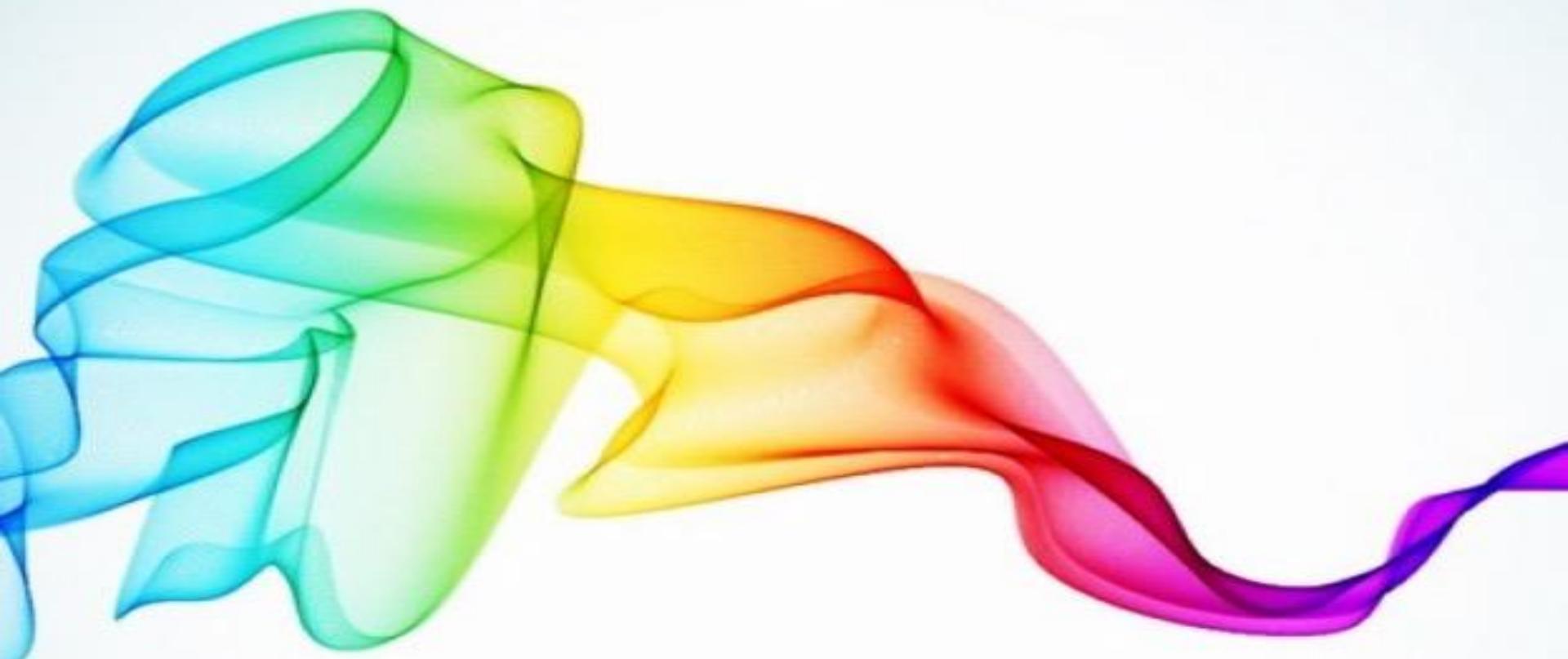


# **SWAB LAB**



**An autoclave is an instrument used to sterilize equipment and supplies by subjecting them to high pressure steam at 121° C temperature for around 15-20 minutes depending on the size of the load and the contents**

**TIME  
TEMPERATURE  
PRESSURE**



If you are getting  
a tattoo or  
piercings, be  
sure to ask about  
autoclave  
settings...!



# Premise of the Swab Lab

You will swab an everyday, ordinary object, you will put your sample on growth media/petri plates, we will incubate these plates and you will come back in to see the evidence of bacterial (sometimes fungal too) growth.

Demonstrating the *Ubiquity of Microbes*  
- the **CENTRAL THEME** of this class!

# Microbes are Ubiquitous

The phrase “Ubiquity of Microbes” refers to the concept that microbes can be found just about everywhere (core concept of this course!!!)

Microbes occupy an amazing diversity of niches; some live in hot springs where temperatures exceed the boiling point, while others have been found living inside solid rock - MICROBES ARE EVERYWHERE!!! (core concept of this course!!!)

# Microbes are Ubiquitous

Some microbes are found in cores of ice, almost 12,000 feet below an ice sheet in the Antarctic

Some live by deep sea hydrothermal vents at extraordinary pressures and temps up to 221°F

Bacteria have even been revived after being suspended in amber (fossilized tree sap) for tens of millions of years!



# **Microbes are Ubiquitous**

**Essentially, the only places where microbes are not found are places where they are intentionally excluded – and even then it's not always doable**

**Many different industries (manufacturing plants - computer components, chips, instruments used in healthcare setting) spend millions and millions of dollars on “clean rooms” designed to minimize and attempt to eliminate microbial contamination**

**\$\$\$ *Cha-ching!* – if you become an expert at clean room technology you can make a decent salary**

# After Incubation Review of Plate

Look at your plate to examine the following:

*How many colonies are there?*

*How many different kinds of colonies?*

*What color, shape and size are colonies?*

*Flat or round, dull or shiny, smooth or granular?*

It is assumed that each colony arose from a single bacterium that multiplied after incubation, 24-48 hours, and are thus genetically identical (same single ancestor)



# Microbial Cultures

# Microbial Cultures (your plate)

Culturing microbes means providing optimal controlled lab conditions, conducive for growth  
*(environmental, nutritional etc.)*

Cultures are prepared in order to GROW more microbes than what's obtained in an original sample - via REPLICATION due to fantastic conditions provided

# Ideal Growth Environments

Lab conditions are designed to provide an ideal growing environment:

- nutrients
- temperature
- lighting
- gas concentrations

Consideration is also given to: pH, salinity, and barometric pressure, etc. (other growth factors)

# What are Cultures Used For?

Microbial cultures are used to determine the type of organism, its abundance in a sample being tested, or both i.e, “*what kind(s) of bacteria and how much is there?*”



# You will be given Petri Plates



Empty Plate Before  
Agar is Added

Petri dishes (PLATES) will be filled with growth media called agar (a seaweed gelatin product filled with nutrients)

We will swab two different plates for this lab (*each with a different kind of agar*) Each type specializes in growing certain kinds of bacteria

One plate has agar known as  
**Blood Agar Plates (BAP)**

One plate has agar known as  
**Eosin Methylene Blue (EMB)**

# BLOOD AGAR PLATES - BAP



Red blood cells on an agar plate are used to diagnose infection. On the left is a positive *Staphylococcus* infection, on the right a positive *Streptococcus* culture.

# EIOSIN METHYLENE BLUE (EMB)



*E. coli*



*K. pneumoniae*

## Blood Agar Plates (BAP)

BAP plates are used to  
detect pathogenic bacteria (skin, throat)

## Eosin Methylene Blue (EMB)

EMB plates are used to  
detect bacteria associated with fecal  
contamination

# Blood Agar Plate (BAP)

Bacterial growth medium (made from agar and a percentage of sheep blood) that helps distinguish between pathogenic bacteria and non pathogenic bacteria (*often skin bacteria*)



## Blood Agar Plate (BAP)



*Left:* Plain agar plate, no blood added

*Right:* Agar plate with 10% sheep blood

# Why Use Blood Agar Plates?

To detect pathogenic bacteria (often from skin)

This type of pathogenic bacteria often have enzymes that lyse (*rupture*) red blood cells

These enzymes are known as hemolysins and the process is hemolysis

Bacteria that can lyse blood cells are often pathogenic (*strep throat*)

Blood agar plates help to visually detect these kinds of bacteria by looking at hemolysis in agar



Hemolysis:  $\alpha$   $\beta$   $\gamma$

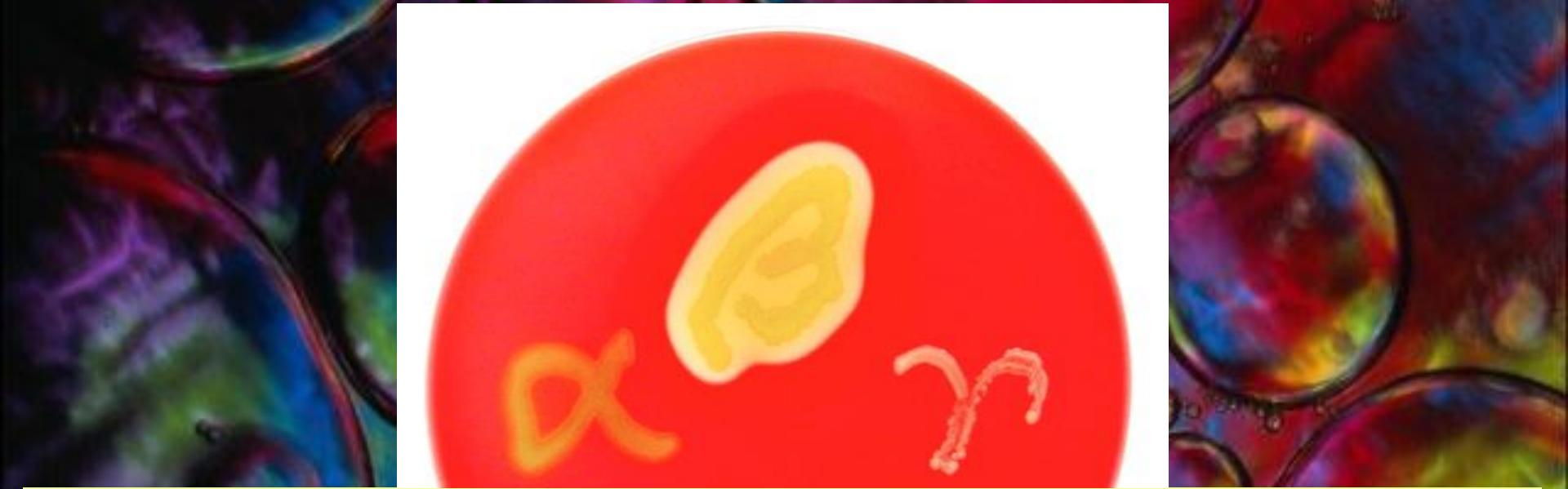
Seen in plate agar

**Blood Agar Plates detect hemolysis of red blood cells**

**$\beta$  (beta) hemolysis shows complete lysis of red blood cells (agar is clear around colony)**

**$\alpha$  (alpha) hemolysis partially lyses hemoglobin and agar appears greenish**

**$\gamma$  (gamma) hemolysis (non-hemolytic) refers to a lack of hemolysis (no clearing of agar)**



**β - Beta** hemolysis shows complete lysis of red blood cells (agar is clear around colony)

**α - Alpha** hemolysis shows a partial hemolysis of red blood cells (agar appears green/brown)

**γ - Gamma** hemolysis refers to NO hemolysis (no clearing of agar/no change)

# BAP - Beta Hemolysis $\beta$

$\beta$ -hemolysis means the bacteria's hemolytic enzymes break down red blood cells

$\beta$ -hemolysis pattern result in the agar displaying clear halos around each bacterial colony

Generally considered pathogenic since it is able to lyse open red blood cells!



# BAP - Alpha Hemolysis $\alpha$

$\alpha$ -hemolysis - means bacterial enzymes *partially* break down red blood cells

This results in media that appears discolored yellow/green/brown around the colony and indicates incomplete hemolysis

Potentially pathogenic, able to partially lyse red blood cells



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# BAP - Gamma Hemolysis $\gamma$

$\gamma$ -hemolysis means NO hemolysis at all

The bacteria have no effect on red blood cells and there is no change to the color of the agar

Unable to lyse red blood cells so likely not pathogenic



Eosin Methylene Plates (EMB) are used to detect FECAL microorganisms – Basically EMB plates are used to detect POOP

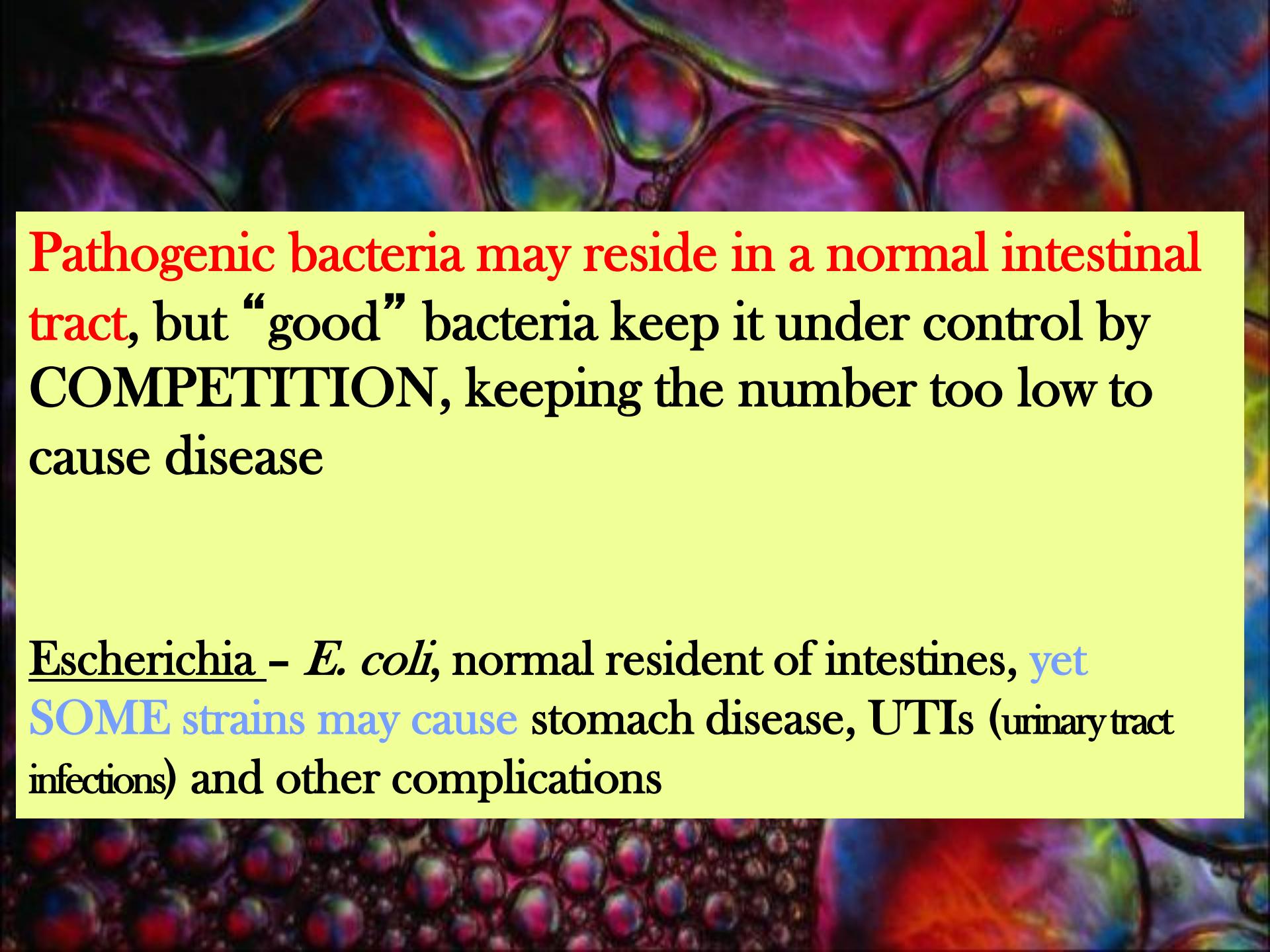
Fecal microbes, also known as ENTERIC BACTERIA, are grown on EMB PLATES

enteric bacteria (reside in intestines)

*...meaning we are looking for fecal bacterial contamination in the area you swabbed, as well as NORMAL gut bacteria*

Many are part of the normal flora in mammals

We will demonstrate the FECAL ORAL ROUTE!!!



**Pathogenic bacteria may reside in a normal intestinal tract, but “good” bacteria keep it under control by COMPETITION, keeping the number too low to cause disease**

Escherichia - *E. coli*, normal resident of intestines, yet **SOME** strains may cause stomach disease, UTIs (urinary tract infections) and other complications

# What EMB plates **MIGHT** reveal regarding fecal contamination of fomites swabbed - called: Presumptive results

1. Positive for Fecal Contamination
2. Negative for Fecal Contamination
3. Positive for *E. coli* contamination

ANY or all of the above conditions can  
occur on your plate!

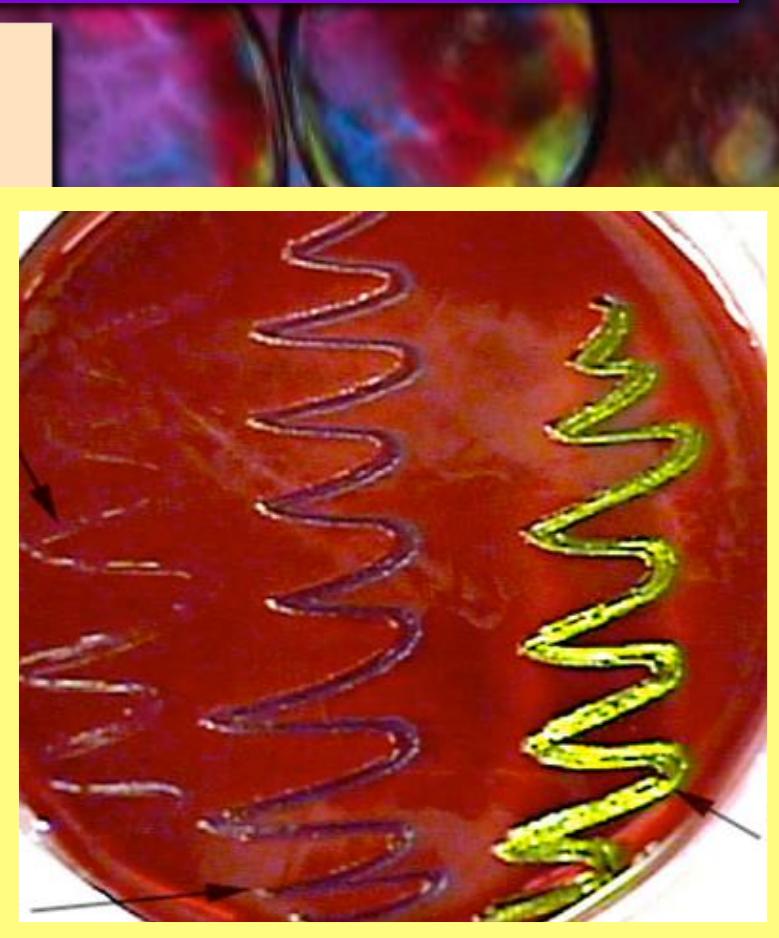
# EMB Plates After Incubation

1. Colorless colonies

(suggests unlikely fecal contamination)

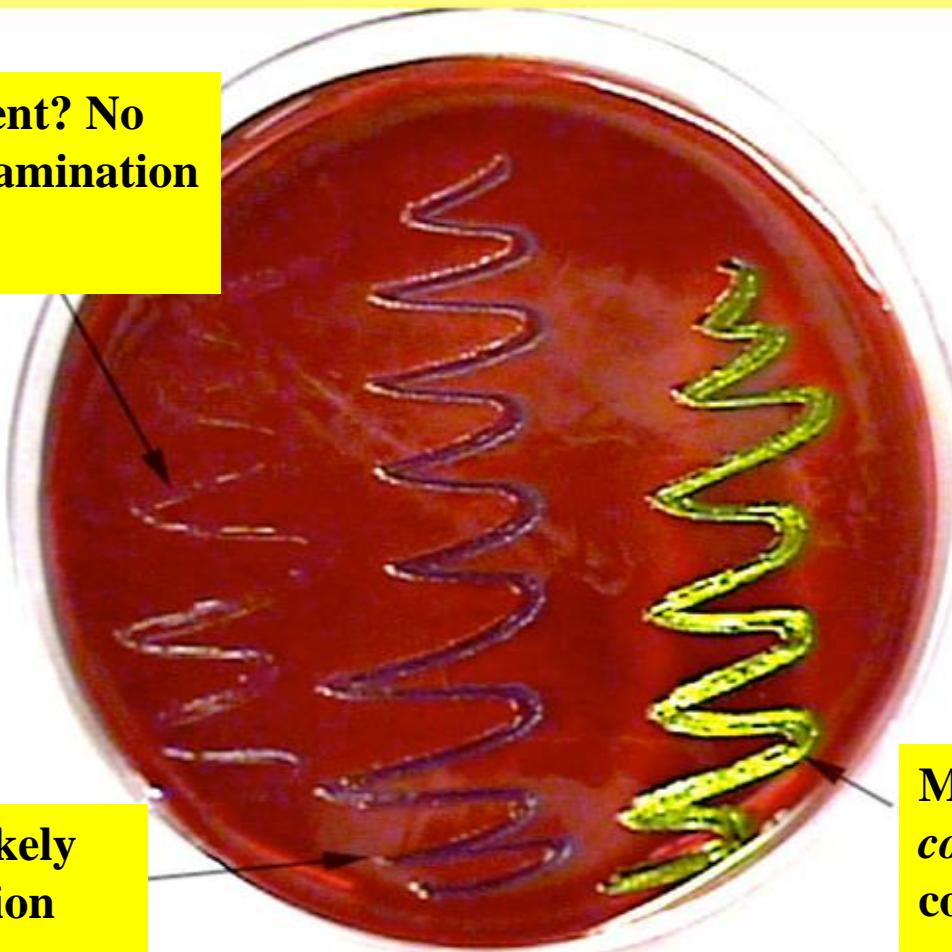
2. Dark centers and clear or pink/purple borders colonies (suggest possible fecal contamination)

3. Green metallic sheen (likely *E. coli* contamination)



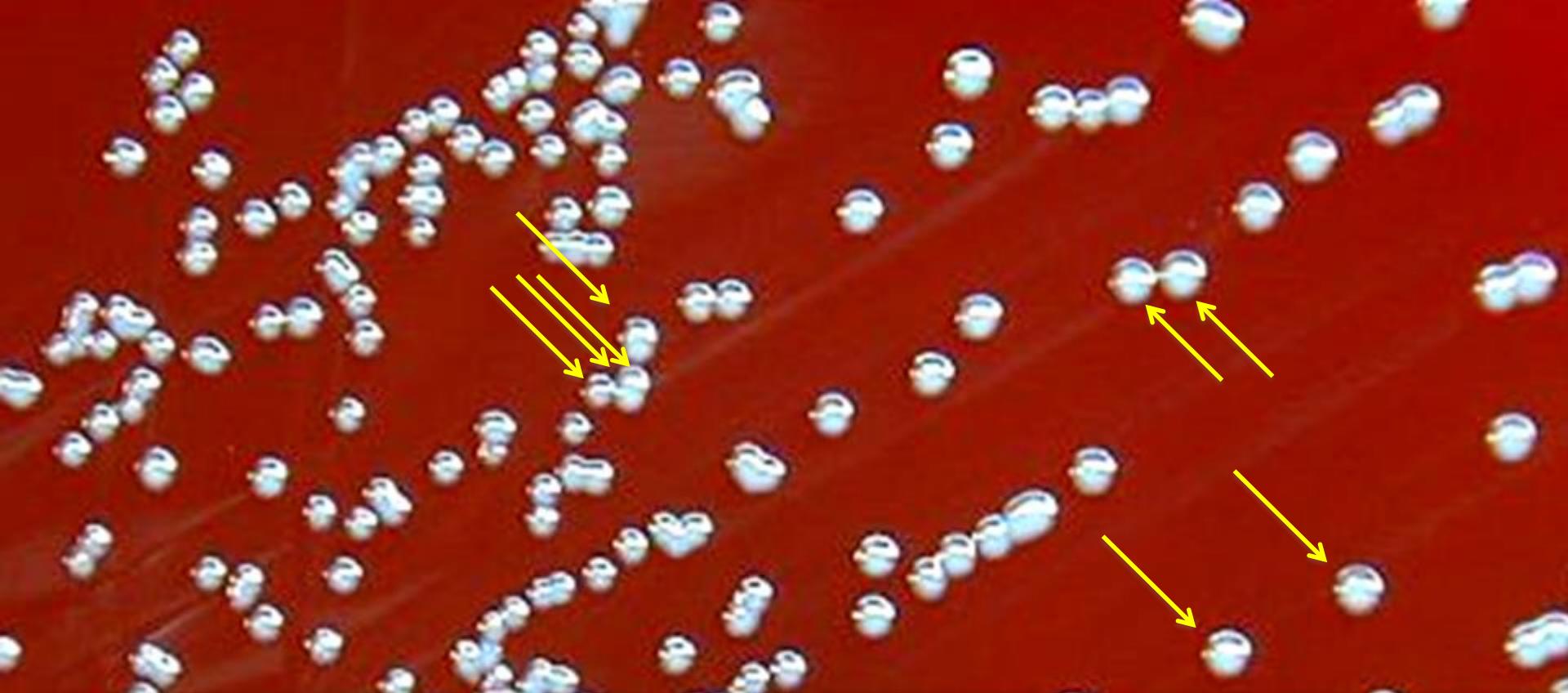
# Evidence or Lack of Fecal Contamination

Transparent? No  
fecal contamination



Purple/Dark? Likely  
fecal contamination

Metallic Green? *E.  
coli* fecal  
contamination



*What is a bacterial COLONY?* A cluster of organisms growing on the surface solid medium such as agar. It is assumed that all the organisms within the colony descend from a single ancestor and are genetically identical

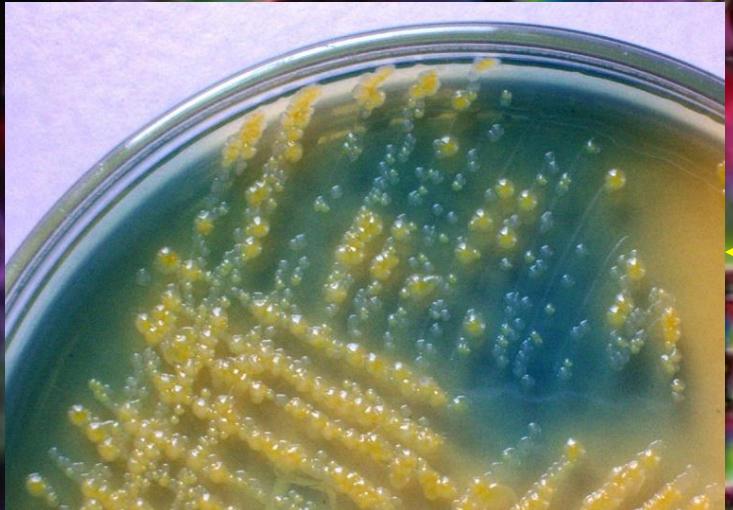


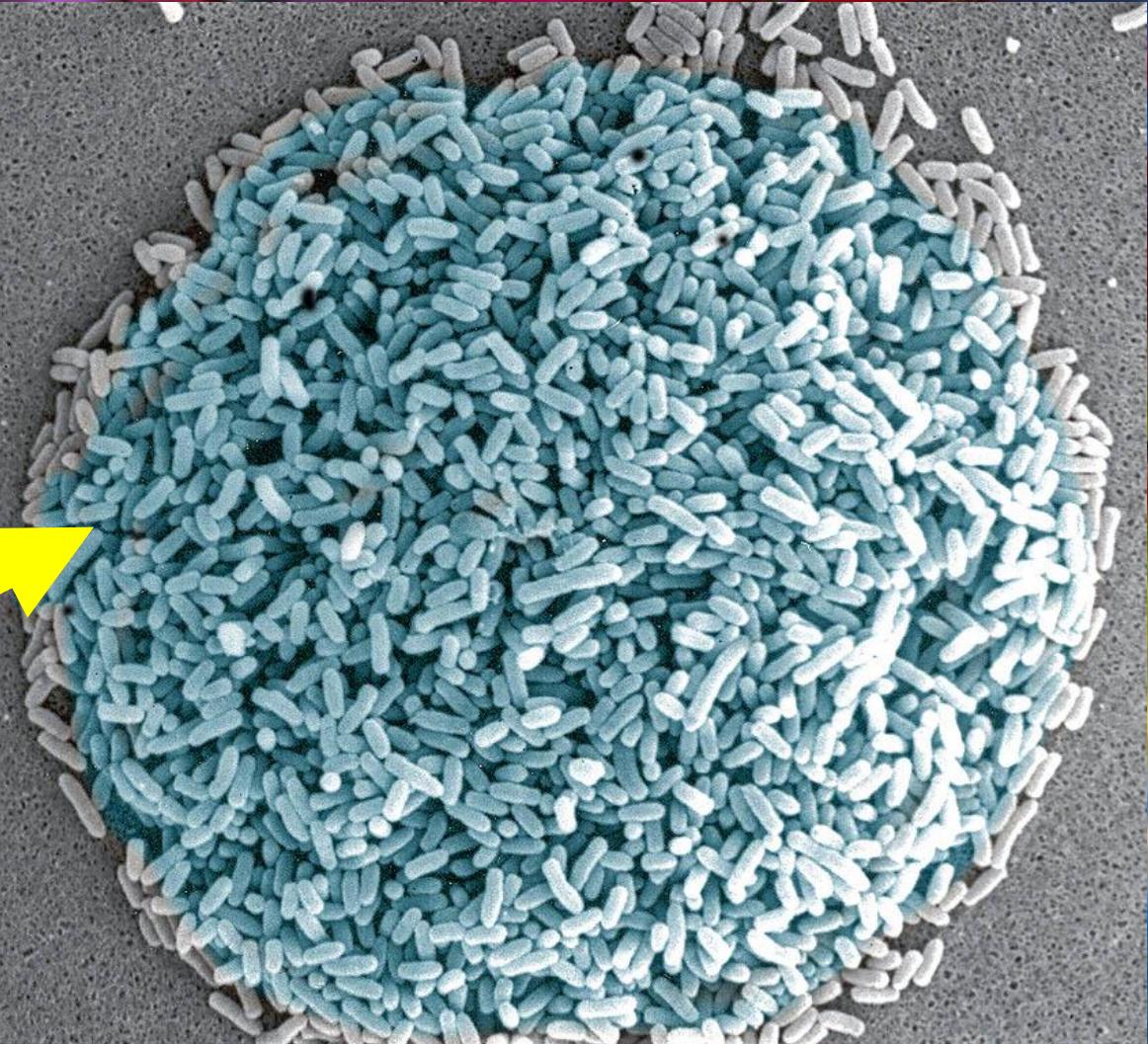
Plate with yellow & blue  
bacterial colonies



It is assumed that each colony arose from a single bacterium that multiplied after incubation (usually 12-48 hours)

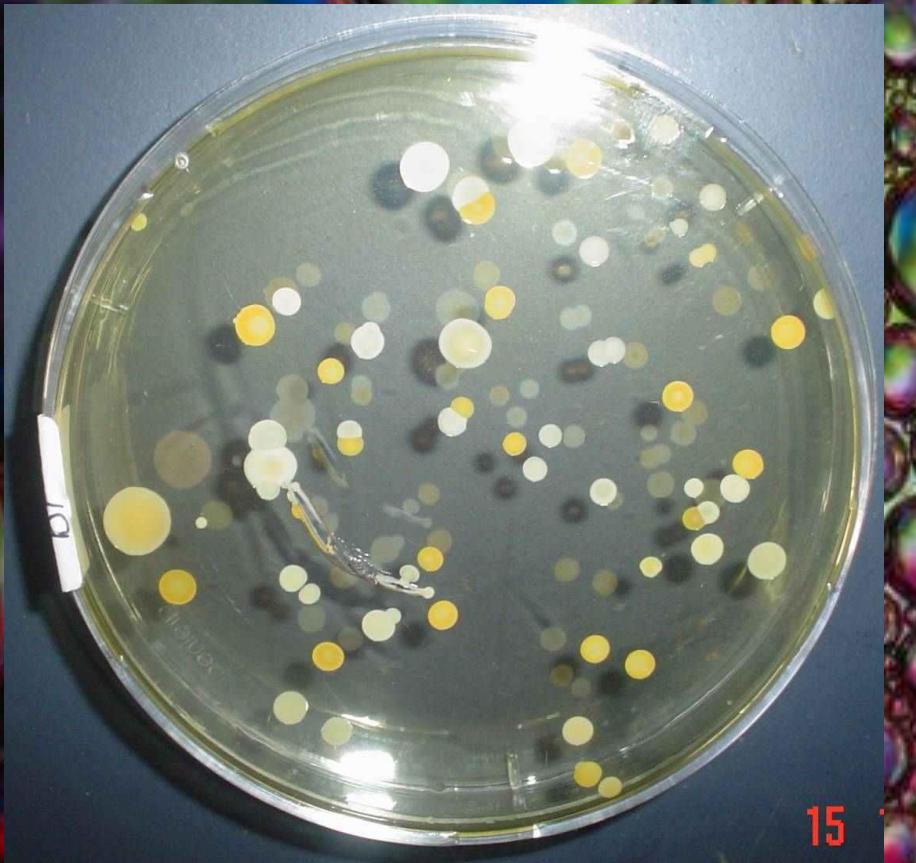


Blue  
Bacterial Colony



Scanning Electron Microscopy  
image of single bacterial colony,  
showing individual bacteria

# Counting Bacterial Colonies



15



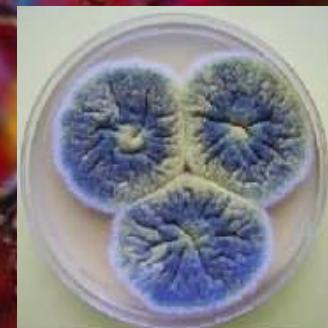
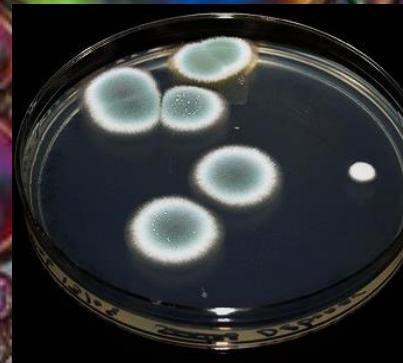
# Bacterial Colonies



# Mold/Fungus Colonies



# Mold/Fungus Colonies



# KINDS - TYPES of colonies

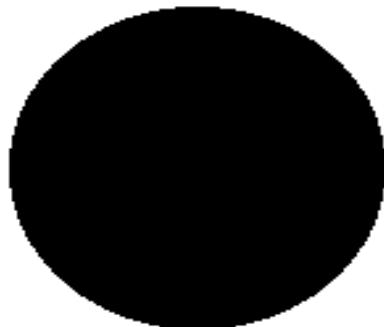
Bacterial colonies on agar may look different from each other

If they look different, it suggests that each colony is made from different KINDS of bacteria

Colonies that LOOK different, ARE DIFFERENT - *color, size, shape, glossiness, texture, height, etc.*

# Bacterial Colony Morphology

## Form



Circular



Irregular



Filamentous



Rhizoid

## Elevation



Raised



Convex



Flat

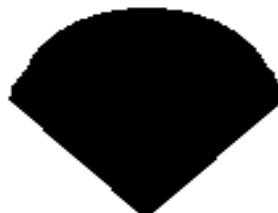


Umbonate



Crateriform

## Margin



Entire



Undulate



Filiform

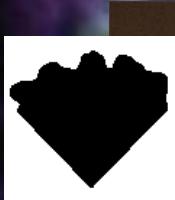


Curled

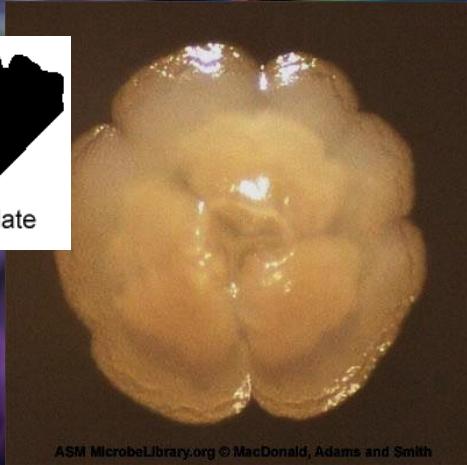


Lobate

# Close Up of Colony Morphology



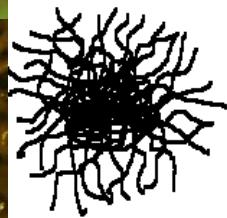
Undulate



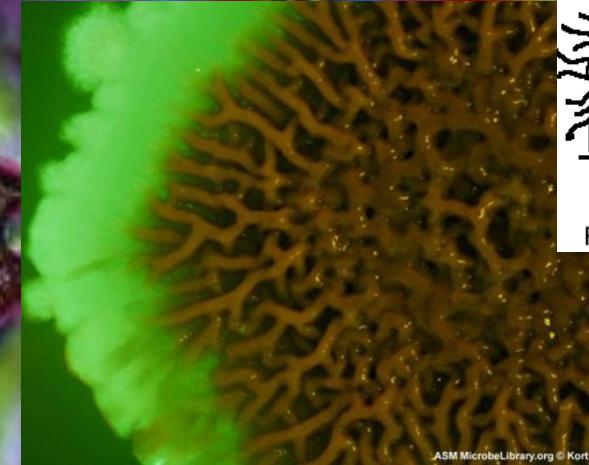
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Filamentous



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Lobate



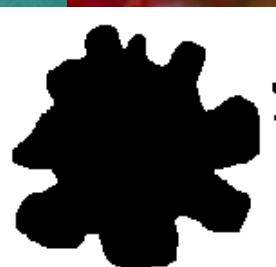
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Filiform

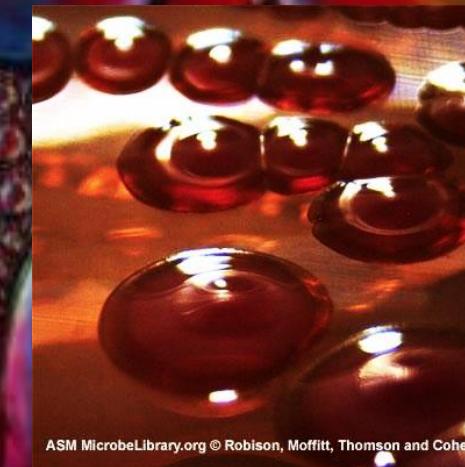
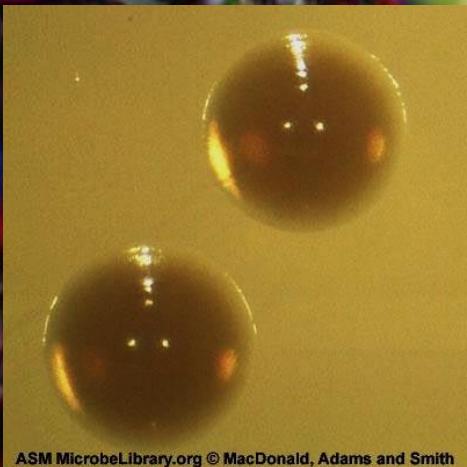
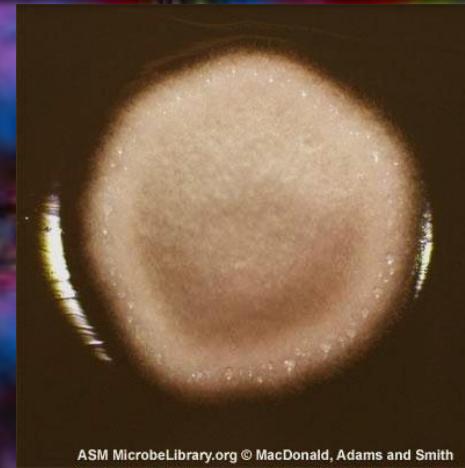
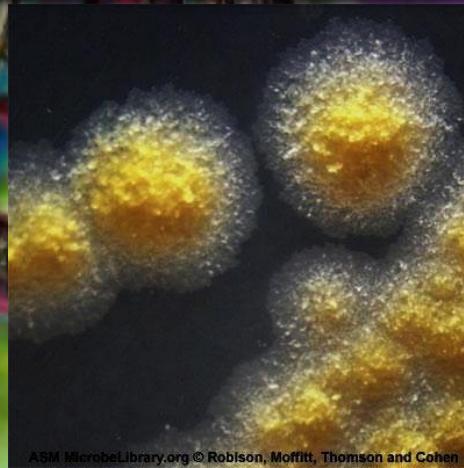
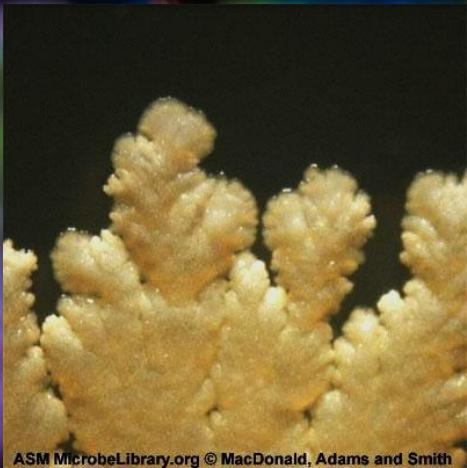


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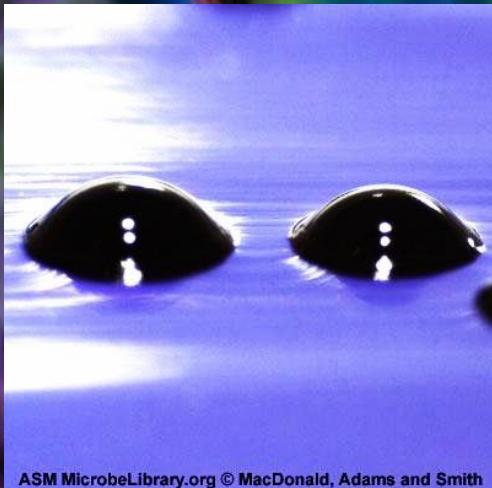


Irregular

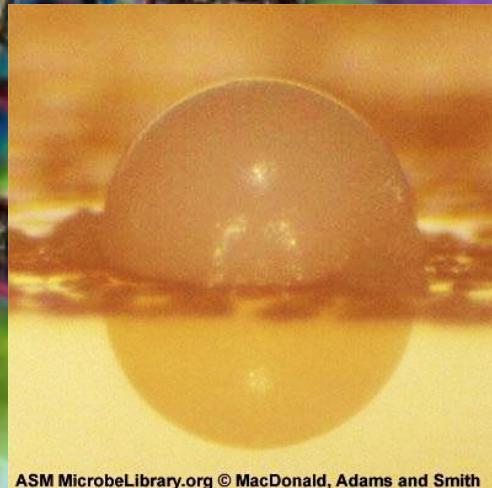
# Close Up of Colony Morphology



# Close Up of Colony Morphology



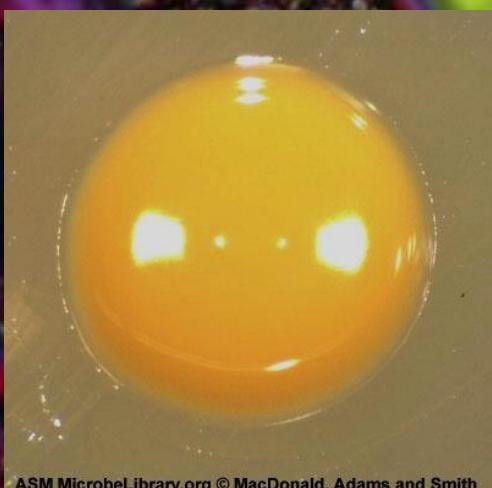
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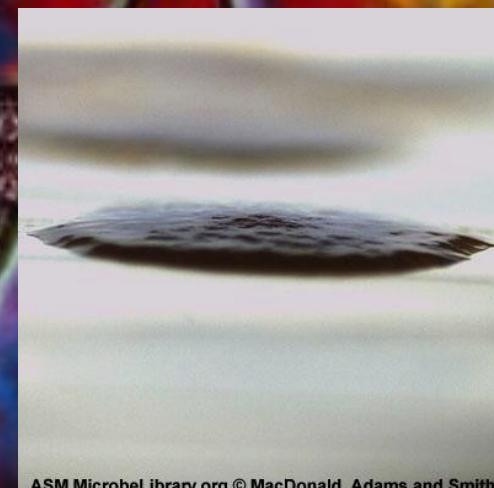
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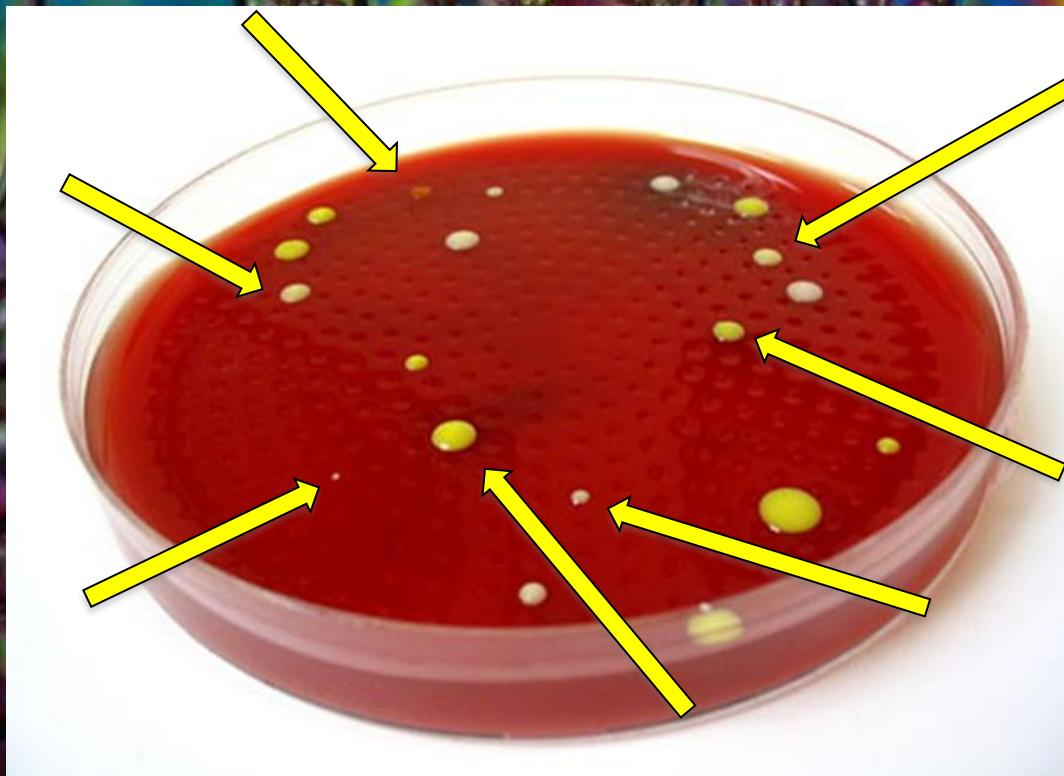


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Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell, therefore a colony constitutes a clone of bacteria all genetically alike.

at least **SIX** types of colonies  
based on color, size, etc.



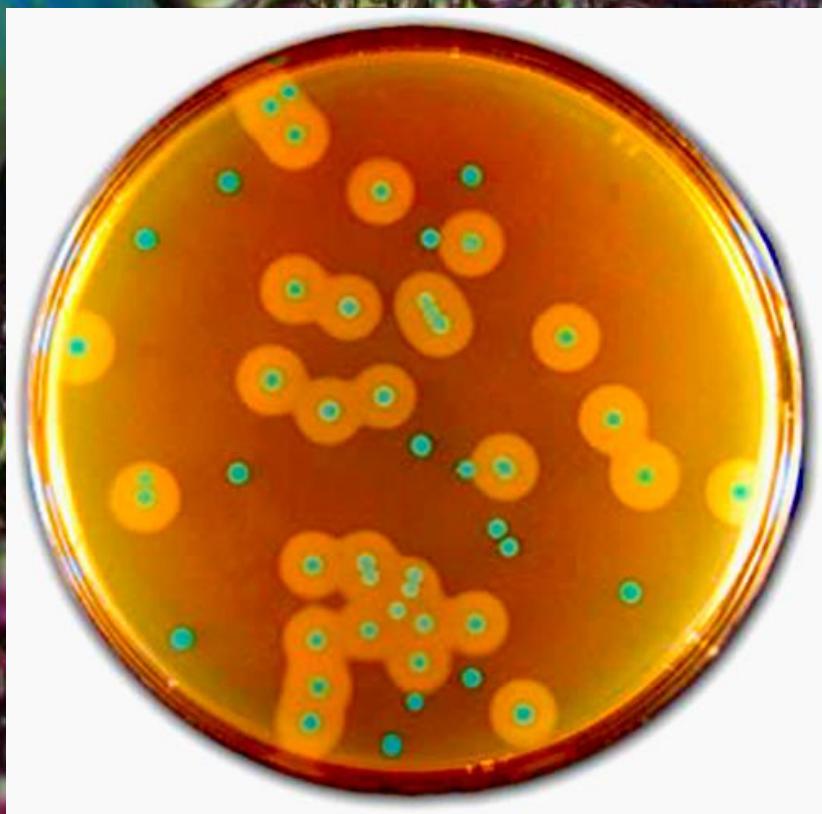
many kinds of colonies



many kinds of colonies



# Two kinds of colonies



*Thursday 11/15 do not forget:*

***SWAB***

***Masking Tape***

***Sharpie (indelible marker)***

***PPT***