

# Penn State **Extension**

## Quad Plate: Easy Identification Guide Gram-negatives



**STEP 1: Are the colonies...**

**Bright Pink**

or

**Off-White**



Bright pink colonies indicate a coliform type of bacteria

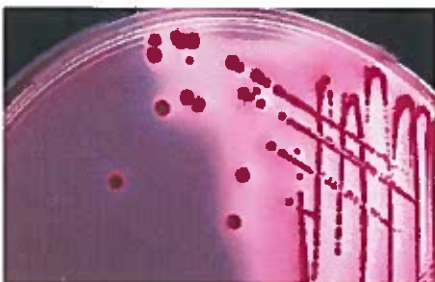
Off-white colonies indicate a non-coliform type of bacteria

Coliforms (*E. coli*,  
*Klebsiella* spp.,  
*Enterobacter* spp.)

Non-Coliforms (*Serratia*,  
*Pseudomonas*, *Proteus*)

**Step 2: Record on identification sheet colonies as either coliform or non-coliform**

Sample		Coliforms		Non-Coliforms			Bacteriophage	
Date	Location	Coliforms	Non-Coliforms	Coliforms	Non-Coliforms	Bacteriophage	Bacteriophage	



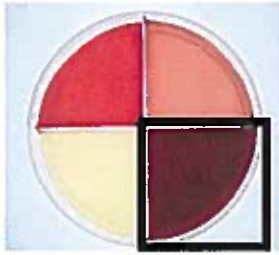
*E. coli*- Bright pink colonies, Small, Dry



*Klebsiella* spp. - Bright pink colonies, Larger, Wet, "snotty" looking

**Step 3: Record bacteria on treatment sheet**

Clinical Mastitis Records						
Northwest SARE Grant						
Date	Cow ID	Quarter	Bacteria 1	Bacteria 2	Treatment	Days



## Streptococci

**STEP 1: Are the colonies...**

**Esculin Positive**

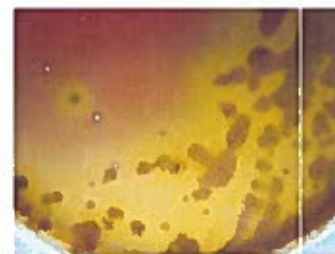


**Black colonies with NO zone of clearing (hemolysis) indicate they are esculin positive colonies**

Esculin positive bacteria (*Strep uberis*)

or

**Esculin Negative**



**Black colonies with a zone of clearing around them indicate esculin negative types of bacteria**

Esculin negative bacteria (*Strep agalactiae*, *Strep dysgalactiae*)

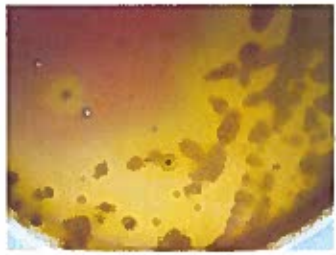
### Step 2:

**Record on identification sheet colonies as either esculin negative or esculin positive**

Date	Cow ID	Quarter	Bacteria 1	Bacteria 2	Treatment			Days	Treatment Outcome
					1st	2nd	3rd		



**Esculin positive colonies are *Strep uberis***



**Esculin negative colonies are either *Strep dysgalactiae* or *Strep agalactiae***

### Step 3:

**If Esculin negative colonies are present use "All Bacteria" sheet to determine bacterial species, otherwise record colonies as *Strep uberis* on treatment sheet**

<b>Clinical Mastitis Records</b>						
Northeast SARE Grant						
Date	Cow ID	Quarter	Bacteria 1	Bacteria 2	Treatment	Treatment Outcome





# All Bacteria (blood agar)



Part 1: If you saw growth on Gram-negative quad, you should also see growth on blood quad

## Coliforms



Large, grey/off white colonies, wet, "snotty" looking

## Non-Coliforms



*Proteus* – swarms plates



*Pseudomonas* – Looks similar to coliforms, smells like grapes



## *Strep dysgalactiae, Strep uberis*



Small, grey/off white colonies  
\*No HEMOLYSIS

## *Strep agalactiae* SUSPECT



Small, grey/off white colonies  
\*\*HEMOLYSIS



## *Staph spp. (CNS)*



Larger, white/cream/yellow colonies  
\*No HEMOLYSIS

## *Staph aureus* SUSPECT



Larger, white/cream/yellow colonies  
\*\*HEMOLYSIS