

# Understanding the Basics of Environmental Monitoring



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# Microbiological Surface Monitoring Systems

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- **Surfaces types include walls, floors, doors, windows, equipment, and tanks**
- **Types of Test Methods**
  - RODAC, Replicate Organism Detection And Counting Plates
  - Flexible Films
  - Swabs
  - Surface Rinse Methods



# Microbiological Surface Monitoring Systems

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## ■ RODAC Plates

- A 50 mm plate is filled with media so that the agar extends above the plate
- The surface of the media is rolled against a flat surface to sample
- The contact surface area of a RODAC is 25 cm<sup>2</sup>
- This is the most common method for sampling surfaces



# Microbiological Surface Monitoring Systems

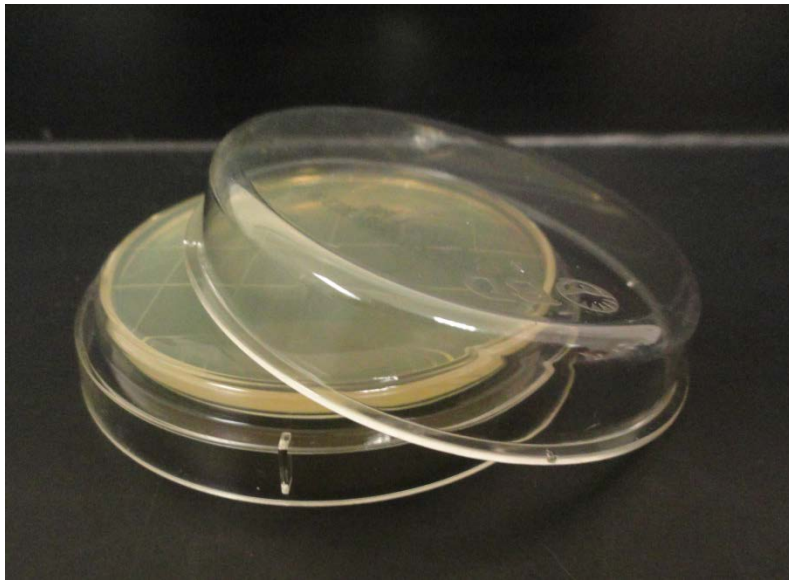
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- **RODAC Plates**

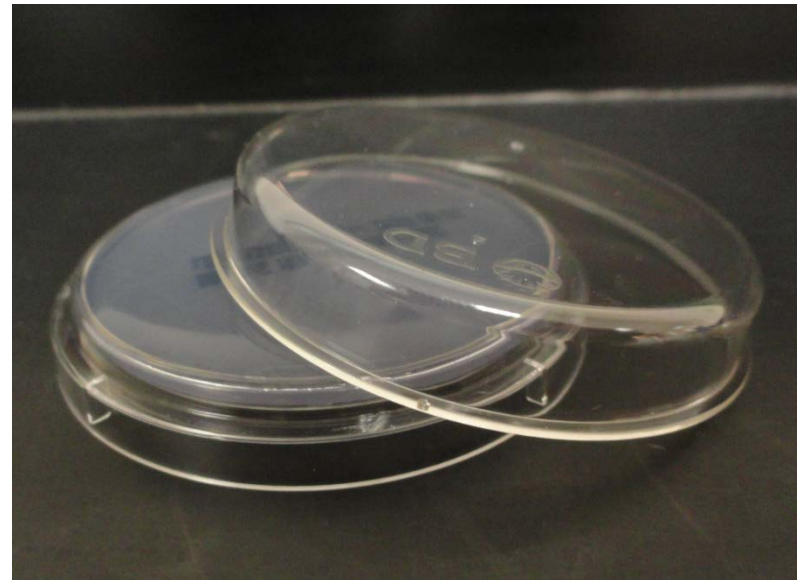
- Automated sampling devices are available
- Neutralizers are usually within the media
- Available, single, double or triple bagged and gamma irradiated
- Recommend not using plates poured in a Grade A area, then bagged aseptically

# Microbiological Surface Monitoring Systems

- **RODAC Plates**



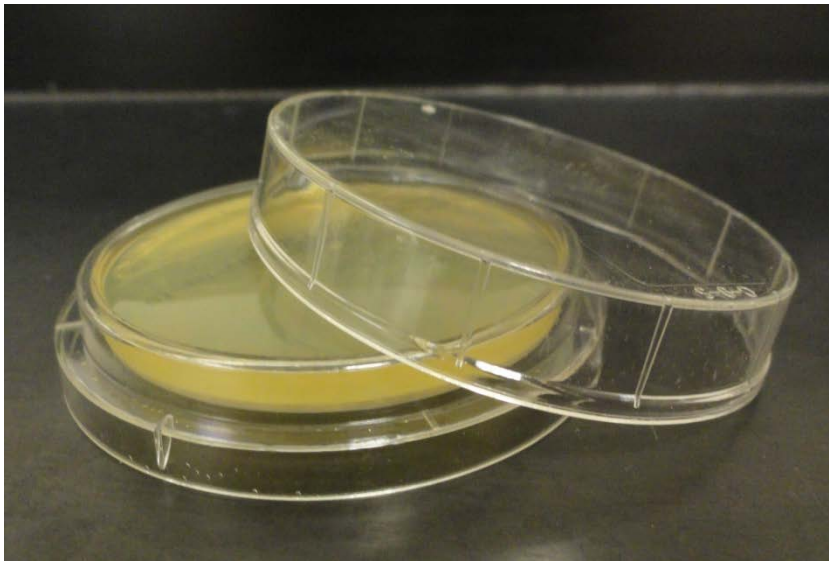
Poly-80 & Lec



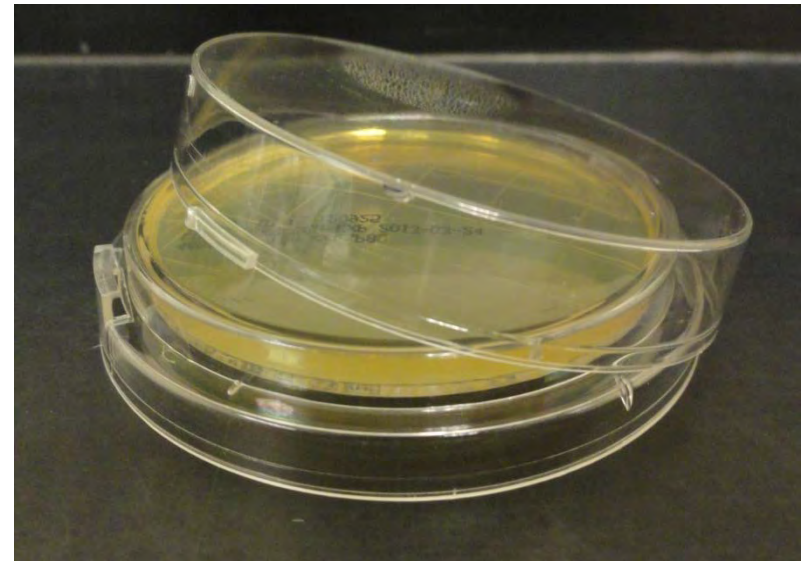
D/E Neutralizer

# Microbiological Surface Monitoring Systems

- **RODAC Plates**



Tacky Plates



Locking Plates

# Microbiological Surface Monitoring Systems

- **RODAC Plates Results**



First Sample



Second Sample

# Microbiological Surface Monitoring Systems

- **RODAC Plates Results**



Third Sample



Fourth Sample



# Microbiological Surface Monitoring Systems

- **RODAC Plates Results**



Fifth Sample



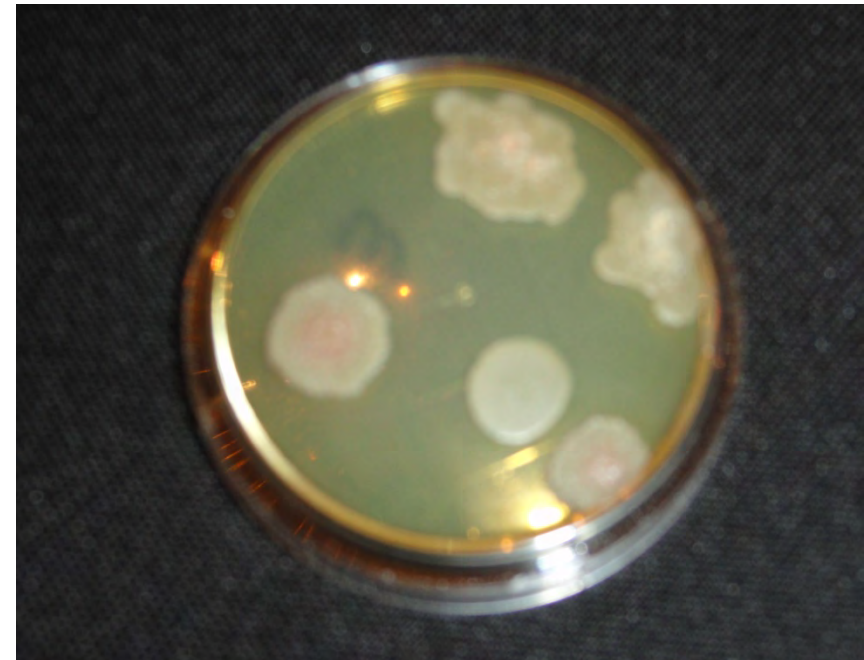
Sixth Sample

# Microbiological Surface Monitoring Systems

- **RODAC Plates Results**



Seventh Sample



Eighth Sample



# RODAC

## Contact Plates

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- Advantages

- Ease of use
- Fast
- Economical

- Disadvantages

- Not suitable for irregular surfaces
- Media residue must be removed from the sample site
- Recovery rate very low



# Microbiological Surface Monitoring Systems

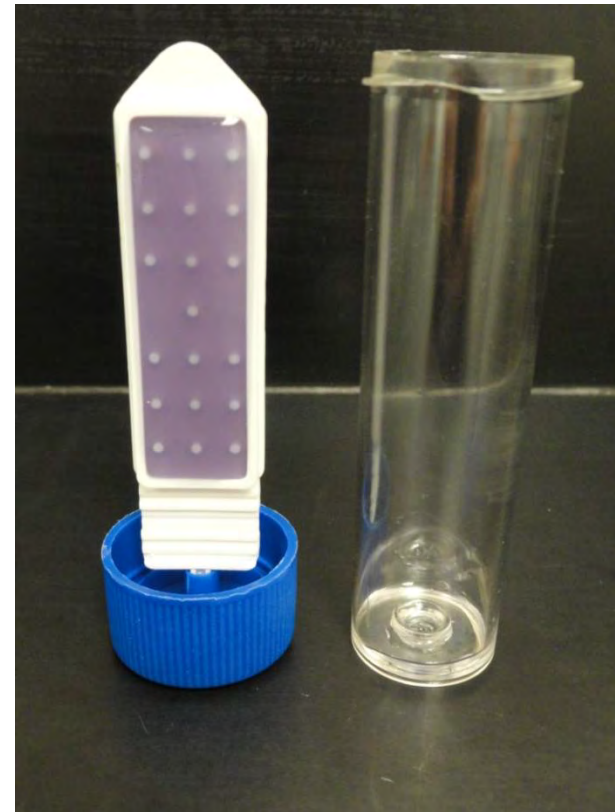
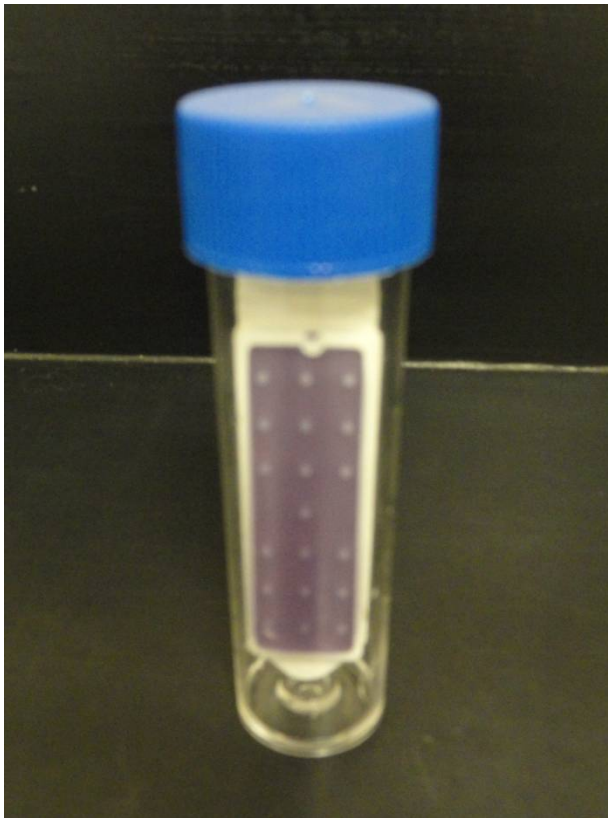
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- **Flexible Film**

- Media is deposited on a flexible material and used like a contact plate
- Can be used on irregular surfaces
- There are several versions, most have a surface area of 25 cm<sup>2</sup>

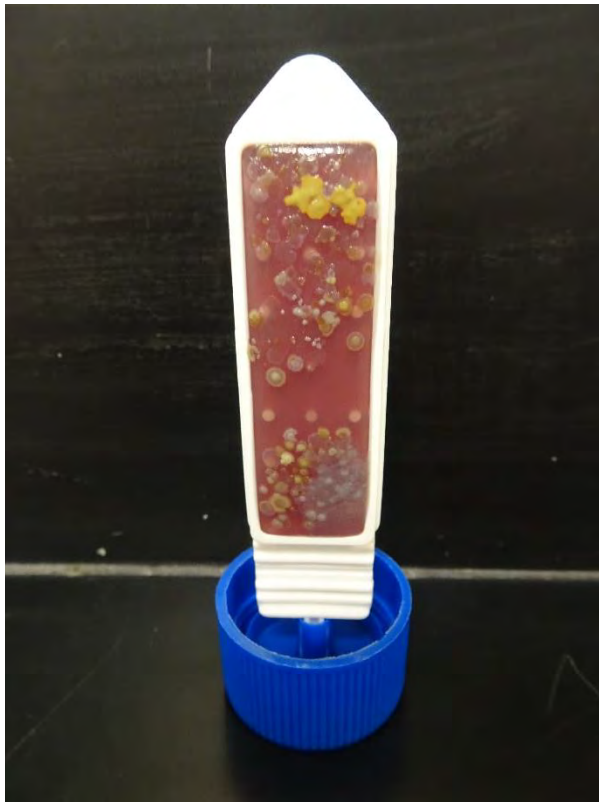
# Microbiological Surface Monitoring Systems

- **Flexible Film**



# Microbiological Surface Monitoring Systems

- **Flexible Film**





# Flexible Film Contact Strips

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## ■ Advantages

- Ease of use
- Economical
- Can bend in several configurations

## ■ Disadvantages

- Not suitable for irregular surfaces
- Media residue must be removed after sampling
- Recovery rate very low



# Microbiological Surface Monitoring Systems

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- **Swab Testing**

- Used for sampling irregular surfaces
- A sterile dry/moist swab is rubbed over 25 cm<sup>2</sup>, if possible
- Swabs may be made of Cotton, Dacron, Calcium Alginate or acceptable material





# Microbiological Surface Monitoring Systems

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- **Swab Testing**

- Organisms are transferred from the surface tested to the swab or paddle
  - ❖ Liquid Method
    - ✓ The swab is broken off into 3 - 5 ml of neutralizing broth
    - ✓ The broth/swab is vortexed to remove the organisms from the swab



# Microbiological Surface Monitoring Systems

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- ❖ Liquid Method Continued
  - ✓ The organisms are transferred from the swab to the neutralizing broth
  - ✓ The broth is plated leaving the swab in tube



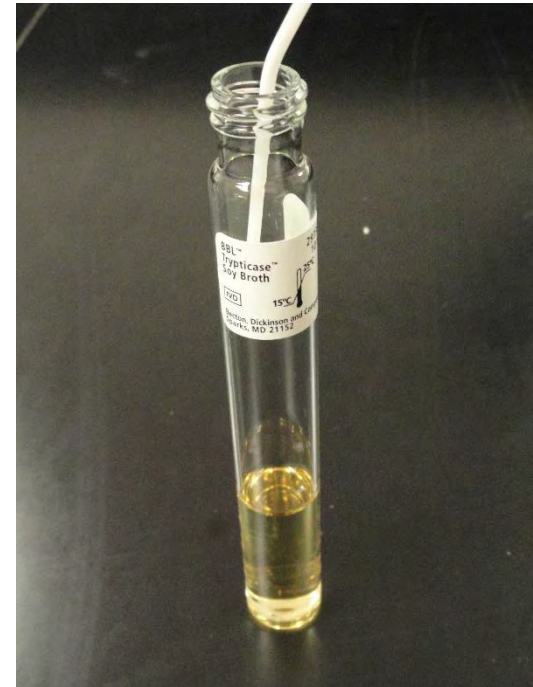
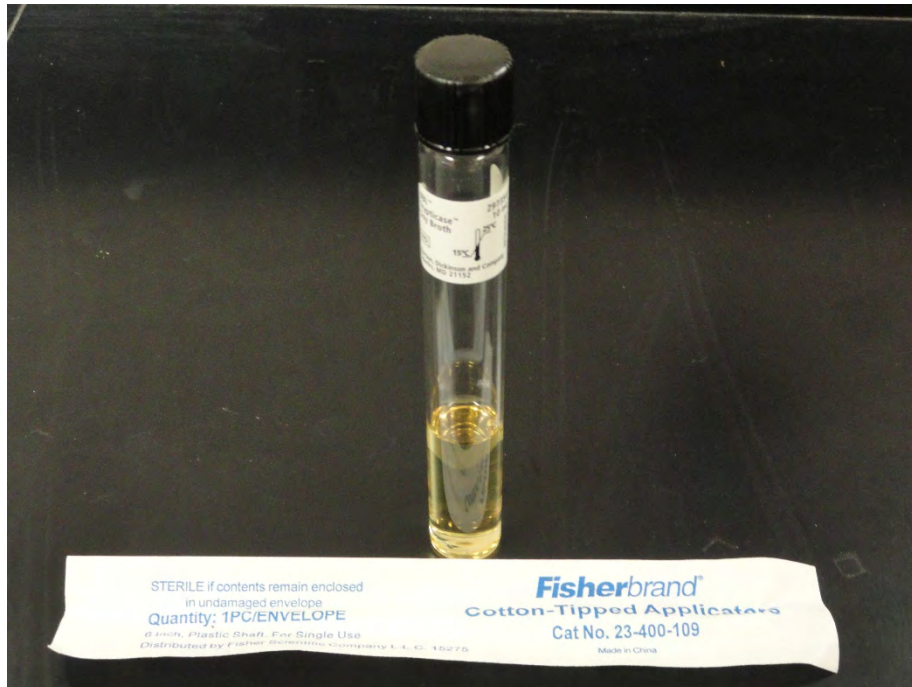
# Microbiological Surface Monitoring Systems

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- ❖ Direct to Broth Swab Method
  - ✓ After sampling the swab is broken directly into TSB
  - ✓ The swab is directly inoculated into the media
    - ❑ This method is qualitative and not quantitative
    - ❑ Fast and easy to use

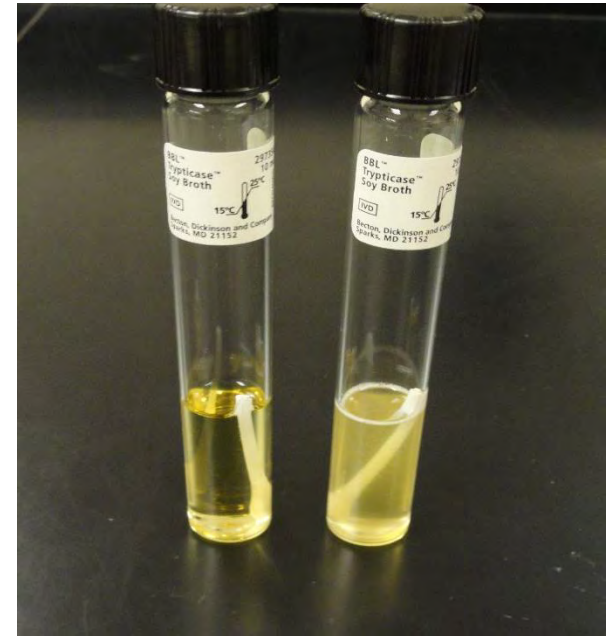
# Microbiological Surface Monitoring Systems

## ❖ Direct to Broth Swab Method



# Microbiological Surface Monitoring Systems

- ❖ Direct to Broth Swab Method





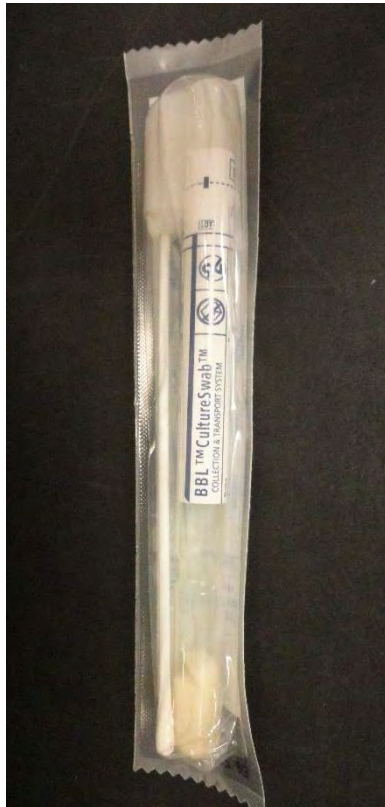
# Microbiological Surface Monitoring Systems

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- ❖ Direct to Agar Swab Method
  - ✓ The swab is directly inoculated on a TSA plate containing a neutralizer
    - This method is quantative
    - Fast and easy to use

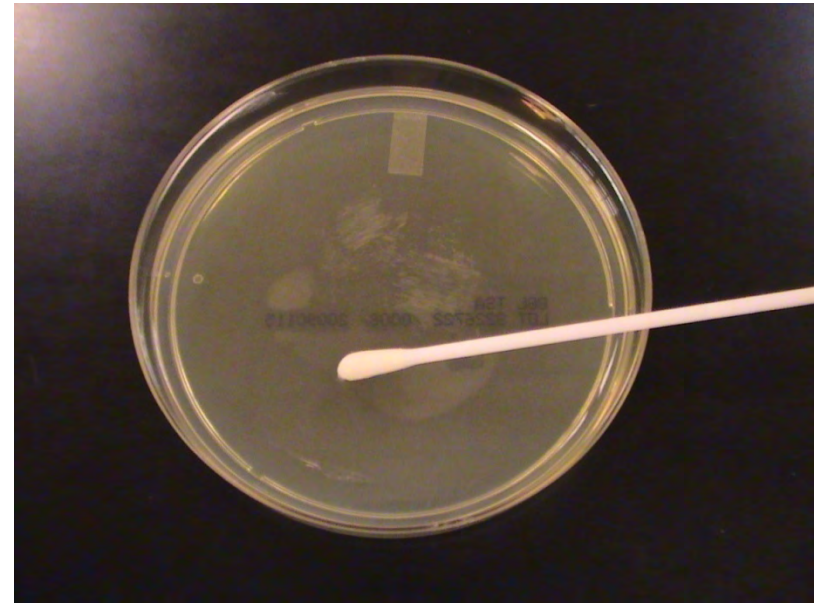
# Microbiological Surface Monitoring Systems

## Direct to Agar Method



# Microbiological Surface Monitoring Systems

## Direct to Agar Method





# Microbiological Surface Monitoring Systems

## Direct Agar Method



First Sample



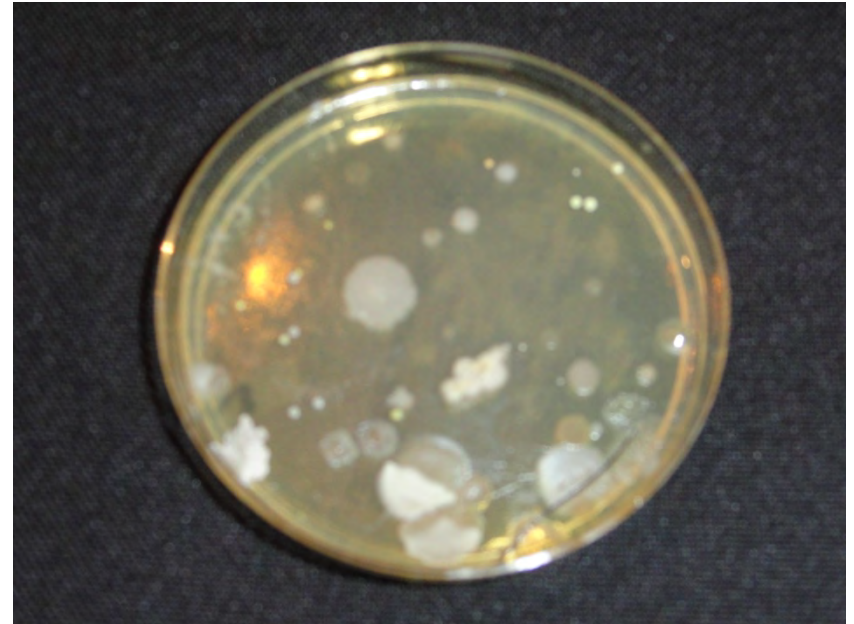
Second Sample

# Microbiological Surface Monitoring Systems

## Direct Agar Method



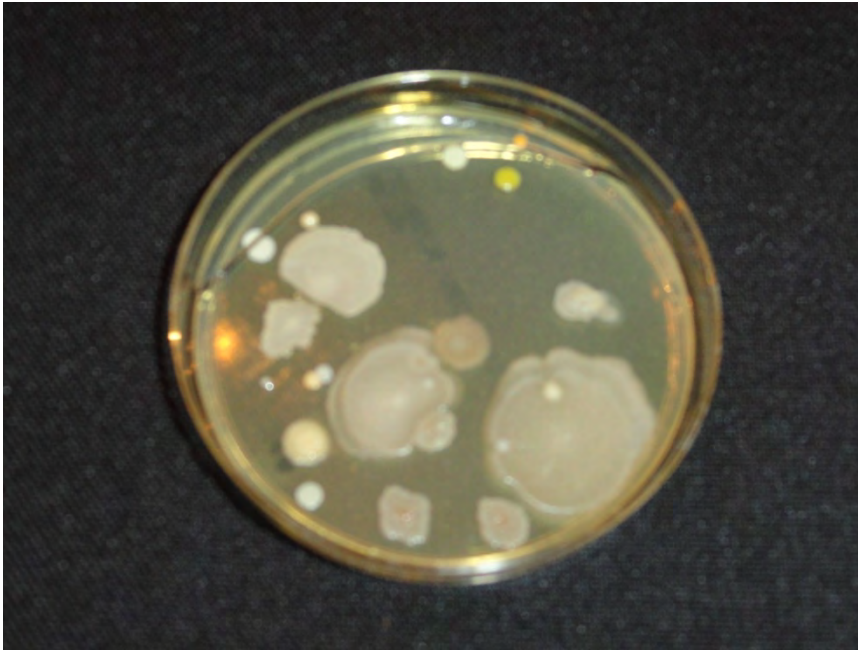
Third Sample



Forth Sample

# Microbiological Surface Monitoring Systems

## Direct Agar Method



Fifth Sample



Sixth Sample

# Microbiological Surface Monitoring Systems

## Direct Agar Method



Seventh Sample  
Note Spreading Bacillus



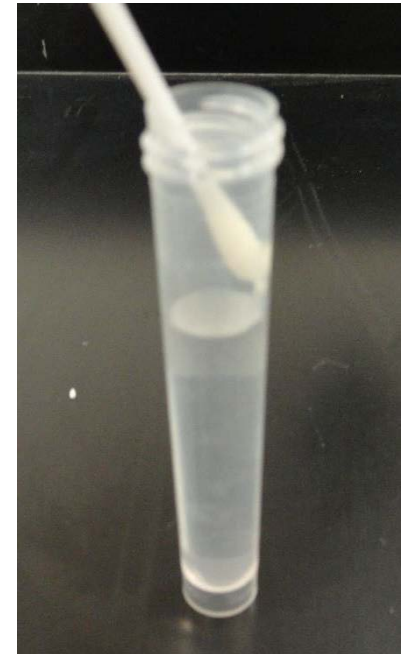
# Microbiological Surface Monitoring Systems

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- ❖ Pour Plate Method
  - ✓ Swab is wetted
  - ✓ Excess liquid is squeezed out
  - ✓ Surface is sampled
  - ✓ Swab is placed into a neutralizing broth
  - ✓ The broth is mixed and poured into an Petri Dish
  - ✓ Molten agar is poured into the plate and mixed

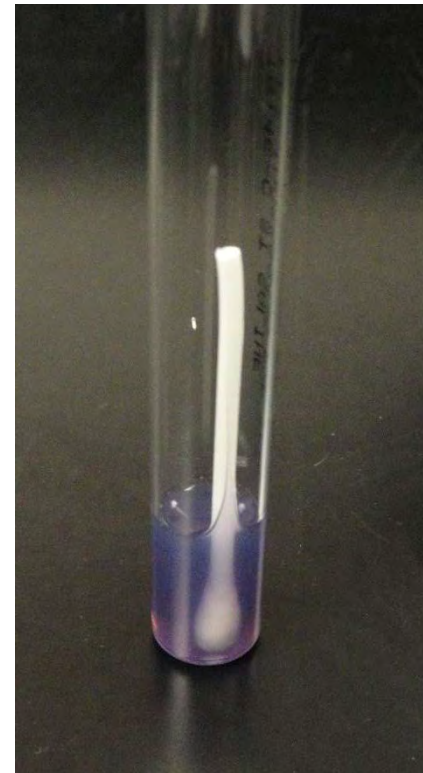
# Microbiological Surface Monitoring Systems

## Pour Plate System



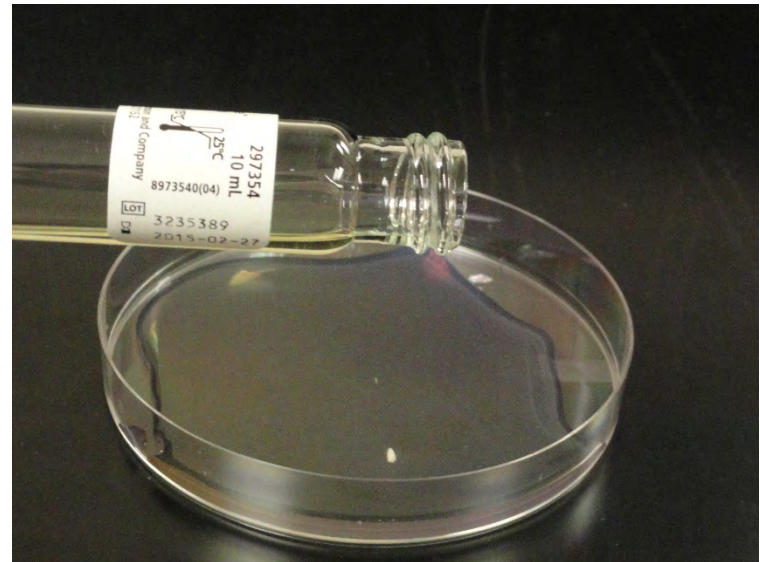
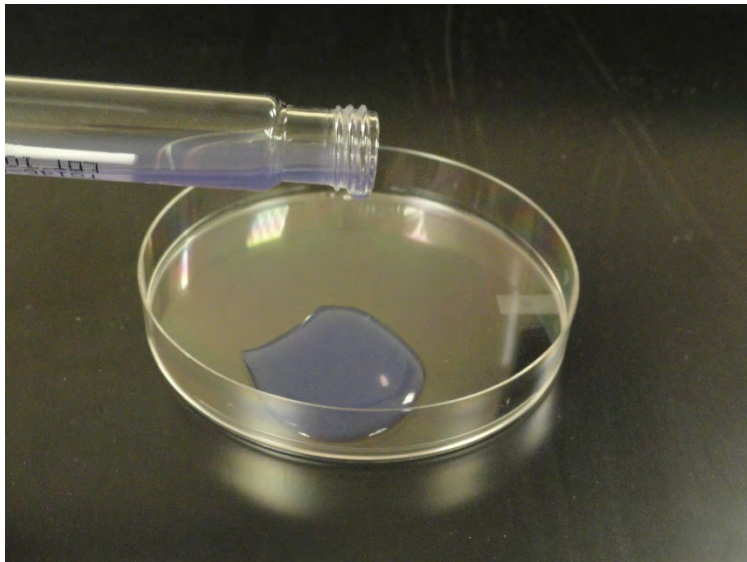
# Microbiological Surface Monitoring Systems

## Pour Plate System



# Microbiological Surface Monitoring Systems

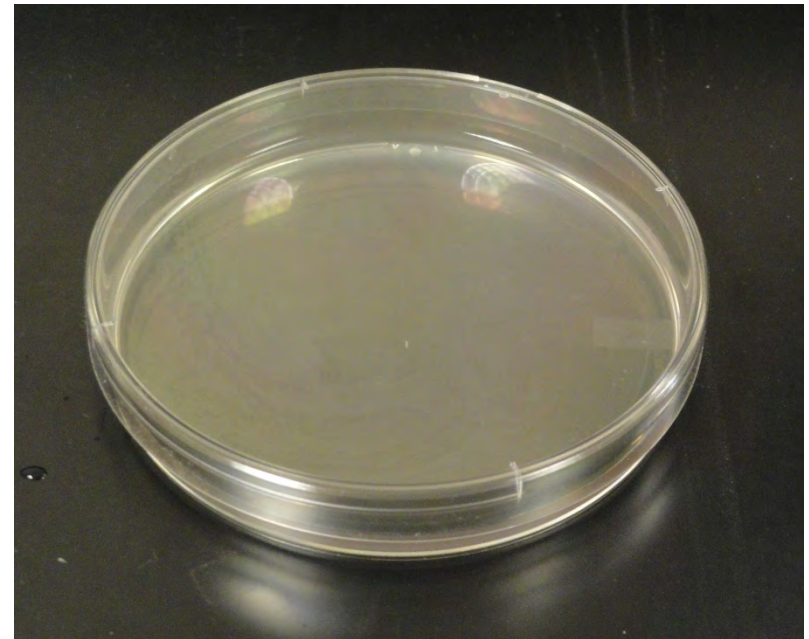
## Pour Plate System





# Microbiological Surface Monitoring Systems

## Pour Plate System

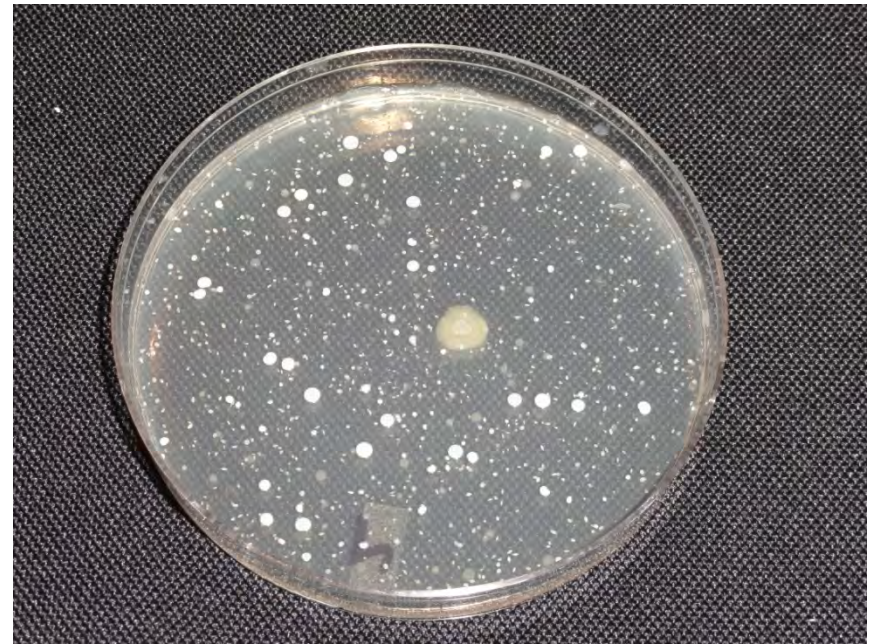


# Microbiological Surface Monitoring Systems

## Pour Plate System



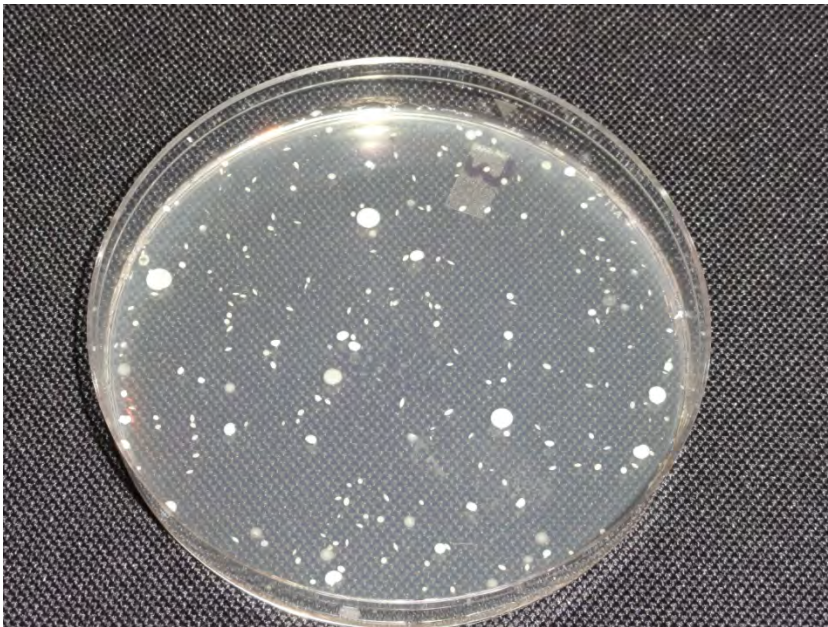
First Sample



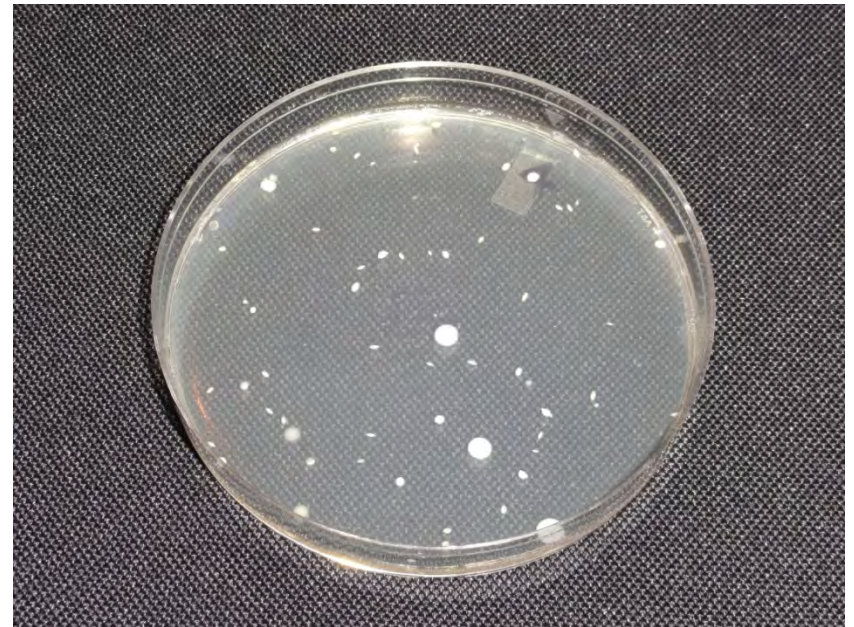
Second Sample

# Microbiological Surface Monitoring Systems

## Pour Plate System



Third Sample



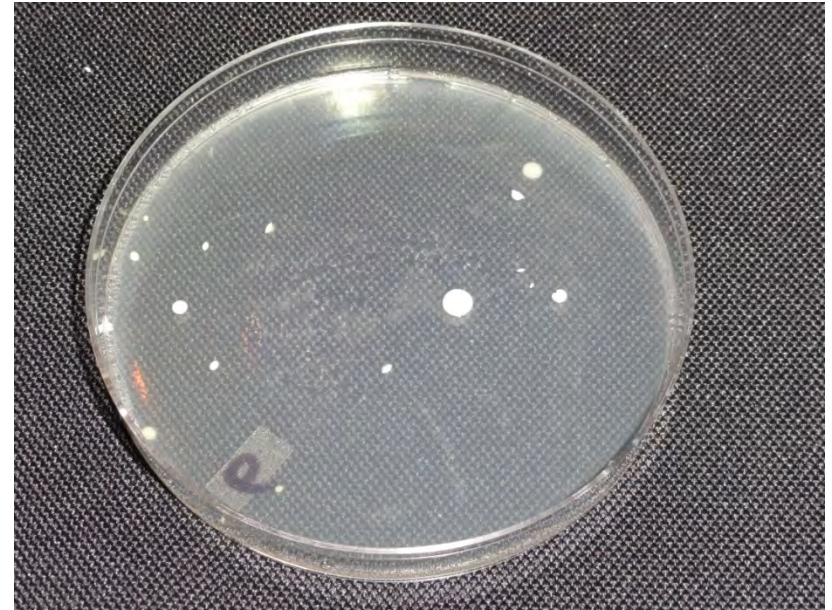
Forth Sample

# Microbiological Surface Monitoring Systems

## Pour Plate System



Fifth Sample



Sixth Sample

# Microbiological Surface Monitoring Systems

## Pour Plate System



Seventh Sample

Note growth around the edge of swab



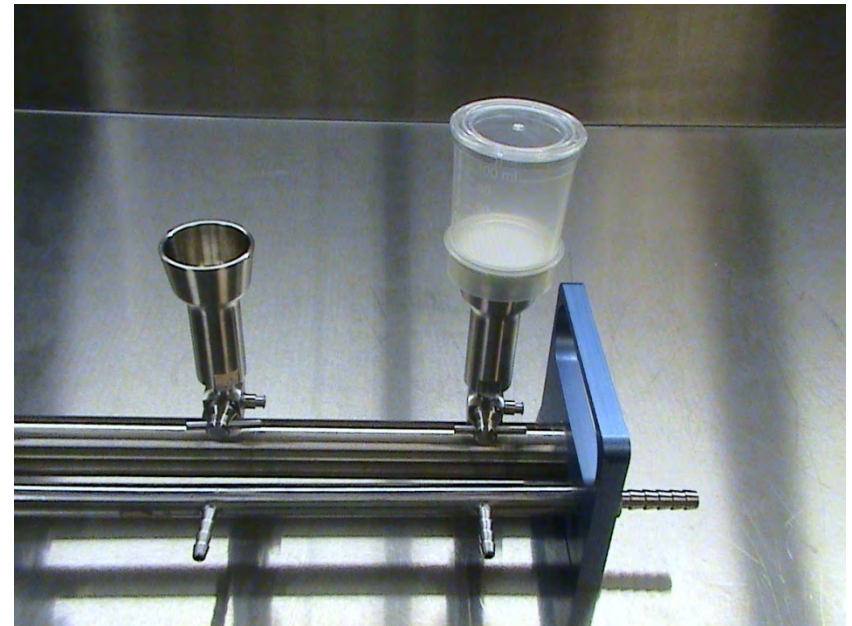
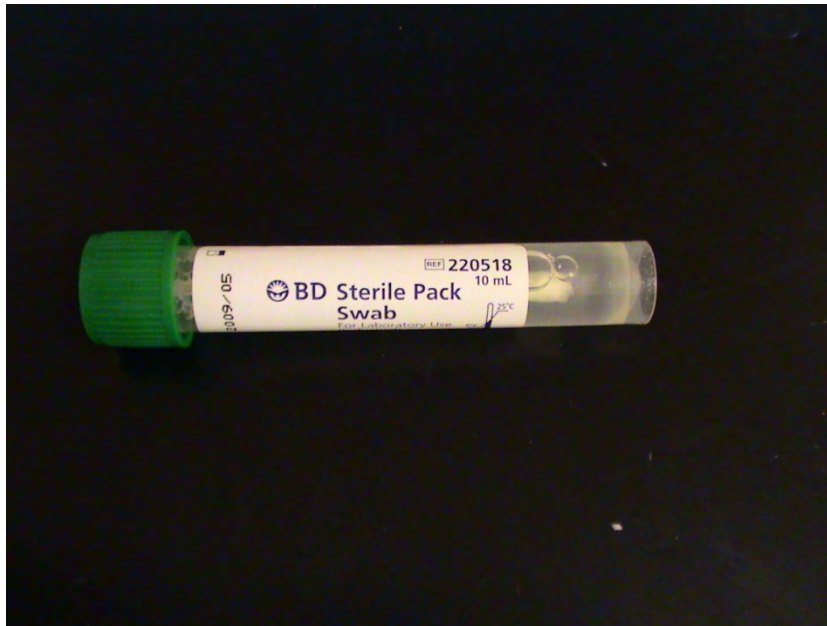
# Microbiological Surface Monitoring Systems

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- ❖ Liquid Method
  - ✓ The broth can be processed as follows
    - Membrane Filtration
    - Spread plate

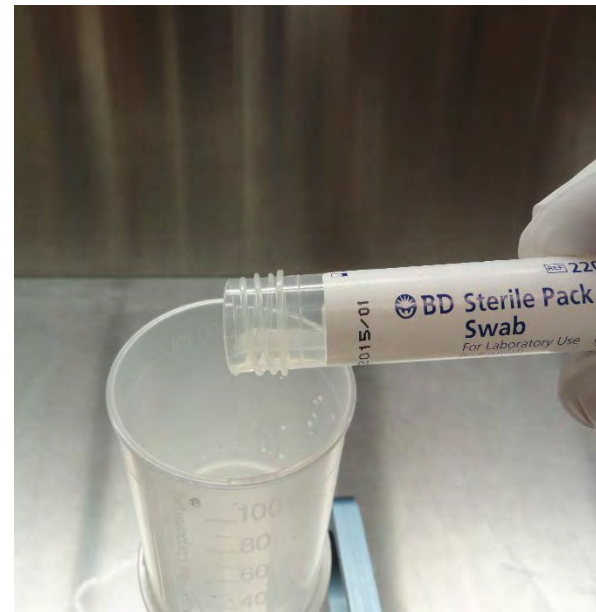
# Microbiological Surface Monitoring Systems

## Membrane Filtration



# Microbiological Surface Monitoring Systems

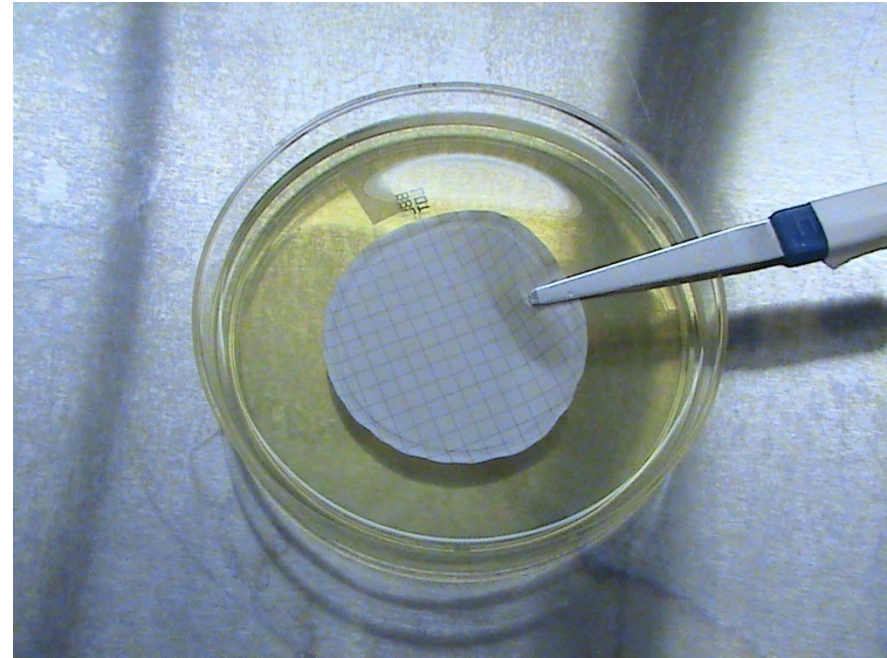
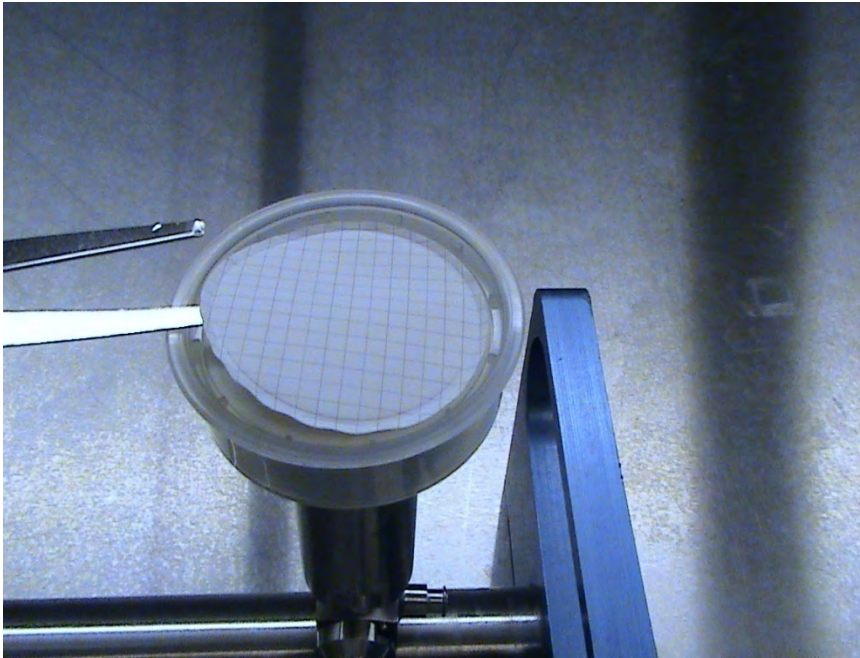
## Membrane Filtration





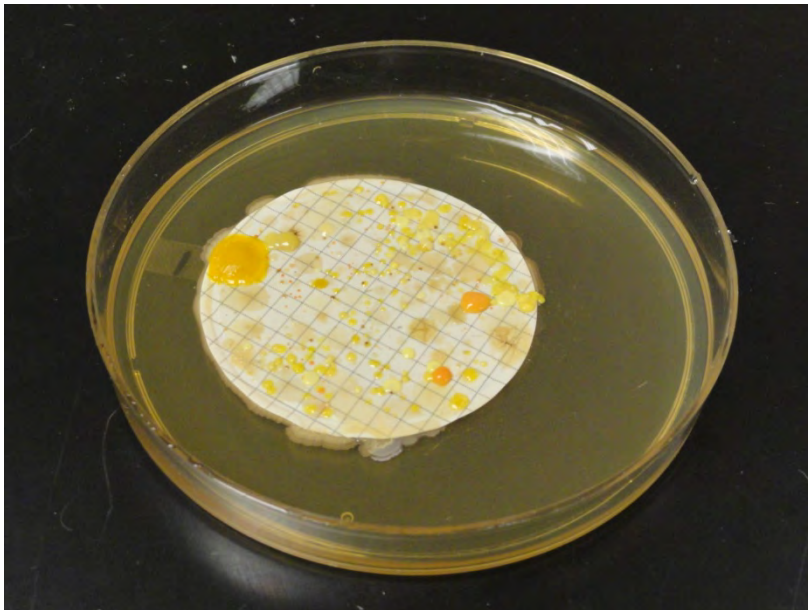
# Microbiological Surface Monitoring Systems

## Membrane Filtration

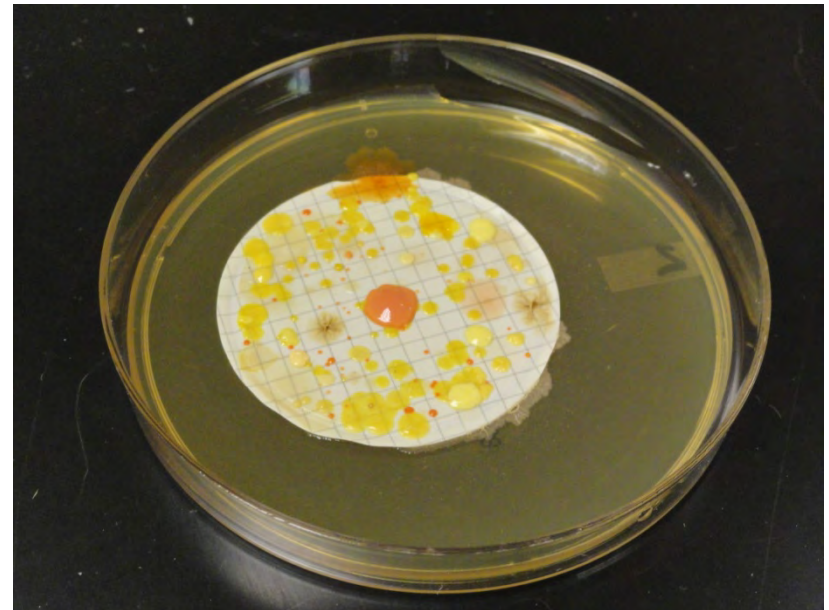


# Microbiological Surface Monitoring Systems

## Membrane Filtration



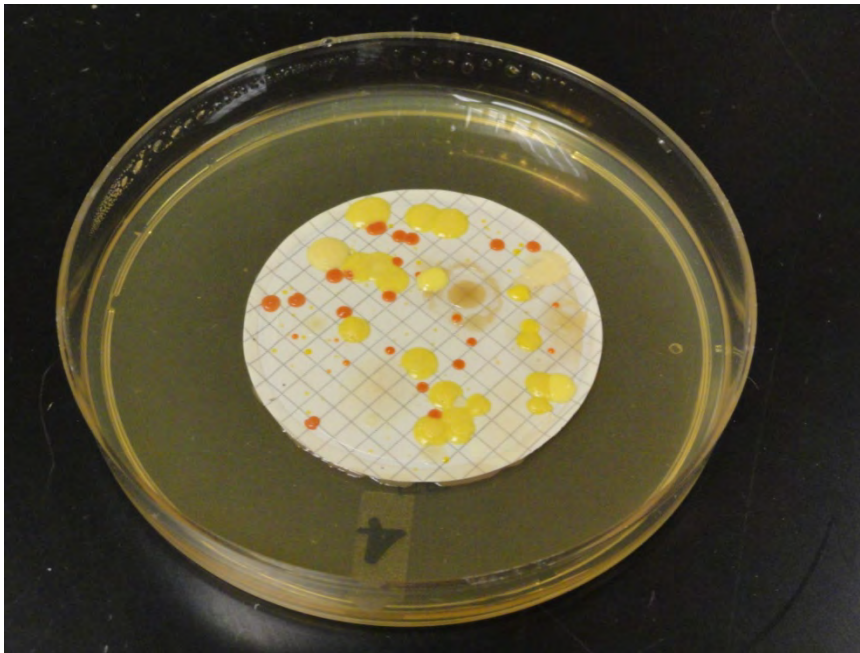
First Sample



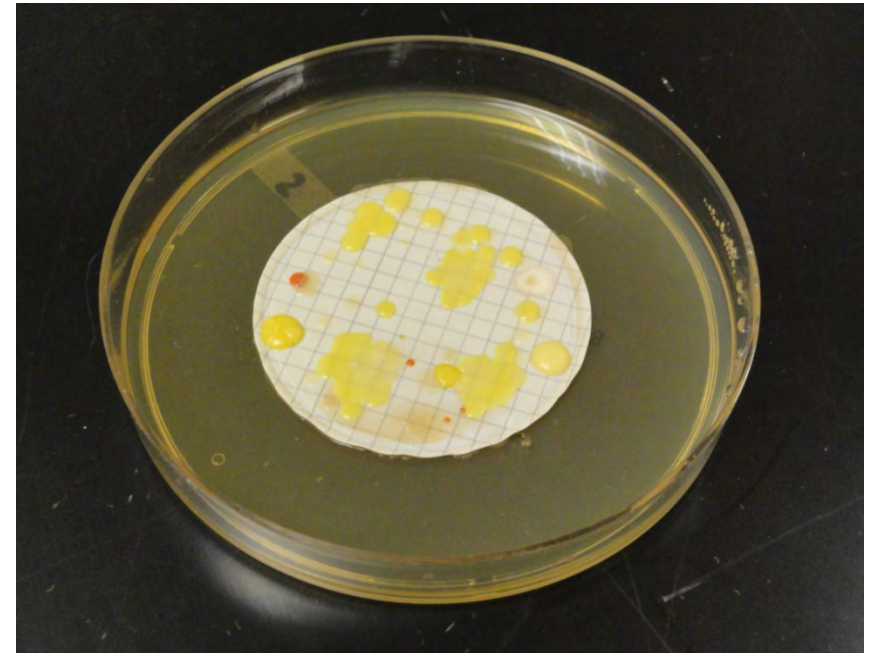
Second Sample

# Microbiological Surface Monitoring Systems

## Membrane Filtration



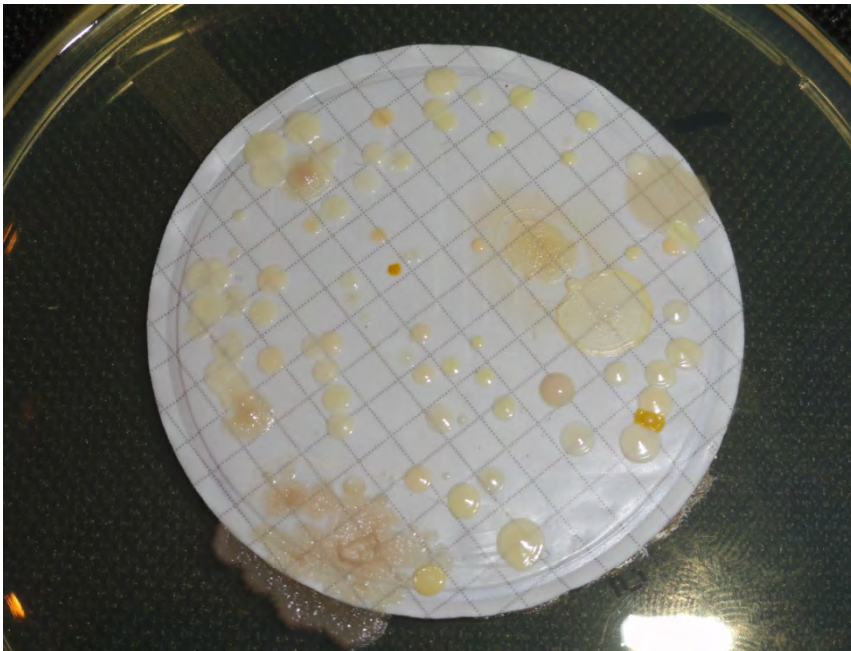
Third Sample



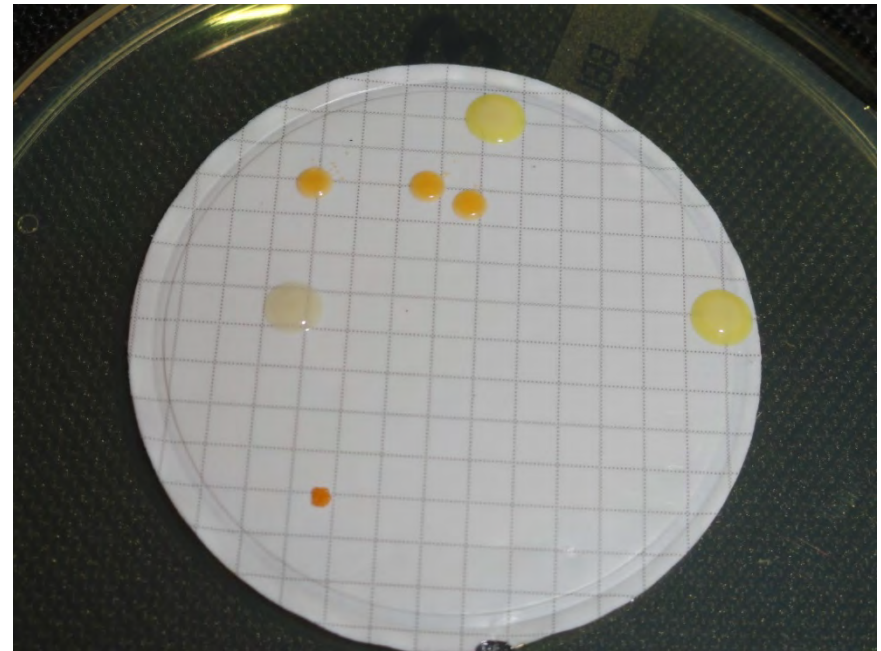
Forth Sample

# Microbiological Surface Monitoring Systems

## Membrane Filtration



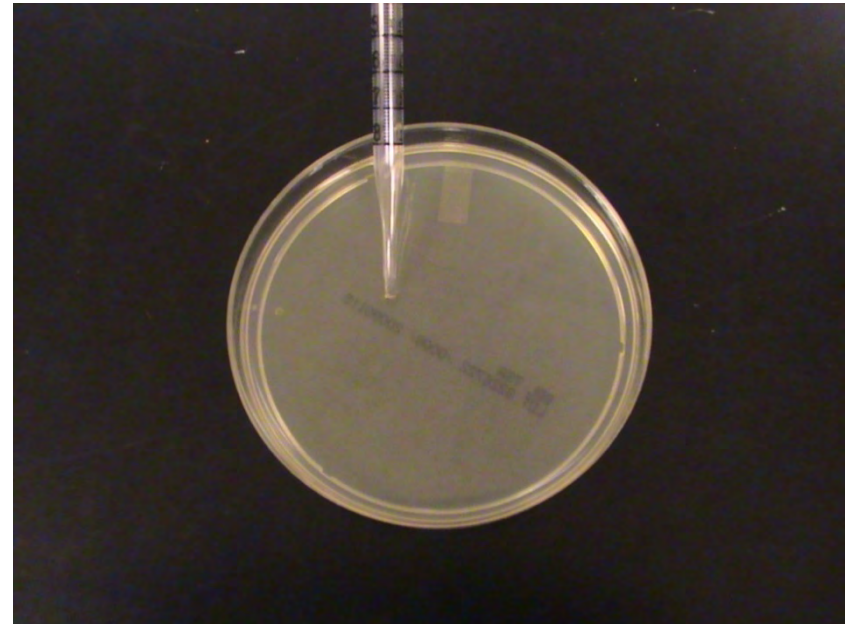
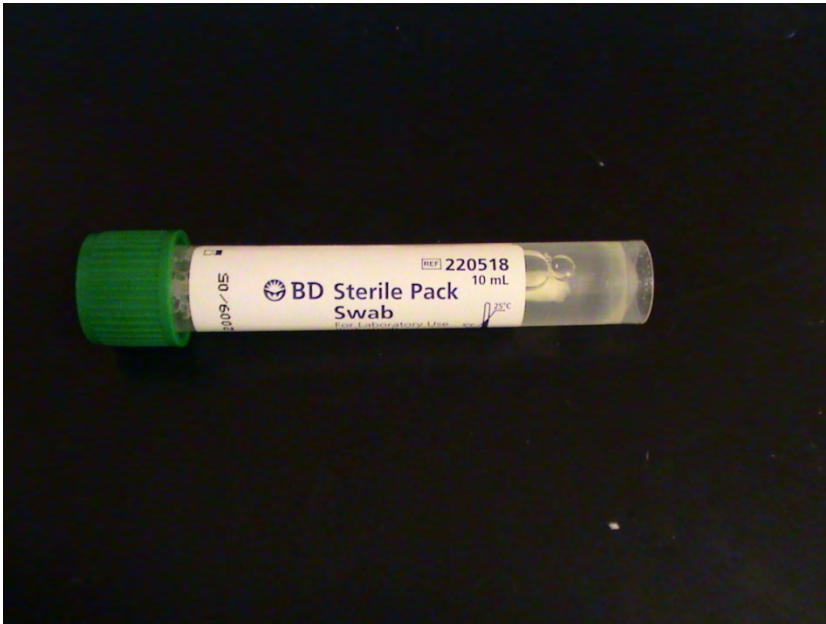
Fifth Sample



Sixth Sample

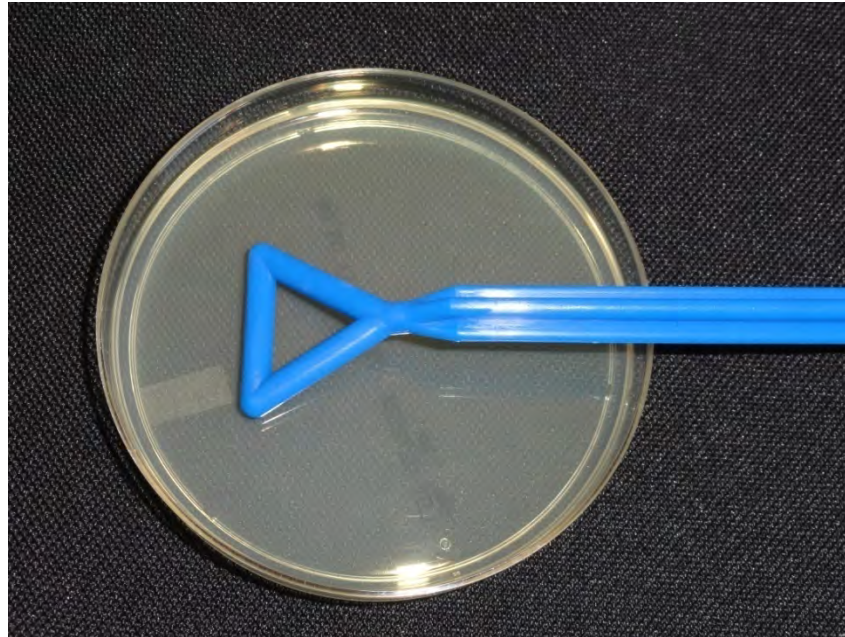
# Microbiological Surface Monitoring Systems

## Spread Plate System



# Microbiological Surface Monitoring Systems

## Spread Plate System



# Microbiological Surface Monitoring Systems

## Spread Plate System



First Sample



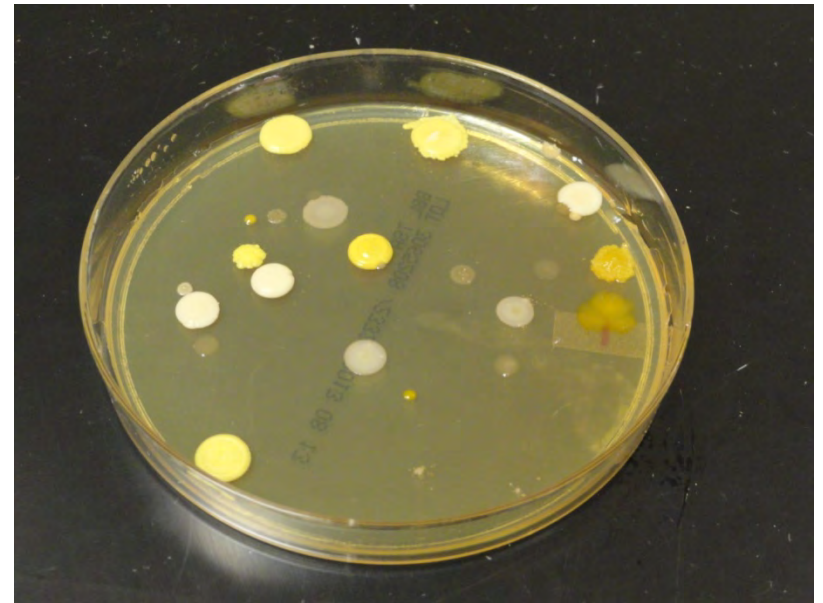
Second Sample

# Microbiological Surface Monitoring Systems

## Spread Plate System



Third Sample

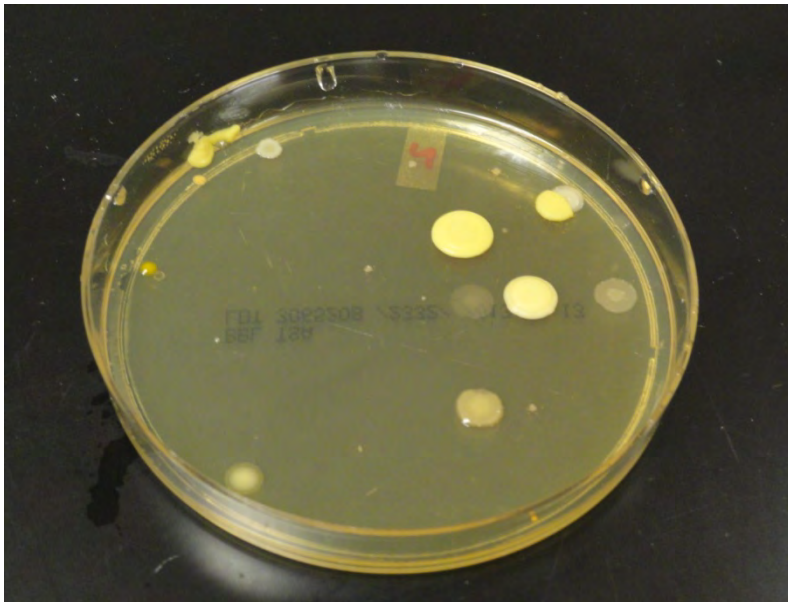


Fourth Sample

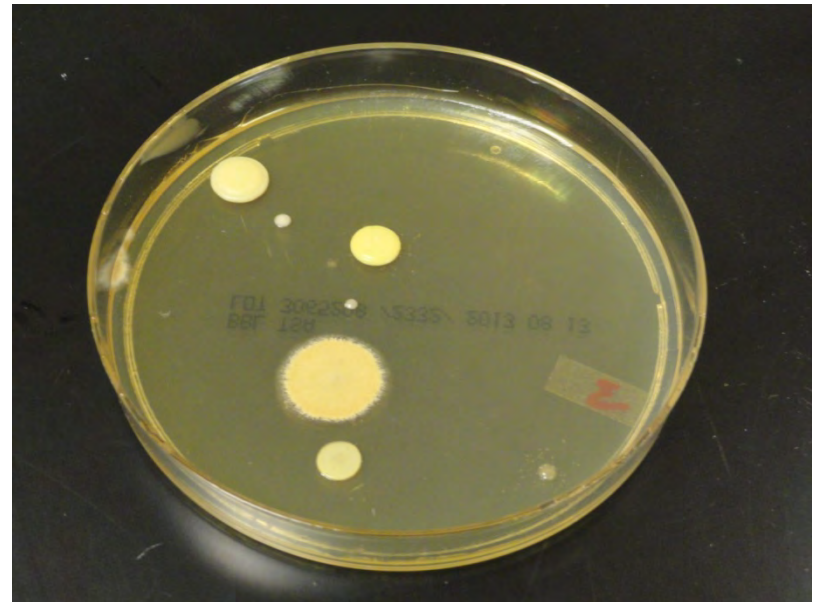


# Microbiological Surface Monitoring Systems

## Spread Plate System



Fifth Sample



Sixth Sample



# Swab Testing for Irregular Surfaces

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## ■ Advantages

- Useful for irregular surfaces
- Can be used on flat surfaces

## ■ Disadvantages

- Sampling techniques could effect results
- Requires manipulation to culture sample
- Time consuming
- Variable based on operator techniques
- Recovery rate very low



# Microbiological Surface Monitoring Systems

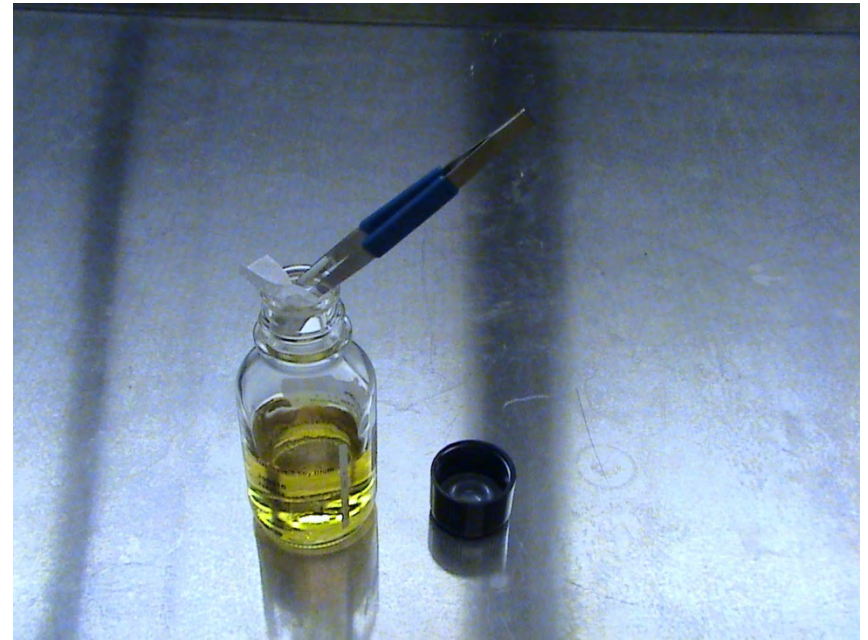
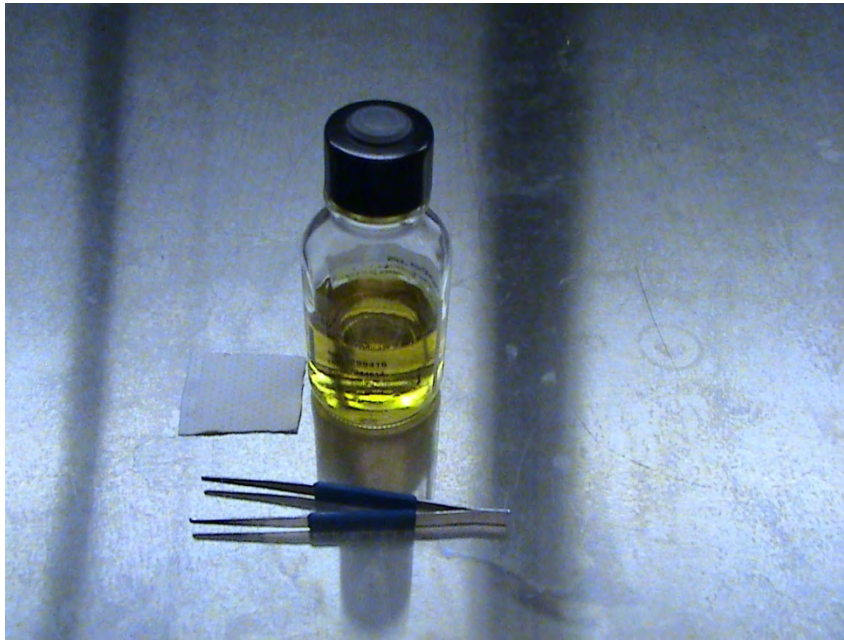
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- **Surface Wipe Method**

- Wipe the testing area with a sterile wet wipe to pick up any CFU's present
- Place sterile wipe into TSB
- Incubate

# Microbiological Surface Monitoring Systems

## Surface Wipe Method



# Microbiological Surface Monitoring Systems

## Surface Wipe Method





# Surface Rinse Method

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## ■ Advantages

- Easy to use
- Can sample a large surface area
- Sample flat and irregular surfaces

## ■ Disadvantages

- Can get messy
- Requires a large volume of media in the sampling area
- Media can tip over
- Placing sterile wipe into the media can be problematic



# Surface Rinse Method

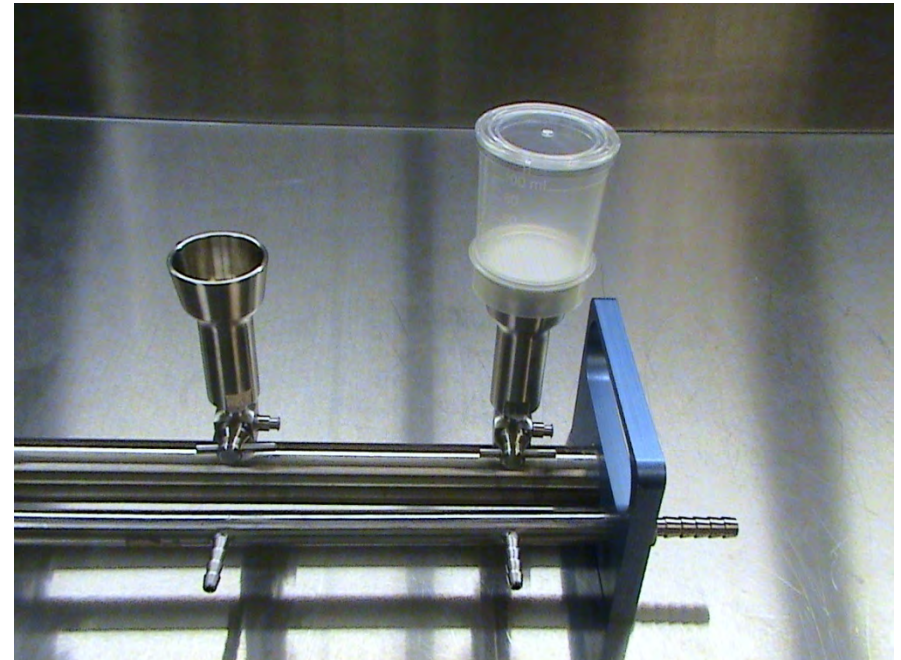
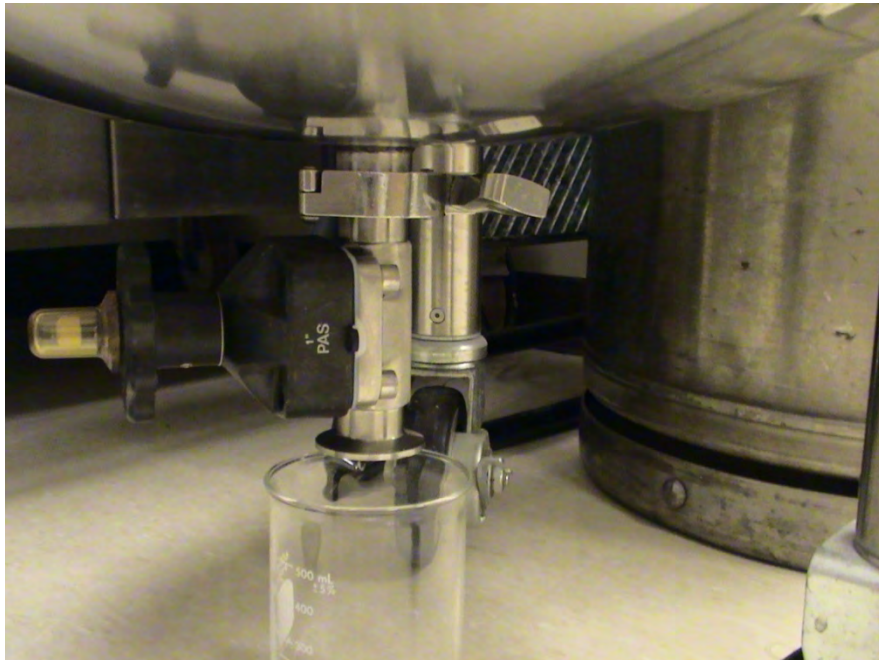
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- **Surface Rinse Method**

- In this method, the container/surface is rinsed with water
- The rinse water is membrane filtered
- The filter is placed on a agar surface
- Used mainly for interior of tanks

# Surface Rinse Method

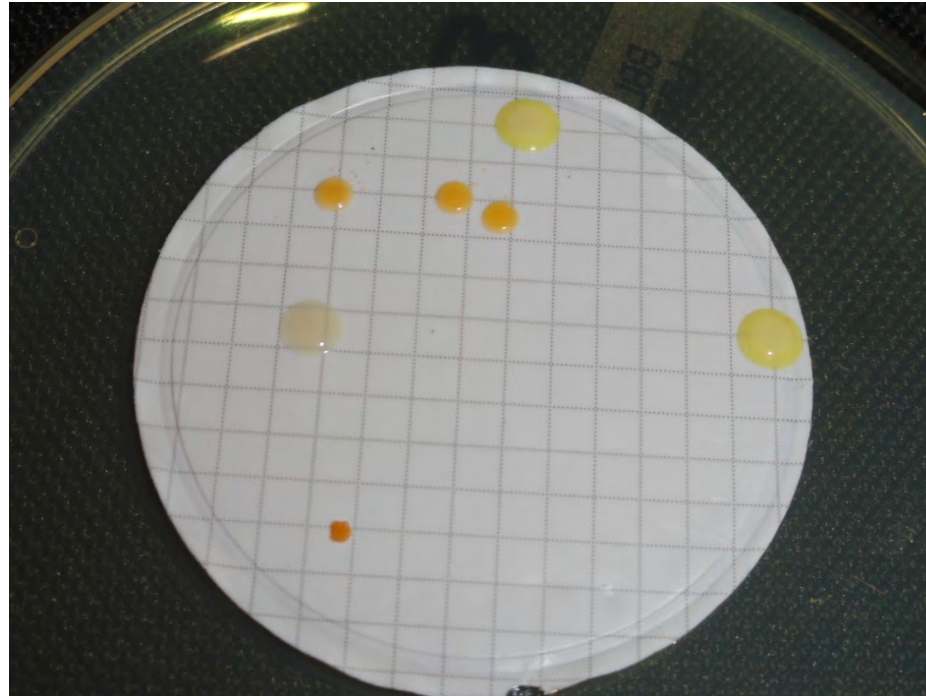
## Surface Rinse Method





# Surface Rinse Method

## Surface Rinse Method





# Surface Rinse Method

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## ■ Advantages

- Useful for large surface areas, where interior surface bioburden is needed.
- Some examples are kettles, tanks and equipment.
- Useful for irregular surfaces.

## ■ Disadvantages

- Not suitable for most applications.
- Requires extensive manipulations.
- Techniques and sample processing can affect results.



# Hot Topics of Interest

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- **Yeast and Mold Testing**
  - Can be combined with bacterial monitoring
  - Performed with routine monitoring or at a minimum quarterly
  - Take a second sample using selective agar
    - ❖ Sabaraud Dextrose Agar (SDA)
    - ❖ Potato Dextrose Agar (PDA)
    - ❖ Rose Bengal Agar (RBA)
  - Incubation 20 – 25 °C



# Hot Topics of Interest

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- **Incubation Times and Temperatures**

- Samples should be placed into the incubator in less than 4 hours after sampling
  - ❖ This time needs to be qualified
- The lower temperatures require longer incubation
- Incubation duration between 48 – 168 hours



# Hot Topics of Interest

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- **Incubation Times and Temperatures**
  - Incubation temperatures
    - ❖ 20 -25 °C
    - ❖ 27.5 – 32.5 °C
    - ❖ 30 - 35°C
    - ❖ 20 -25°C and 30 - 35°C



# Hot Topics of Interest

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- **Anaerobic Monitoring**
- **Media Types and Vendors**
- **Contamination Control**
- **Qualification/Validation of Methods**



# Hot Topics of Interest

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## ■ Takeaway Message

- Ensure proper viable air sampler is being used based on exhaust air
- Confirm all sample locations are justified based on the validation data
- Routing sampling is not performed in static conditions
- Ensure all testing methods are optimized