Methods in Immunology for *Brucella abortus*

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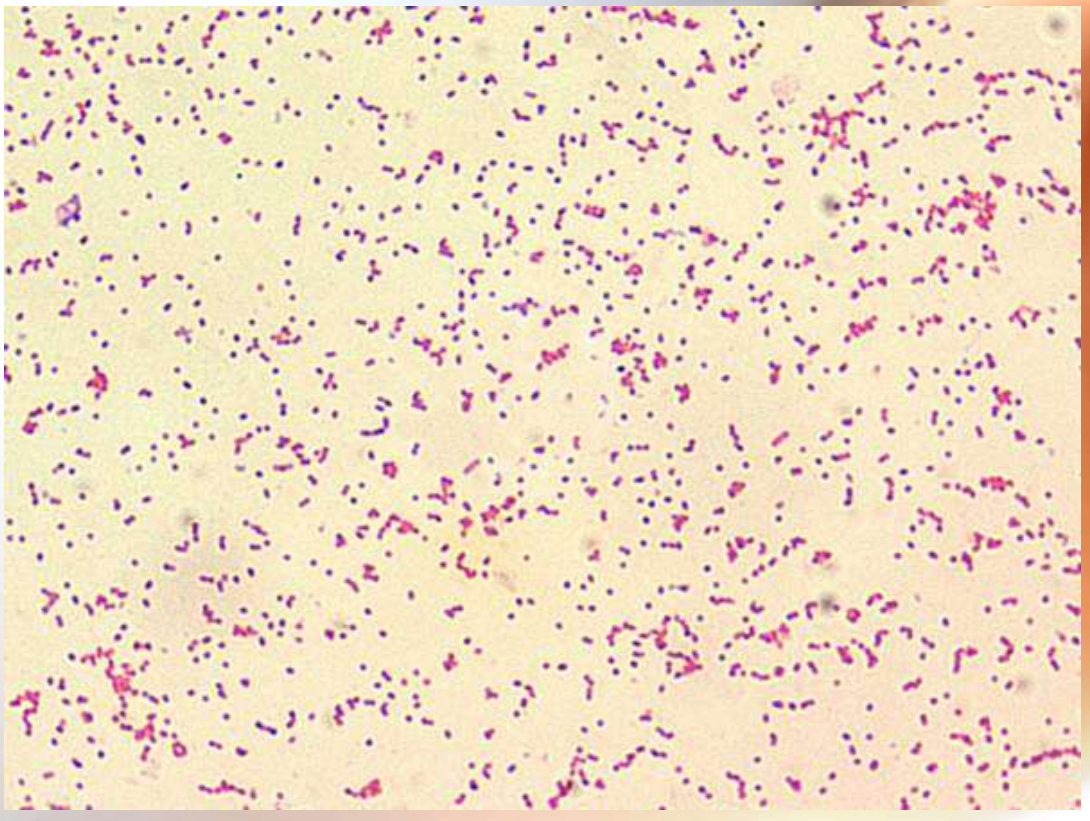
What is *Brucella abortus?*

- Gram (-) bacteria
- Found to cause spontaneous abortion
- Zoonotic
- Important in both veterinary medicine as well as human medicine



Why it's a problem?

- Endemic in the Greater Yellowstone Area
- Crosses from wildlife to cattle and domestic ungulates
  - Economic loss
- Zoonotic and is classified as by USDA/CDC as a "select agent"

Introduction to Project

- Entered the lab of Dr. Adamovicz with the project of developing and fine tuning methods to isolate bovine PBMCs from blood
- To adapt assays to measure CMI to bovine brucellosis

Specific Aims

- Develop an MOI for the 2 vaccine strains of *B. abortus*
  - *RB51 and S19*
- Develop PCR primers and protocols to detect and quantify cytokines from PBMCs
- Help establish the lab and find things that go wrong (critical thinking and troubleshooting)

Methods

Density gradient enrichment

Whole PBMC

T-cell Isolation

Stimulation and Cytokine Analysis

Cryopreservation

Future: Macrophage Killing Assay, MOI and T-cell interactions

Cryopreservation

Stimulation

The bacteria

Brucella Growth

- Agar plates – stock cultures from Gerry Andrews Lab
- Vaccine Strains

Batch Growth

- Liquid cultures in BHI broth
- Used to define a growth curve

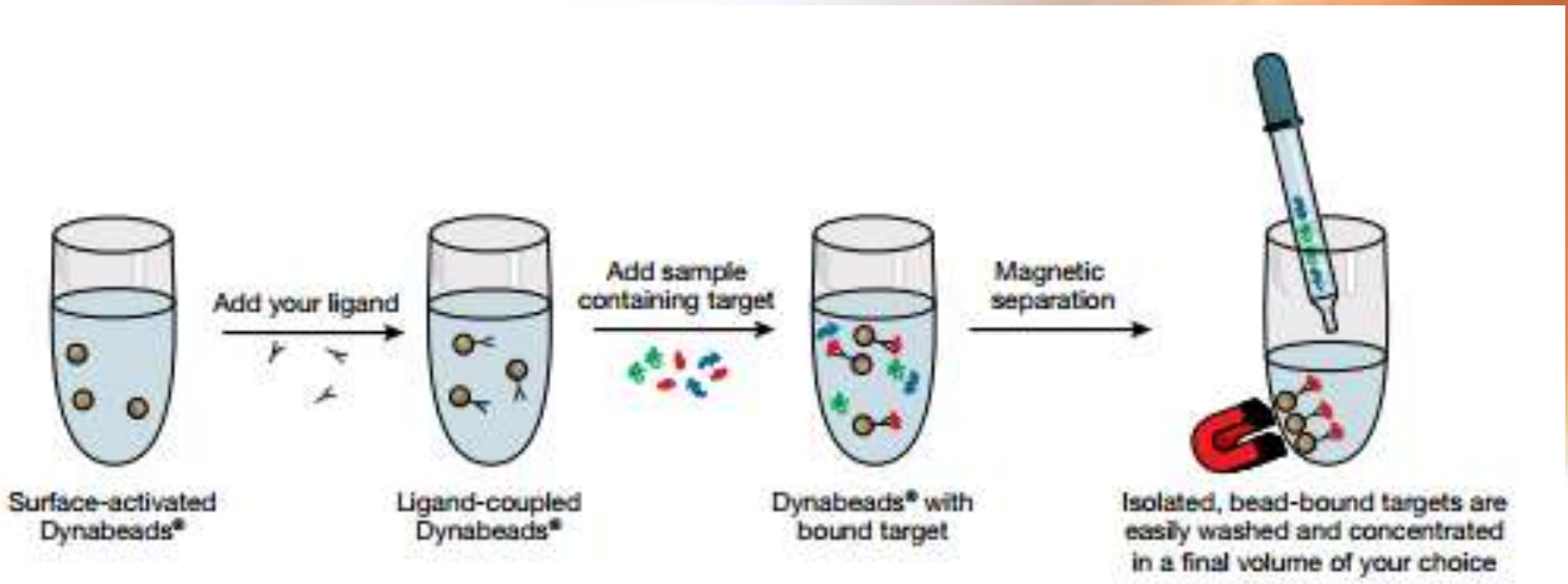
Freezing of bacteria cultures

- In glycerol
- Frozen as working stocks

PCR

- Cells stimulated with *B. abortus* or another agent (SEB, peanut lectin, etc.)
  - Cytokines are secreted due to the stimulation
- The level of cytokine expression is related to the amount of mRNA produced by the PBMCs
- Reverse transcriptase and PCR allow for quantitation of the cytokines.
- Use HPRT as a control and relative marker

Magnetic Bead enrichment



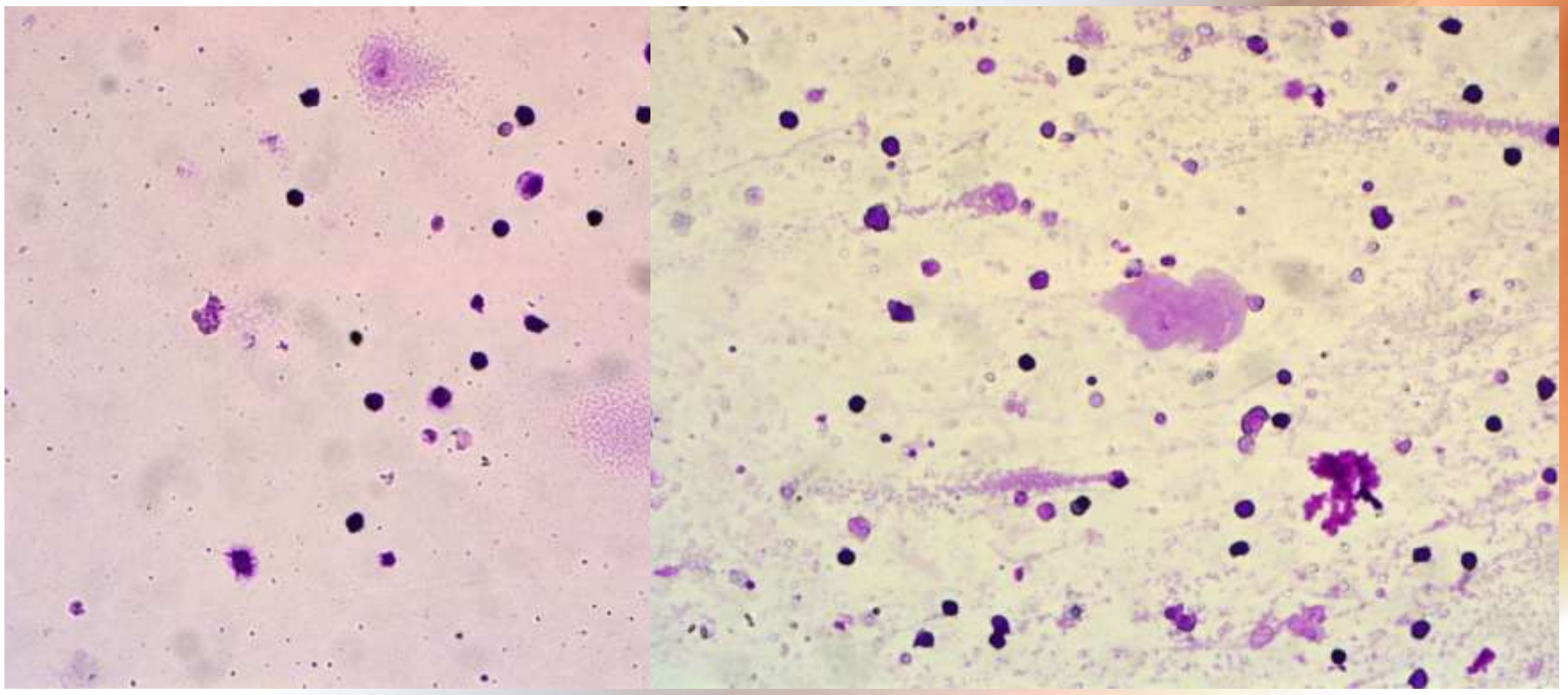
Multiplicity of Infection

- PBMCs are put in culture with varying amounts of *B. abortus* RB51.
- The cells become infected and the amount of infection is measured by lysing the cells and growing the remaining bacteria on plates.
- Infect, wash, lyse, plate

Results

Viability of PBMCs in Culture

- Found to be above 50% viable for 3 days in culture

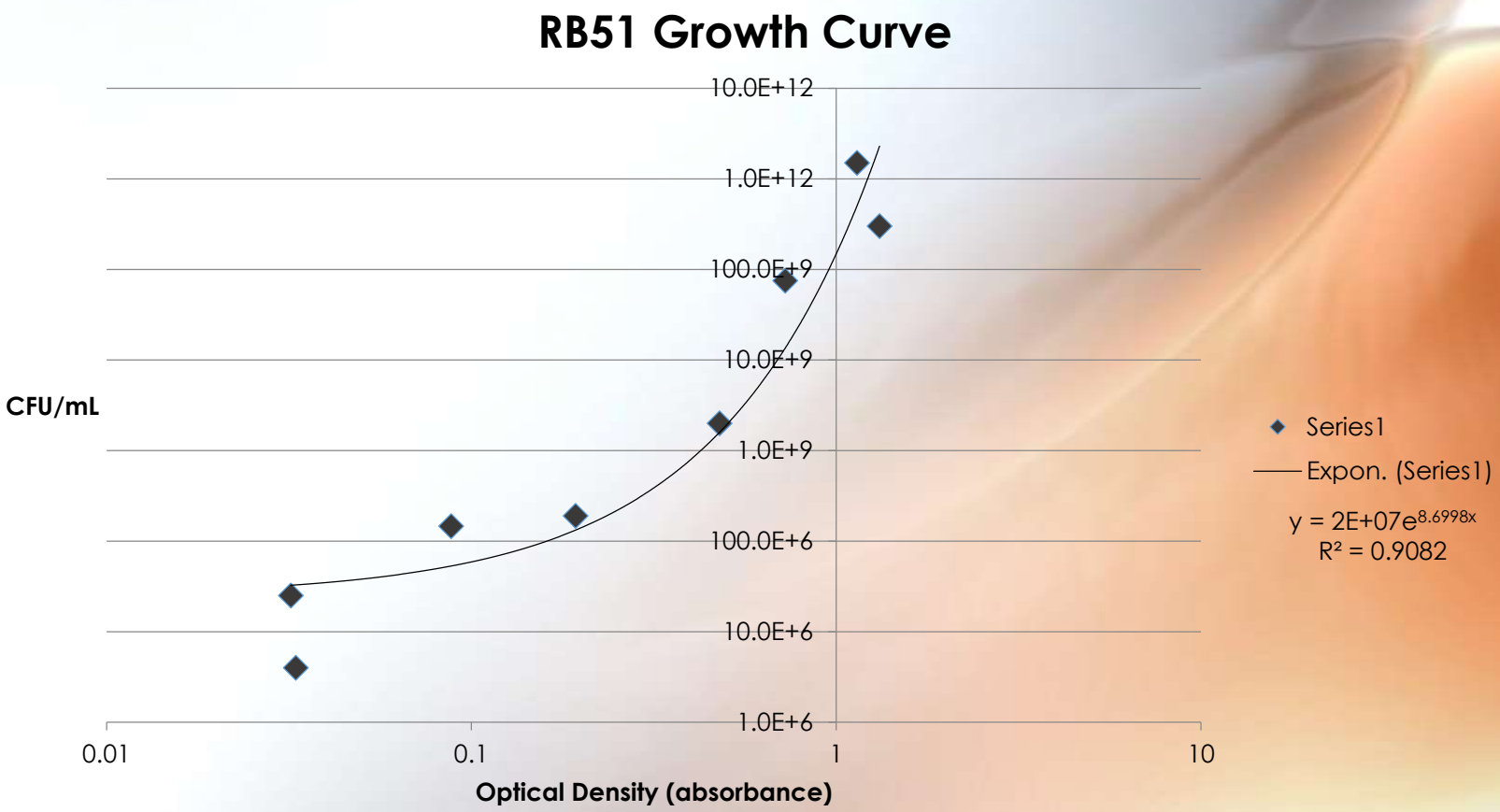


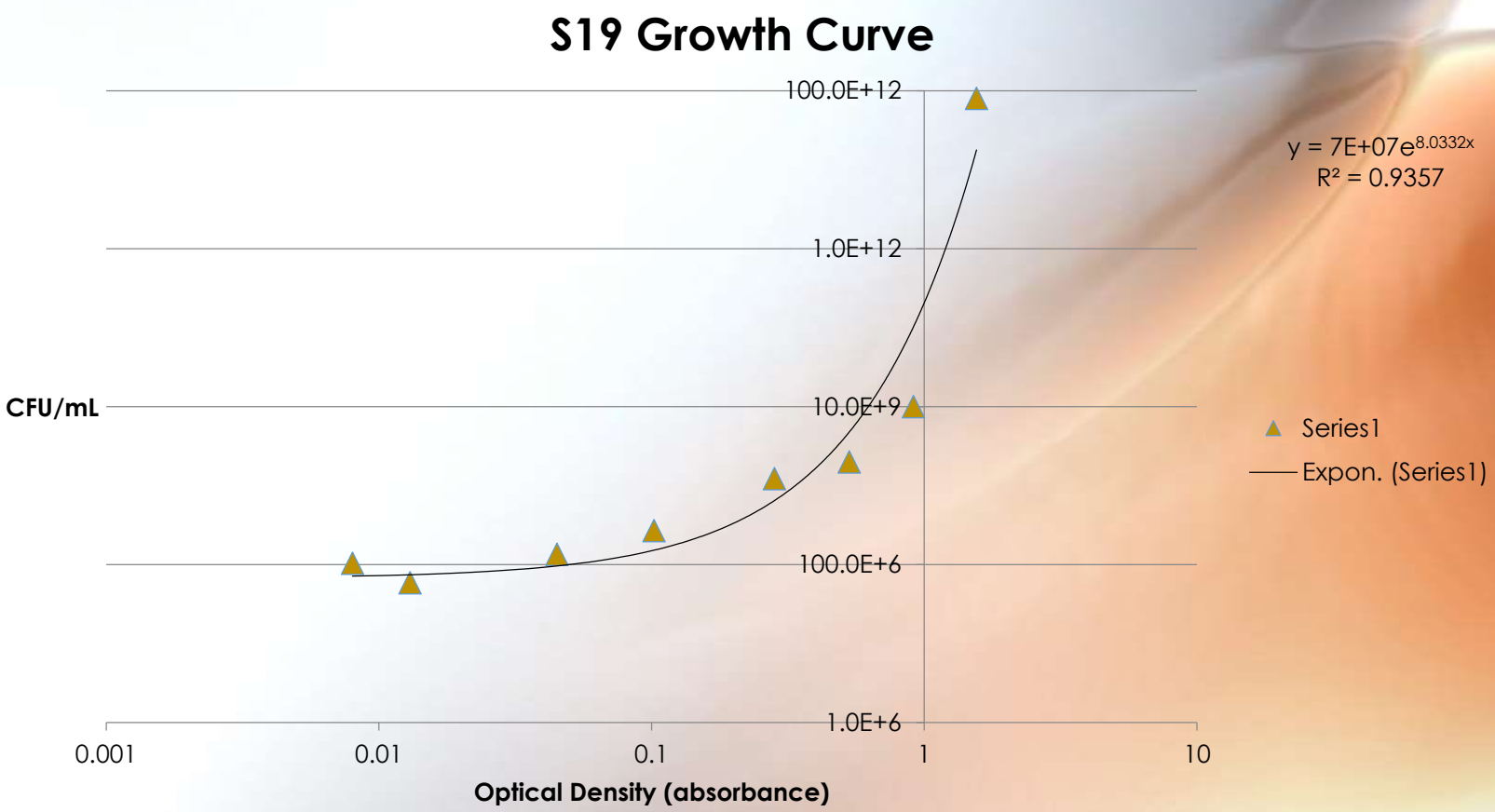
Frozen *Brucella*

- In 20% glycerol

Strain	Plate count 1 at dilution factor of 10^7	Plate count 2 at dilution factor of 10^7	Average	CFUs/ml
RB51	133	129	131	1.31×10^{10}
S19	300	218	257	2.57×10^{10}

Brucella Growth Curve



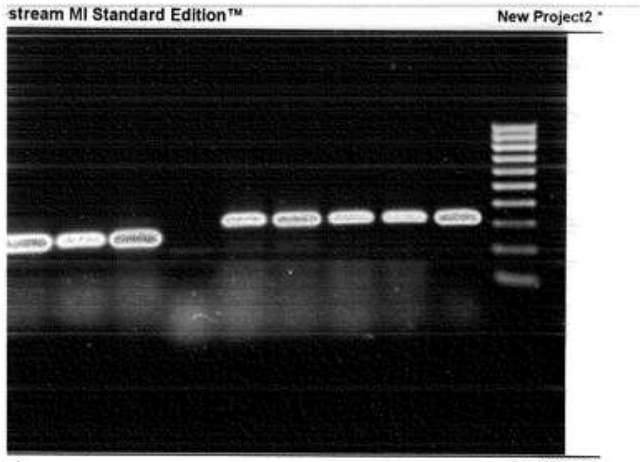

S19 Growth Curve
100.0E+12
1.0E+12
10.0E+9
100.0E+6
1.0E+6
CFU/mL
0.001
0.01
0.1
1
10
Optical Density (absorbance)
y = 7E+07e8.0332x
R² = 0.9357
Series1
Expon. (Series1)

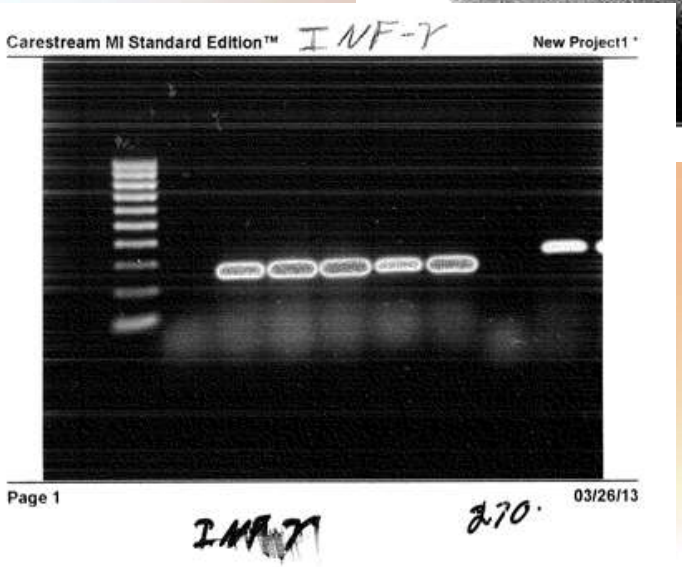
MOI

- Currently around 50 bacterial cells/ bovine PBMC
- Waiting to receive macrophage cell line to retest/confirm/re-establish

Cytokine PCR

- Troubleshooting was an interesting process of elimination





More PCR pictures

Future aspects of the project

- The methods developed will be used in a RB51 vaccine study in cattle
- Challenging PBMCs with the vaccine strains and looking at cytokine profiles
  - Also, in development are a set of macrophage killing assays
- Testing samples for cytokine and immune responses to the vaccine

Conclusions

- The MOI of 50 bacterial cells to one macrophage leads to reproducible cell killing
- The methods developed will help give quantifiable data in studies involving *Brucella abortus*
- Methods development is a vital part of the laboratory experience
- Troubleshooting is science in the raw

Acknowledgements

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Questions?