Bovine Whole Blood Bactericidal Competence of *E. coli* Protocol Development: Undergraduate Research Experience

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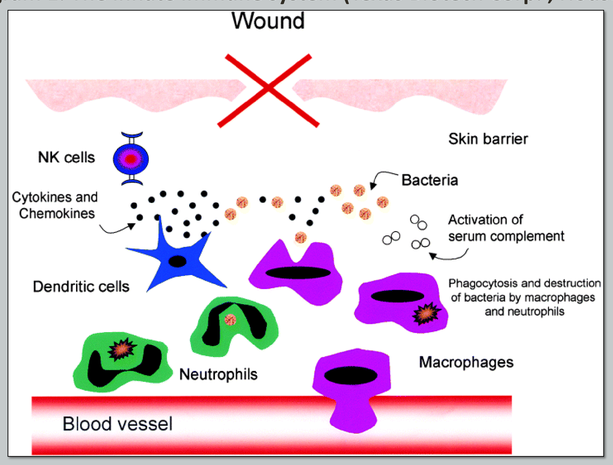
Background

The world population is projected to reach approximately 9 billion people by the year 2050. To meet the nutritional demands of a growing global population, agriculture industries will have to become efficient in the use of natural resources (soil, air, and water) in food production. Producing sustainable animal products is a complex challenge and animal health greatly contributes to the efficiency of this process. Therefore, the ability to quickly identify animals prior to becoming ill is key to maintaining high standards of animal well-being and efficiency in the food chain. One tool that can be used to measure the immunity of livestock is to measure the bactericidal competence of E. coli by innate immune cells in the blood. This assay, also known as 'Whole Blood Killing' (WBK), can examine if the innate immune function of animals is suppressed by comparing the number of bacterial colonies formed when an animal is stressed (i.e. during disbudding, castration, or weaning) versus when the animal is not stressed

Diagram 1. The innate immune system (Texas Biotech Corp. , Houston TX)



As an undergraduate in the College of Agricultural Sciences and Natural Resources (CASNR) and Department of Animal Science, I am interested in developing research experience in the field of animal well-being. Specifically, I wanted to learn about research tools that can be used to help welfare experts and producers evaluate animals and preventatively assess their health status. Therefore, the goal of my research experience was to assist in the development of the WBK assay in order to 1) help establish a research tool for animal health assessment in the Calvo-Lorenzo laboratory and 2) develop laboratory skills and obtain knowledge on the influence of stress on the physiological responses of animals.

Objectives

- Develop a laboratory protocol for the WBK Assay
- Establish a research tool for animal health assessment in the Calvo-Lorenzo Laboratory
- Obtain laboratory skills
- Acquire knowledge on the influence of stress on the physiological responses of animals

Materials and Methods

Tryptic Soy Agar (TSA) Plate Preparation

- Prior to blood sample collection, TSA powder must be weighed, mixed with distilled water, and autoclaved.
- In the hood, pour TSA into sterile petri dishes, place lids, stack lids, and place into incubator (lid side down) overnight at 37°C.
- Tape, label, and place plates into the refrigerator.
- Dispose any contaminated plates into biohazard waste containers.

Photo 1. TSA

Bacteria Pellet Rehydration

- Prior to rehydration, Phosphate Buffer Saline (PBS) Solution was measured and incubated for 30 minutes at 37°C .
- *E. coli* 51813 bacteria pellets were taken out of the refrigerator and allowed to rise to room temperature.
- In the hood, one bacteria pellet was placed into the PBS and immediately incubated for 30 minutes at 37°C.
- After incubation, the mixture was vortexed until the bacteria pellet was completely dissolved while simultaneously trying to keep froth at a minimum.

Photo 2. *E. coli* 51813 pellet

WBK Assay

- Fresh whole blood samples were collected in heparin tubes from adult dairy cows at the OSU Dairy Facility.
- Blood samples were diluted with Roswell Park Memorial Institute (RPMI) media at specific ratios (Table 1.) and equal concentrations of *E. coli* 51813 added.
- Control samples were also prepared, which consisted of RPMI mixed with *E. coli* 51813 in the same concentration as the blood samples.
- The blood and control samples were then plated onto tryptic soy agar (TSA) in duplicates.
- All TSA plates were incubated for a 24 hour period at 38.5°C.

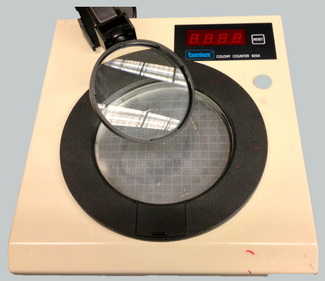
Table 1. Dilution of whole blood

Ratio	Plate Label	Dilution	Blood (µL)	RPMI (µL)
Control	A	0	0	200
1:2	B	50%	100	100
1:4	C	25%	50	150
1:10	D	10%	20	180
1:20	E	5%	10	190

Counting Colonies

- After the incubation period, the number of colony-forming units (CFU) was counted for all samples using the Bantex Colony Counter 920A.
- The percentage of bactericidal activity of the blood samples was compared to that of the control samples.

Photo 3. Bantex colony counter



Results

Table 2. WBK Colony Forming Unit (CFU) counts.

Plate Label	Count (CFUs)	Duplicate Differences
A1 (Control)	81	32
A2 (Control)	49	
B1	26	12
B2	38	
C1	54	3
C2	51	
D1	38	16
D2	54*	
E1	63	51
E2	114	

*Plate possibly contaminated with another species

Discussion

- Large variability was observed between duplicates.
- Potential contamination may have occurred.
- Further adjustments in the protocol methods will be needed to minimize the variation and any potential contamination issues.

Conclusions

- Potential success of this protocol is possible with further adjustments.
- Other considerations needed to optimize this protocol include:
  - Determining the best blood dilution for effective bactericidal activity
  - Determine optimal blood + bacteria incubation time
- As this protocol continues to be refined, it will serve as an important research tool that can provide a simple, quantitative approach to determine if an animal can effectively kill bacteria present in the blood during stressful situations that might lead to poor health status.
- Developing this assay and protocol has enabled me to receive useful training in preparation for graduate school, in addition to enhancing my learning experience in the field of animal well-being.

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