

# The impact of freezer temperature, storage duration, and glycerol cryoprotectant usage on clinical mastitis culture results

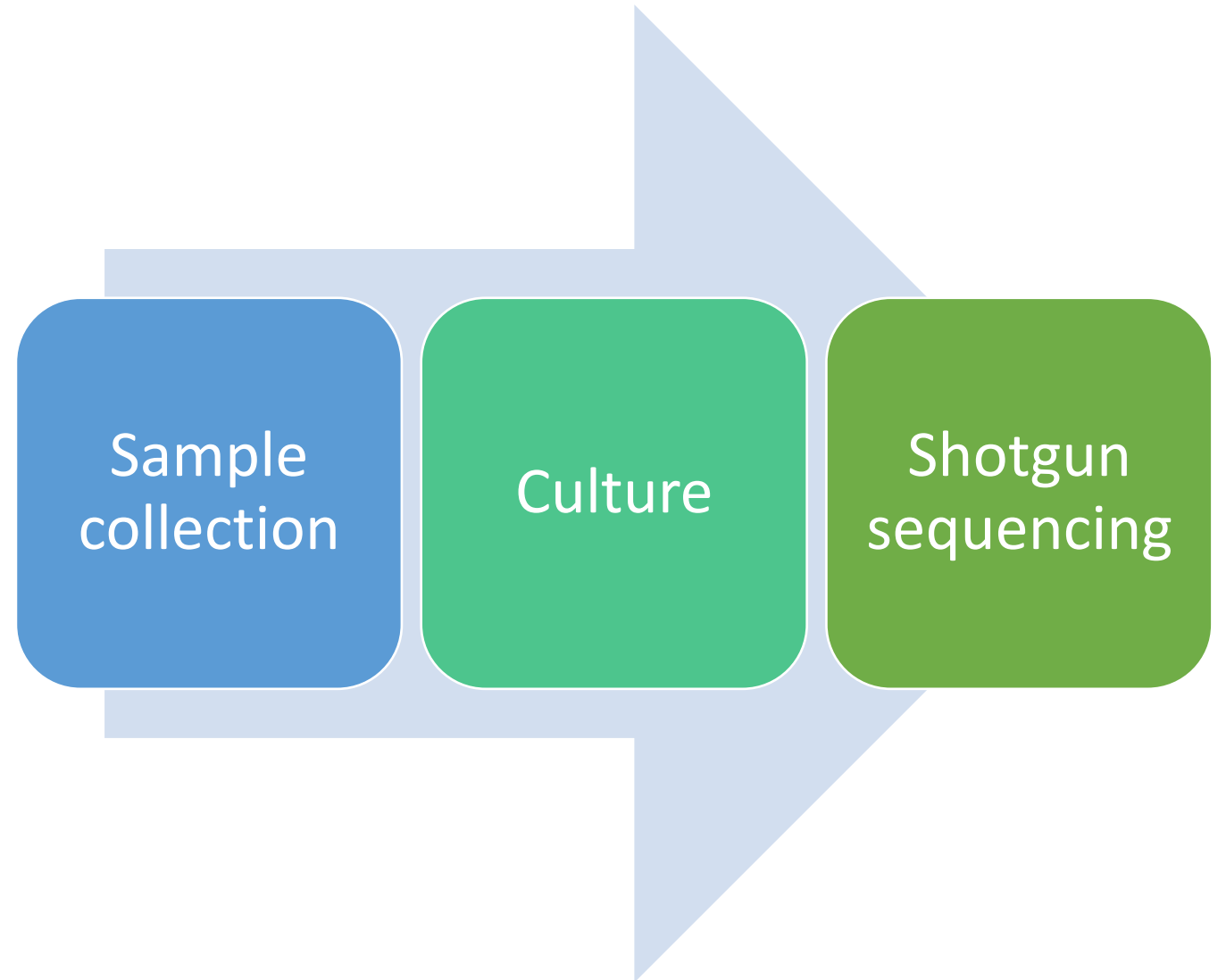
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# Project overview

- Compare healthy and mastitic milk microbiomes
- Explore fungal-bacterial interactions
- Investigate association between fungal microbiome and recurrent mastitis



Hannah Dairy Research  
Foundation





## Healthy

- No history of Mastitis
- SCC < 100,00 cells/ml



## Subclinical Mastitis

- SCC > 200,000 cells/ml
- No clinical symptoms
- Quarter affected confirmed with CMT



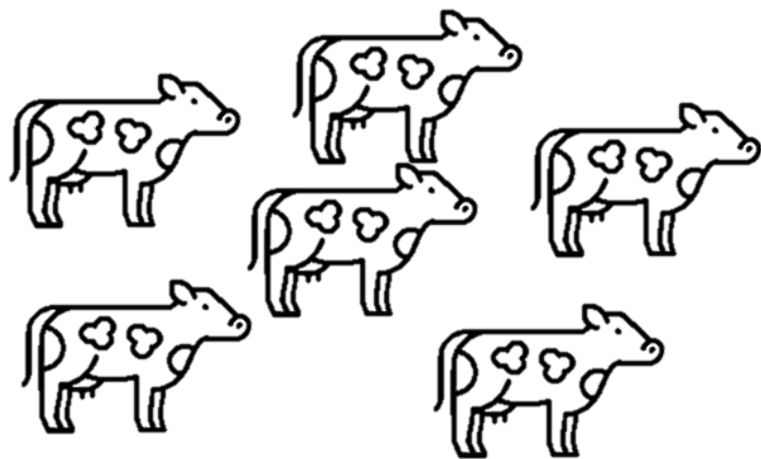
## Clinical Mastitis

- Clinical symptoms identified at milking
- No previous mastitis

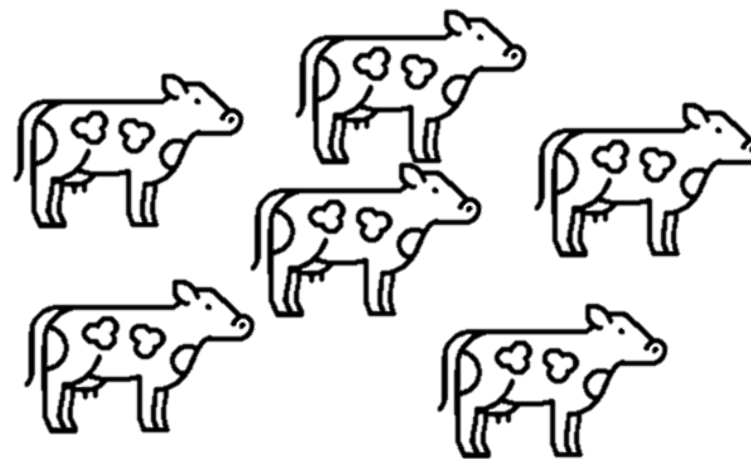


## Repeated Mastitis

- Clinical mastitis at milking
- History of Mastitis



Top 5% milk fat and protein  
SELECT



National Average  
CONTROL

# Langhill Groups



Top 5% milk fat and protein  
SELECT

National Average  
CONTROL

# Langhill Groups

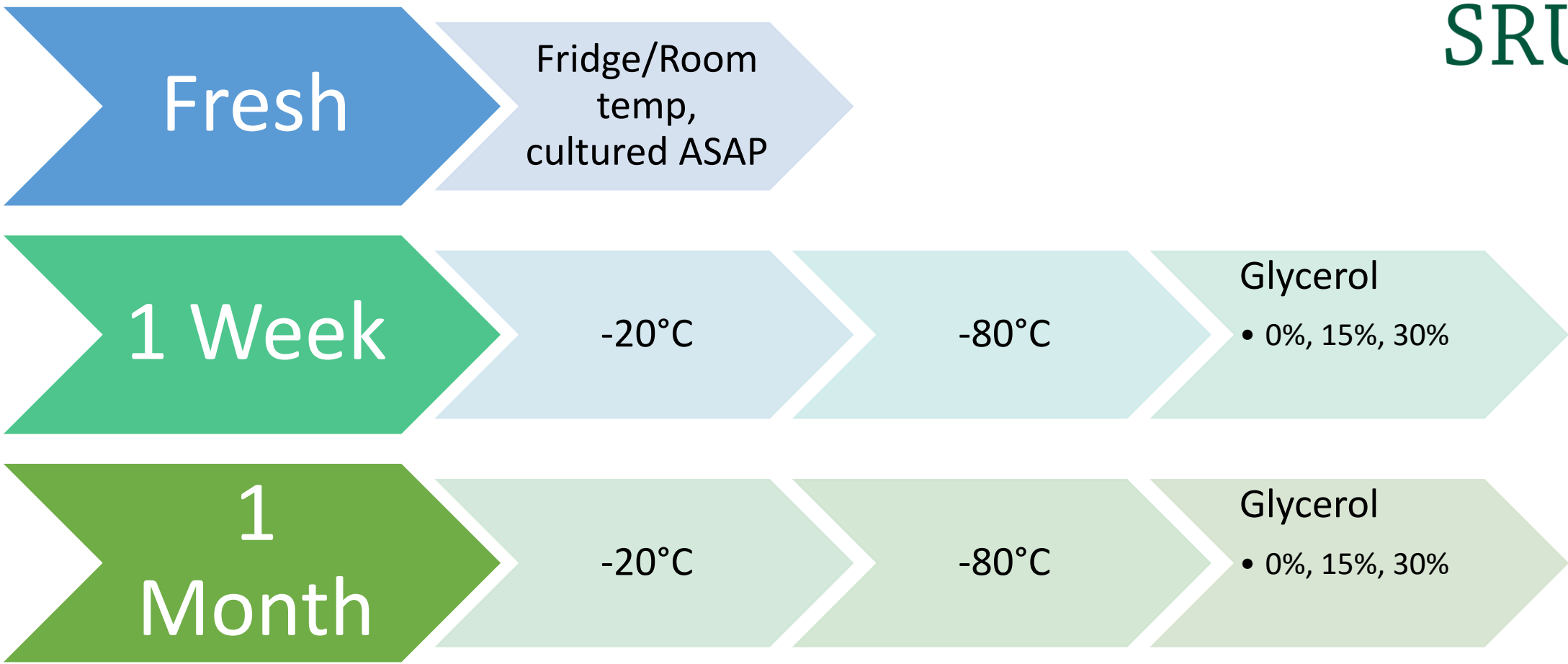
10 in each group

Healthy and SC cows matched to CM cows for

- DIM (within 10 days)
- Genetics
- Feed group

Healthy cows mix of heifers and multiparous

## Enrolment Groups



Freezer Test

Duration	Temperature (°C)	Case 1	Case 2	Case 3	Case 4 (1 – FR, 2 – BR)
Fresh	Room Temperature	<i>Serratia liquefaciens</i> & <i>Pichia fermentans</i>	Sterile	Mixed growth	(1) <i>Bacillus cereus</i> ; (2) sterile
1 Week	-20	<i>Serratia</i> spp.	Sterile	<i>Streptococcus dysgalactiae</i>	(1) <i>Bacillus cereus</i> ; (2) sterile
	-80	Sterile	<i>Klebsiella</i>	<i>S. dysgalactiae</i>	(1) <i>Bacillus cereus</i> ; (2) sterile
1 Month	-20	Sterile	Sterile	Not Cultured	(1) Mixed growth, <i>Bacillus cereus</i> , <i>Enterococcus</i> spp; (2) sterile
	-80	Sterile	Sterile	Not Cultured	1) Mixed growth, <i>Bacillus cereus</i> ; (2) sterile

Gram +  
Gram –  
Yeast



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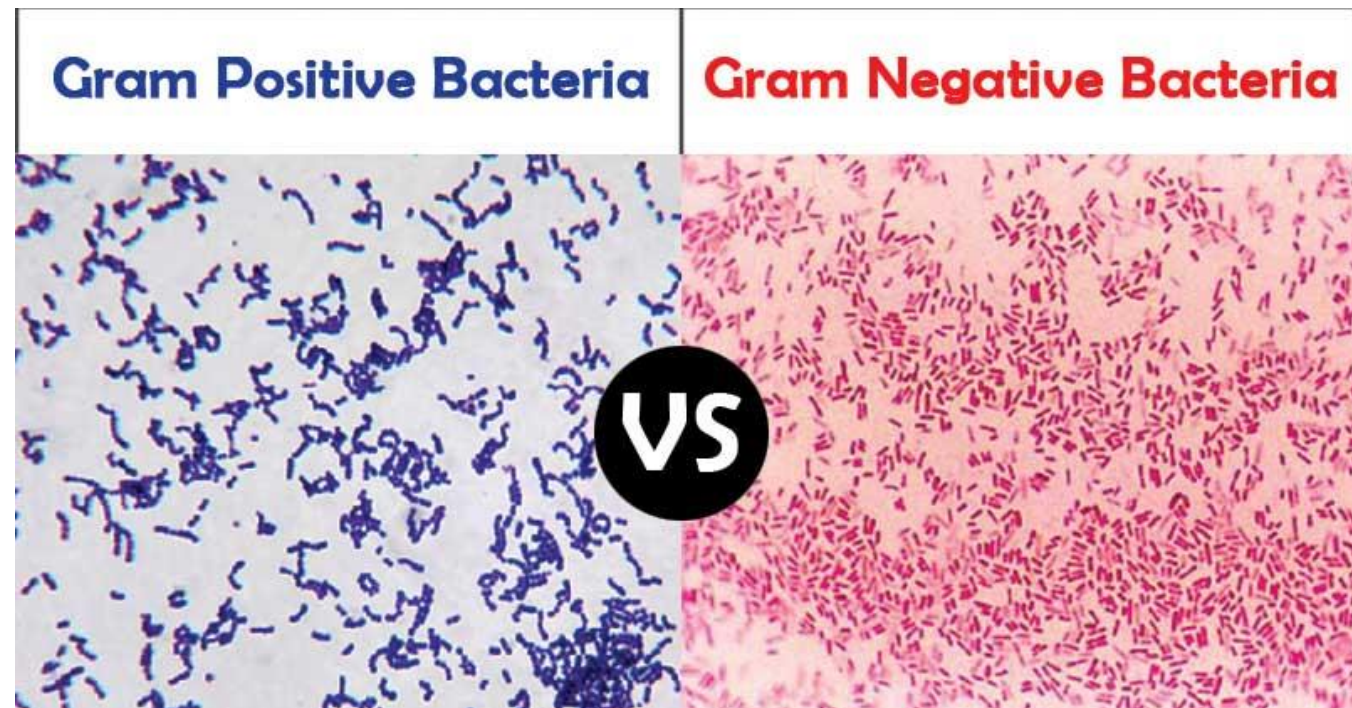
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Freezer/Glycerol Concentration	Culture - Case 4	Culture - Case 5
Fresh (5°C fridge) / 0%	<i>Streptococcus dysgalactiae</i>	<i>Trueperella pyogenes</i>
-20°C for 1 week / 0%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
-20°C for 1 week / 15%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
-20°C for 1 week / 30%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
-20°C for 1 month / 0%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
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-80°C for 1 month / 0%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
-80°C for 1 month / 15%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>
-80°C for 1 month / 30%	<i>S. dysgalactiae</i>	<i>T. pyogenes</i>

Gram +  
 Gram –  
 Yeast

# Summary

- Increased freezer temperature and duration
  - Negative Impact on Gram – bacteria and yeast
  - No impact on Gram + bacteria
- Impact of glycerol
  - Inconclusive
- Next steps
  - Expand trial to include more Gram –
  - DNA sequencing



# HoloRuminant

- Following milk microbiome of all Langhill cows over 2 lactations
  - 5 days in milk
  - Clinical mastitis
  - Subclinical mastitis
- 16S sequencing
- Analyse changes in microbiome
  - Cow factors
  - Mastitis status







**QUEEN'S  
UNIVERSITY  
BELFAST**

Thank you for your attention

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