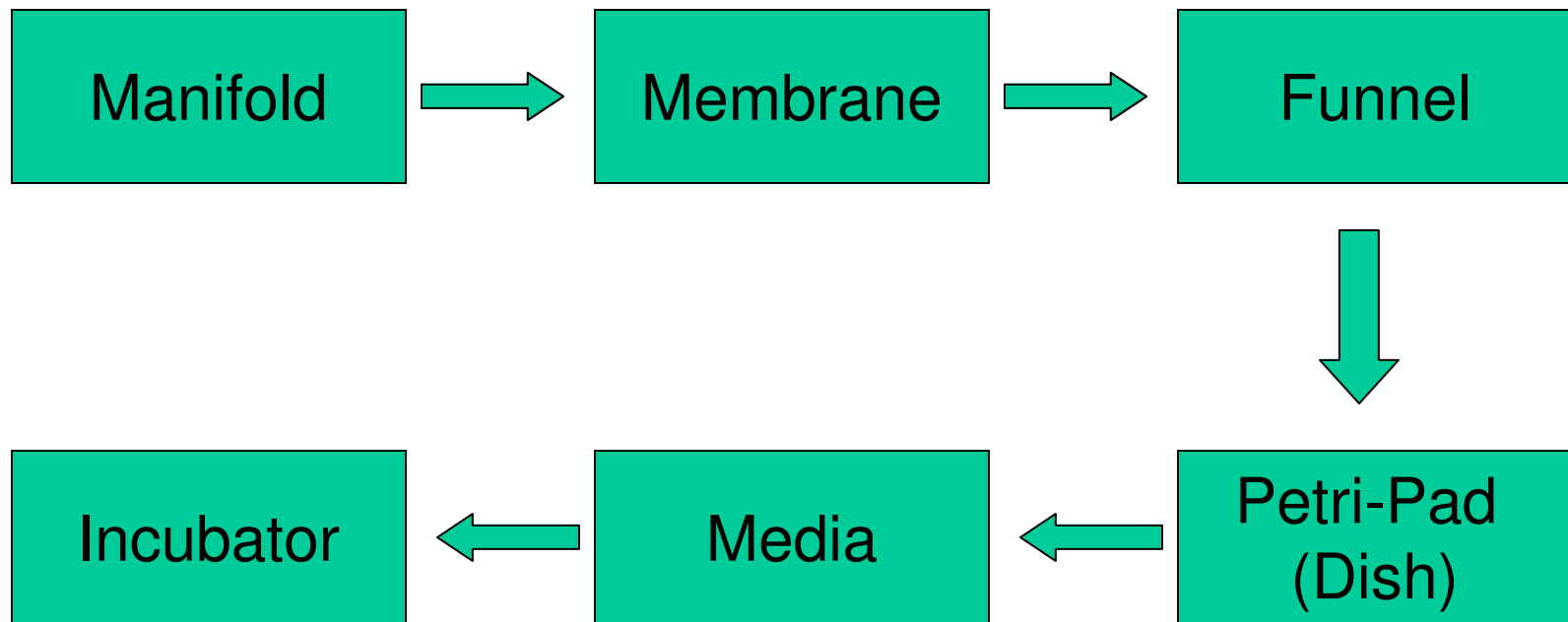
Bad Micro-Tests

- Dirty Funnels





Micro Plating Steps

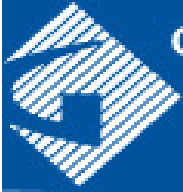


**Several steps may be combined
with new innovative products**



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Plating Demo



Why monitor microbes in the winery?

- We know microbes cause wine spoilage
- We know microbes are harbored in the winery

Assessment Category	Average Microbial Load (CFU)
Crush Equipment	198
Cellar Equipment	136
Oak Cooperage	128
Bottling Equipment	82
Infrastructure	190
Atmosphere	45
Water	55

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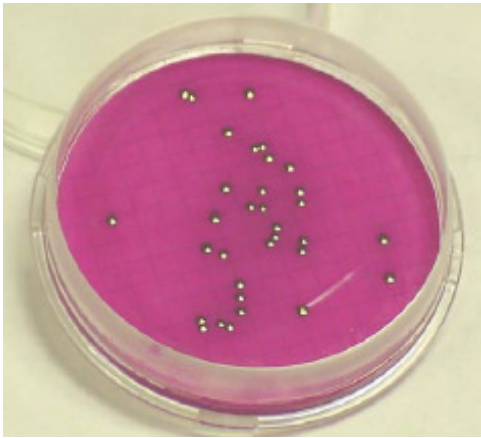


Monitoring the Winery: Process Sanitation

- **Process sanitation** reduces unwanted microbes to at or below an acceptable level
 - **Cleaning** – Removing some contaminants
 - **Sanitation** – Greatly reduces micro load
 - **Sterilization** – Completely eliminates microbes from process
- Three steps to winery sanitation
 - **Clean...Rinse...Sanitize**



Process Sanitation



- All contact surfaces must be sanitized at start-up
 - Hot **180+ F° water**
 - Ensure each surface reaches temperature
- If line remains down for extended period it should be re-sanitized
 - Typically 1-2 hr specification



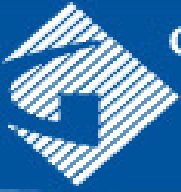
Sanitation Audits

- **Regular audits** should be performed to ensure sanitation cycles are effective and being carried out correctly
 - **ATP** and **Swab/Samplers** to check piping and equipment
 - **Temperature checks** on suspect areas
 - **Visual inspections** of hard to clean areas
 - **Procedure checks** and operator training



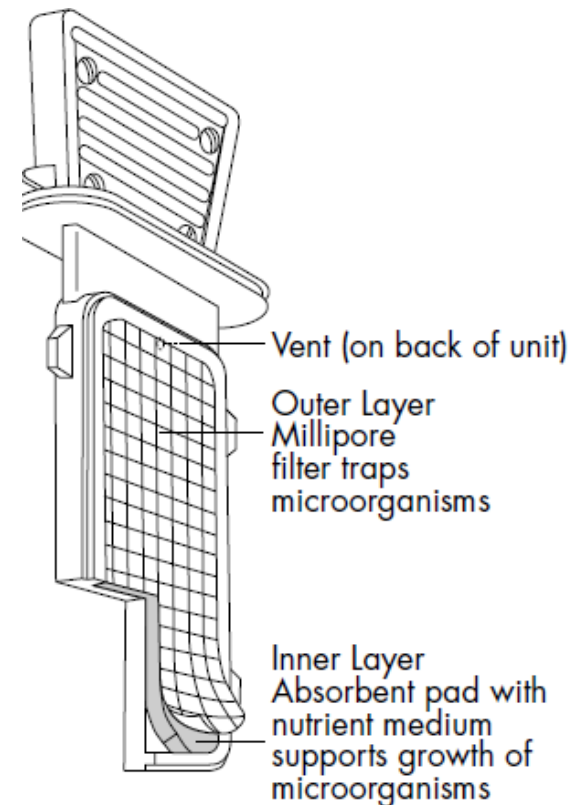
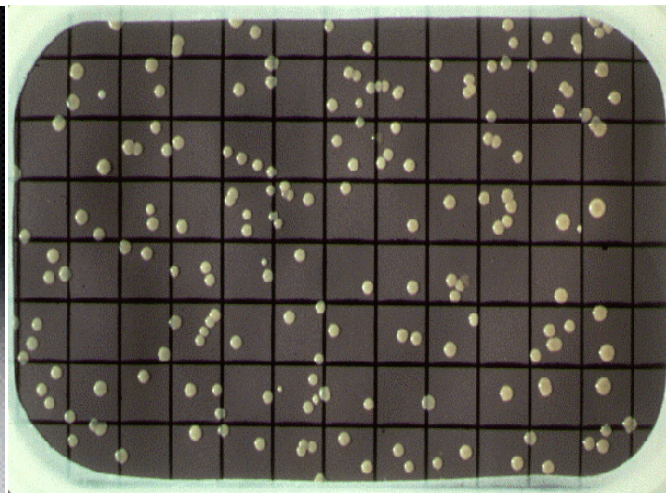
Tools to Test the Winery

- **Swabs/Samplers**, presence/absence **ATP** tests can directly test surfaces
- **Air monitoring** can test ambient contamination
- **Standard plating** can be performed on sanitation water effluent or raw water



Selective Swabs

- Membrane paddle with dehydrated media
- Use with sterile swabs to get actual enumeration and type of micro contamination from surfaces
 - ATP does not give micro type
- Great troubleshooting tool
- Entire unit is incubated





Hot Spot “Mapping”

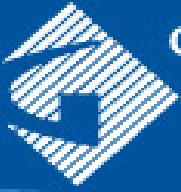
- Tracking contamination points or failed swab tests can create a map of where contamination is occurring
- Focus improvements
- Create check lists or check points during or after sanitation





Sanitation Watch Outs

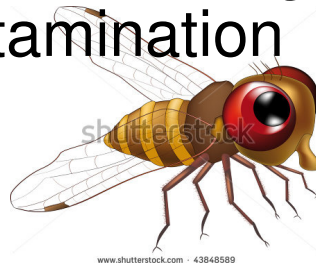
- Not opening all valves
- Not closing valves ***while still under flow***
 - This, often combined with drain placement, is a leading cause of periodic micro hits
- Not sanitizing the top of containers (surge tanks, filler bowls)
- Failure to reach temperature due to dead legs
- Outside of filling spouts (sanitation cups)
- Not using adequate water flow
 - Typically the same flow rate the process is run at should be the CIP/sanitation flow rate
 - There should be pressure on the line, occasionally throttle back valves to create a little back pressure for better cleaning/sanitation



Common Contamination Concerns

- **Floor Drains**

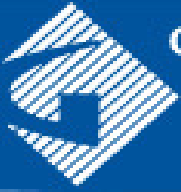
- Many drains have positive pressure (watch for steam) which push microbes up and out into the bottling area
- Unfortunately many lines are designed so that sampling and drain valves are located directly above floor drains
- Opening without flow or not closing under pressure during CIP will cause contamination





Common Contamination Concerns

- **Dead legs**
 - Long T's for drain/sample valves
 - Old piping, reclaim or recycle lines
- **Filling spouts**
 - Sanitation cups allow the outer rim and portion of the filling spout to be cleaned
- Drip and drain lines, gas lines, addition lines



Biofilms

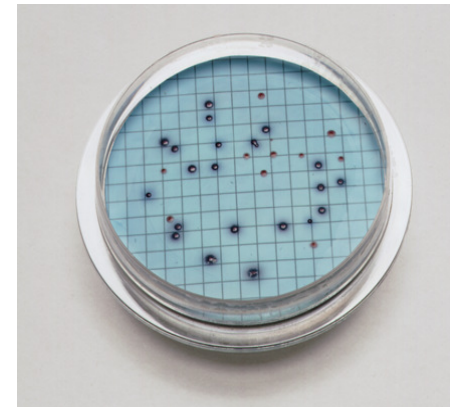
- Poorly performed or too infrequent of sanitations can lead to the build up of biofilms
 - Biofilms harbor and protect microbes from sanitation
 - Typically in hard to clean areas
 - Difficult to remove
 - Prevention is key

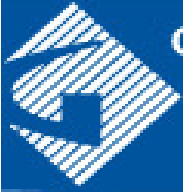




Process Water

- **Untreated water** can be a major source of contamination
 - Bottle rinser, spray hoses
- Water should be filtered or treated
 - **0.45 μ m is acceptable** for wineries, however, bear in mind some microbes may grow and be present which would not grow in wine and be a product concern
 - **0.2 μ m eliminates all microbes**
 - **Chlorine dioxide** helps eliminate micro contamination

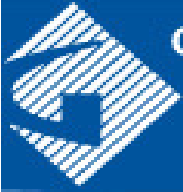




Sanitary Sampling



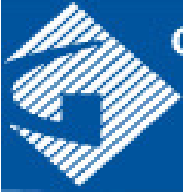
- Only open and close valves while under pressure
- Use smallest valve possible
- **Spray valve outlet with alcohol** and allow a second or two of flow before collecting sample
- Install specially designed **sanitary sampling valves**
- Use **sterile containers** for sample collection
- **Avoid dead legs** between product flow and valve outlet (long T's)



Advances in Rapid Micro

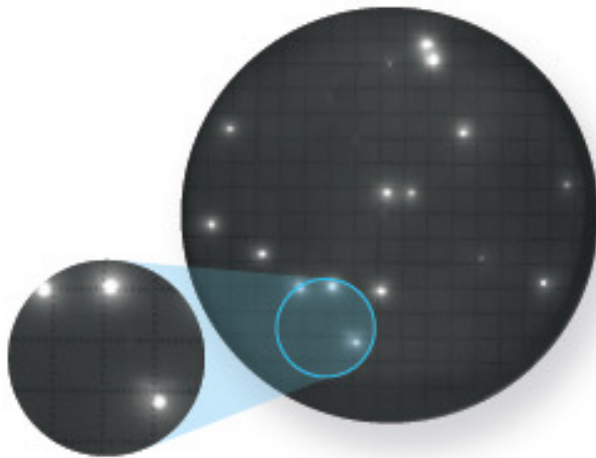
- **EZ-Fluo** Bioluminescent system
- **Reduces plating time** by 1/3 of current method
- Uses existing equipment, only requires the addition of a new reagent and reading the plate under a special reader



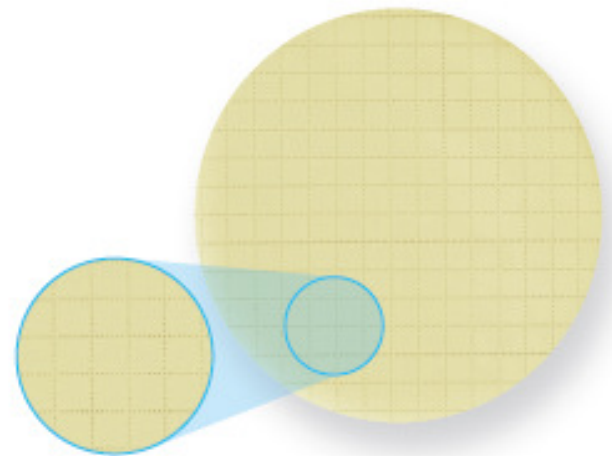


Advances in Rapid Micro

- Perform the current plating process
- Incubate for a reduced period of time (validation for each customer recommended due to media, incubation, product differences)
- Transfer membrane to a new Petri dish (w/ cellulose pad) that has had reagent added
- Re-incubate for approx 30 minutes
- View plate under reader



Visualize plate after staining.
View of membrane in the reader.



Colonies are not visible outside
the reader.



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EZ-Fluo Demo



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Thank you, Questions?