AN ABSTRACT OF THE THESIS OF

Emily Darchuk for the degree of Master of Science in Food Science and Technology presented on May 19, 2015.

Title: Impact of Industry Hauling Practices on Raw Milk Quality in a Commercial Setting

Abstract approved:

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Lisbeth Goddik

Hauling is a critical part of the commercial milk supply chain, yet very few studies have aimed to understand its impact on raw milk quality. Consolidation of the American milk industry has led to the use of tanker trucks for up to 24 h between cleanings, which is the maximum duration permitted by the Pasteurized Milk Ordinance (PMO). As the extended use of tankers has not been previously studied, the impact of this form of hauling on raw milk quality is unknown.

This study focused on the impact on raw milk quality during both short distance and high frequency hauling (up to 9 loads per 24 h) as well as long distance and low frequency hauling (2 loads per 24 h) situations at a commercial facility. Standard tanker use, (cleaned-in-place (CIP) once per 24 h) served as our control and incremental

cleaning treatments were added to the study to understand if any impact could be mitigated by more frequent cleaning. Producer samples were collected from the farm prior to loading milk into the tanker as well as sampling the same milk directly out of the tanker truck prior to unloading at the manufacturer. The study was repeated at multiple facilities in both warm and cool months to understand any impact due to the facility or season. Milk quality was quantified through industry relevant microbiological tests: individual bacteria count (IBC), thermophillic spore count (TSC), and preliminary incubation count (PI).

Within the study we defined a negative impact on milk quality as a statistically significant difference between the tanker and producer samples in any of the three microbial tests conducted. Results from the study showed no clear impact due to hauling in IBC, TSC, or PI counts. This result was consistent across all studies and locations suggesting that hauling does not have a measurable impact on milk quality regardless of the frequency of truck use. As we did not see a negative impact on milk quality due to basic hauling practices (24 h CIP), the addition of cleaning treatments did not appear to provide any benefit.

Tanker surface swabs and ATP swabs were also used to monitor tanker sanitation and the efficacy of cleaning treatments. Both surface and ATP swabs revealed differences between cleaning efficacy at the facilities. Although the differences in efficacy did not influence tanker milk quality within our study, variability in sanitation may provide a source of contamination that could negatively impact raw milk quality in other quality attributes not measured.

Based on this study, the current PMO regulation requiring a CIP every 24 h appears to be effective in mitigating any measurable impact on raw milk quality in both short and long haul situations.

Impact of Industry Hauling Practices on Raw Milk Quality in a Commercial Setting

by
Emily Darchuk

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Emily Darchuk, Author

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CONTRIBUTION OF AUTHORS

Dr. Lisbeth Goddik and Dr. Joy Waite-Cusic assisted with the interpretation of the data and are co-authors of chapter 3 and 4. Gina Shellhammer acted as a contract statistician and contributed to the development of the statistical design as well as the analysis of the data presented in chapter 3. Dr. Hui Feng, Tomomi Fujimaru, Danny Dupree and Christopher Baird assisted with the data collection and sample analysis during both studies.

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1.1   Research Problem

Due largely to the demand of the export market, domestic dairy volumes have increased (USDA, 2012, 2014b). 2014 exports accounted for 15.4% of US milk solids production and were valued at $7.1 billion USD (USDEC, 2015). Although the United States dairy industry has been actively expanding exports, it is still in close competition with other dairy exporting countries such as New Zealand, Australia, and the EU (Blayney and Gehlhar, 2006; FAO et al., 2010; USDA, 2014b). With the goal of continuing to grow exported volume, milk quality is of increasing importance to the US dairy industry due to tighter quality specifications and increased competition when selling into a global market.

Specification limits have been historically difficult for domestic producers to meet, partly due to the added complexity that has resulted from consolidation of the industry (MacDonald et al., 2007; Ollinger et al., 2005; Watterson et al., 2014). Changes within the dairy industry over the past 30 years have been drastic. For example, between 1997 and 2007, there was a 21 percent decrease in the number of milk processing plants; however because of increased scale, these plants processed 26 percent more milk per facility. During this same period, the number of dairy farms decreased by 43 percent, yet total yield per farm doubled (USDA, 2014a).

This consolidation has also impacted how milk is hauled from the farm to the manufacturing plants. Consolidation and price competition has pushed the industry to improve efficiency and reduce resource usage at every step of the supply chain. An example of this is tanker usage, historically, tankers were cleaned after every load, but

the consolidation of the industry has led to longer routes and more frequent use of tankers between cleans.

Within the United States, all Grade A dairy products are regulated by the Pasteurized Milk Ordinance (**PMO**). As stated in the PMO, milk tanker trucks can be used repeatedly for a full 24 hours in-between mandated clean-in-place (**CIP**) treatments (Food and Drug Administration, 2013). Although individual truck utilization varies, routes typically involve frequent tanker use, during which each load of milk has a short duration within a tanker, but a truck sees multiple loads per day, or extended hauls during which each load of milk has a long interval within a truck but only a few loads can be transported each 24 h period. Although permitted by the PMO, the multiple use of tankers between CIP treatments is a new phenomenon for many companies.

The decision on how often to clean tanker trucks beyond regulation is a balance between efficiency and quality. Within industry, the receiving bays can be a bottle neck impacting overall plant efficiency. To reduce this, tanker milk rarely undergoes testing beyond the regulated antibiotic and temperature screenings (Food and Drug Administration, 2013) prior to transferring into a large co-mingled silo. These silos contain milk of different ages, from many producers, and hauled under different conditions, making it impossible to understand if downstream quality issues could be triggered by the hauling process.

Due to this complexity, very few studies have solely investigated the impact of hauling, which requires isolating and collecting samples at the point of receiving. To understand this impact our study investigated hauling by looking at two conditions that large dairy companies encounter today; long haul loads and high frequency tanker use.

1.1   Research Objectives

To understand the impact of frequent use hauling practices on milk microbiological quality within an industrial setting we investigated i) impact of operating trucks for extended duration between CIP treatments; ii) impact of incremental cleaning procedures beyond the standard 24 h CIP, and iii) the differences between manufacturing facilities.

To understand the impact of long duration hauling practices on raw milk microbiological quality we investigated; i) the impact of operating trucks for extended duration between CIP treatments and ii) the impact of incremental water rinse and sanitizer treatment between loads.

2   Chapter 2 – Milk Hauling Overview

Hauling is an important link in the milk supply chain which involves the transfer of milk from the producing farms to a manufacturer or cooperative. The transportation of milk occurs within tanker compartments towed by a truck. Although this is a highly regulated process, variations in procedures and equipment do occur based on scale, region and hauling company. The process outlined within this thesis is representative of the conditions and processes that the milk experienced within this study and is illustrative of the industrialized milk supply chain within the United States. It is also important to note that all dimensions quoted within this thesis should be assumed to be realistic estimates that are either based on measurements taken of an actual tanker or from information provided by hauling industry contacts.

2.1   Milk Industry Background

The US dairy industry has undergone drastic changes over the past few decades. Milk was previously produced on small farms but consolidation has been influenced by an economy of scale that has benefited some larger dairy operations. Although this trend has led to shifts towards larger herd size, the makeup of the dairy industry is varied and can be regionally dependent. Dairy farms now can contain over 37,000 cows, but the majority of operations still contain fewer than 100 head (MacDonald et al., 2007; Charles, 2014). The largest percentage of production comes from farms with over 2,000 head which is also the farm size that has seen the fastest growth (MacDonald et al., 2007). In 2014, the average herd size was 204 cows which was nearly double the average herd size just 10 years prior (Progressive Dairyman, 2015).

This variability and change in herd size has had an impact on milk hauling. As herd sizes grow the volume of milk that can be collected from any given farm increases, allowing for fewer farm pickups per load. The spread of small and large farms has created situations where milk tankers either travel extended distances to pick up a large farm load or collect milk from multiple smaller farms prior to delivery. For large farms, multiple pickups can be required daily.

Hauling companies must work closely with both farmers and manufacturers to manage both the producer and processor milk supply all while scheduling the most efficient tanker routes to reduce resource usage. Although milk can remain in a farm bulk tank for up to 24 h (Food and Drug Administration, 2013) more frequent pick-ups benefit the farmer as tank capacity is limited and premiums are often paid based on producer milk quality at the time of delivery. Concurrently, the receiving bay of dairy manufacturing plants can be a bottle neck in production and deliveries must be staggered throughout the day to balance production, silo capacity, and truck resources. This creates a dynamic environment for the milk hauler, where schedules need to be constantly updated to account for both producer and manufacturer's needs. As haulers are typically contracted, it is in their best interest to identify efficiencies in the process which create situations where a tanker is only cleaned when required by the Pasteurized Milk Ordinance (PMO).

2.2   Hauling Regulations

As defined by the PMO, a bulk milk tanker is a vehicle and associated equipment (tanks, pumps, hoses) used by a hauler to transport raw milk from a dairy farm to a milk plant (Food and Drug Administration, 2013). Milk tanker trucks are regulated by the

PMO and gross vehicle weight (GVW) regulations outlined by each state's department of

transportation which are based on bridge laws. The PMO regulates the materials and

procedures for the hauling process, whereas the GVW outlines regulations based on

safety and road maintenance concerns. Aside from regulation, configurations, usage

and engineering of milk tankers can vary greatly depending on manufacturer and

hauling company.

Within the Pacific Northwest (Oregon and Washington), both single and double

trailer configured trucks are used to haul milk. Within industry, single compartment

trailers are called tanker tubes whereas the double trailers are referred to as farm

transfer systems (FTS) or double-bottoms (Karpoff and Webster, 1984). Both Oregon

and Washington have the same GVW regulations, limiting a vehicle's maximum weight

to be no more than 105,500 pounds (FHWA, 2000; ODOT). Aside from the GVW, the

legal operating weight of a truck is determined by the tire size, wheelbase, and number

of axles, which all impact the manufacturer's design to maximize load efficiency through

reducing truck weight (ODOT; Sharma and Mahoney, 1983; FHWA, 2000). As haulers are

paid partly by how much weight they can haul, the design of milk trucks has been

carefully considered to protect the product while maximizing efficiency.

2.3 Tanker Truck Design

Although the PMO allows some flexibility in the type of material used in the

design of trucks, most tankers are constructed from 300 series AISI stainless steel (Food

and Drug Administration, 2013). Stainless steel is an alloy produced when chromium is

added to iron and carbon to protect the steel from corrosion and oxidation through the

development of a passive layer (Lo et al., 2009; ISSF, 2010) . Within dairy tankers, basic

grade 304 stainless steel is commonly used, this material is also referred to as Austenitic

Cr-Ni stainless steel, with a composition of 18%Cr and 9%Ni (ISSF, 2010).

Milk tankers are designed like a Thermos® with a 10 gage metal end cap, 12

gage metal interior tank and an 18 gage exterior shell. The tanker consists of two

cylinders fabricated from stainless steel metal sheets welded around a 1.5 inch

polystyrene core which acts both as support and as an insulator between the internal

and external diameter of the tank. Polystyrene is an extruded foam in which air is

entrapped within the cell structure which provides an insulating effect (Dow Plastics,

2014). For its weight and price, the combination of polystyrene foam and thin gauge 304

stainless steel is very strong, and offers a smooth internal surface that allows for high

efficacy cleaning utilizing clean in place (CIP) systems.

2.3.1 Tanker Engineering

Tanker trucks are designed to protect milk quality as well as transport large

volumes of milk efficiently from a farm to a plant. Understanding how a tanker is

designed is critical to understanding how hauling can impact milk quality. Two areas of

tanker engineering that are critical to milk quality are the rate of heat transfer between

the cold milk and warmer outside temperatures and the quantity of residual milk that

can remain in a truck following delivery.

Tanker insulation efficiency background. To understand the efficacy of an

insulated truck, a heat transfer formula can be used to determine the theoretical

temperature change that can occur during two conditions the truck can encounter while

on a route (Figure 2.1) giving insight into the rate of heat transfer while a truck is

stationary and in motion.

When using either of these models we are assuming that the tanker compartment is full of milk, creating a negligible head space. This is a condition common in the front compartment as this is filled to capacity prior to transferring milk into the back trailer. Due to this there is often residual head space in the back compartment, the volume of which will vary depending on load number and farm size. As the volume of head space is variable in the back compartment, all calculations were done to estimate heat transfer of milk in the front compartment. In evaluation of these calculations, it is important to note the key assumptions made and the understanding that these are extreme examples of the situations that milk tankers undergo, providing a worst case scenario estimate into the expected rate of heat transfer.

Assumption one: Milk remains in a tanker continuously for 24 h

Due to industry pressure to maximize use of equipment, hauls typically occur consecutively, leaving little time for the tanker to sit empty before picking up the next load. Based on this our calculations assume a tanker is full for the entire 24 h period.

Assumption two: Tankers are continuously in motion or still

Tankers in motion are representative of longer haul situations, during which trucks travel long distances between deliveries. Trucks in motion experience greater temperature changes as compared to stationary trucks which would be more representative of shorter haul situations.

Tanker insulation efficiency calculations. Although some temperature change occurs over time in insulated tankers; these trucks, even when exposed to very warm temperatures (35°C), experience little change to the receiving raw milk temperature as compared to the temperature it was pumped into the truck on the farm (Figure 2.1) .

Based on the Churchill-Bernstein Equation (Perry et al., 1997) , a tanker at constant

motion (60 mph) filled with cold milk (5°C) will gain less than 2°C over a 24 h period. This

same tanker in stationary conditions will gain less than 1°C. Per the PMO, all grade A

milk must arrive at the plant under 7°C , allowing for tanker trucks to be used for

extended periods without issue as long as milk is loaded at cold enough temperatures at

the farm (Food and Drug Administration, 2013).

It should be noted that there are also non-insulated areas of the truck such as

the transfer pump hoses located in between the tanks. As there is no insulation, this

area will see elevated temperatures very quickly. Although this is an area of potential

risk for microbial growth the risk is mitigated through purging the hose with air after

pumping to reduce the amount of residual milk remaining in the line. These results

show that regardless of the hauling situation, milk can remain within refrigerated

temperatures over a 24 h use period, limiting bacterial growth.

Tanker load out efficiency. The other aspect of tanker engineering is

understanding how much residual milk can remain in a truck following delivery.

Calculation of the internal surface area (Figure 2.2) provides understanding into the

volume of residual milk that can build up on the walls of the tanker between washes

and cleans. It is this milk, which harbors bacteria that could directly contaminate future

loads or create long term issues through the formation of biofilms. Milk tankers are

weighed coming into and leaving the plant; so tracking the residual milk left in the

tanker is achievable. As milk is pumped from the tanker into the plant, the only milk

remaining in the truck is within a foam which coats the inside surface of the walls. The

formation of this foam can occur from movement of under filled loads or as a result of

seal issues in receiving pumps or hoses. This foam creates a thin layer across the surface of the tank which later collapses back into milk upon transport. Once foam is formed within a tank, the only way to remove it is with a water or chemical rinse. Typically, the weight of the residual foam is negligible and even at worst case scenario only a few gallons of foam remain in the truck (Hauling Contact, 2014). Based on industry data, typical shrinkage of a load is less than .02% the total weight of a tanker (Industry Sponsor, 2015). After pumping out a 34,000 kg load of milk there will be less than 10 kg of milk remaining in a truck. It is this remaining milk that can grow bacteria or form biofilms, so minimizing the residual milk through a highly effective pumping systems helps to prevent quality issues within the truck and in downstream product.

2.3.2 Tanker Sanitation Concerns

How a tanker is utilized impacts how favorable the conditions can be for biofilm formation and thus the potential for quality defects. Milk tankers provide an opportunistic environment for biofilms to grow due to the surface interface with the milk and tanker walls, extended periods of time the truck is empty but not clean and the varying internal surface temperature that can occur when a truck is empty (Donlan, 2002; Teh et al., 2012). Biofilms are created when a community of bacteria create an exopolysacharide shell which can protect them from harsh conditions such as CIP treatments. Once biofilms form they can be difficult to remove and have the potential to enter the milk plant where they can thrive (Marchand et al., 2012). Thermo-resistant, enzyme and biofilm forming bacteria have also be isolated from the internal surface of a dairy tanker, suggesting hauling could be a potential cause of milk quality issues (Teh et

al., 2011, 2012, 2013, 2014). Although biofilm formation within a tanker is a concern, the risk of their development is less likely as compared to other areas of the plant due to the low temperatures, low shear, and smooth surface area that the raw milk is exposed to within a tanker truck (Marchand et al., 2012). The risk of development is also managed through cleaning treatments, but the overall tanker sanitation is only as good as the cleaning treatments and preventative maintenance that it obtains.

2.3.3 Tanker Cleaning

Milk tanker trucks are required to undergo a CIP treatment after every 24 h of use but are allowed to be used for multiple loads between washes. Washes are loosely regulated by the PMO and trucks must display a wash tag on the exterior of each tank documenting the last time it was cleaned. Within the 24 h period, a truck can be used as needed to haul the milk from the farm to the plant which may involve long hauls, frequent use or extended waiting periods during which the tanker is soiled but empty. The PMO only mandates minimum temperatures and frequency of cleans allowing manufacturers a great deal of flexibility in their choice of chemicals, pressures and frequency beyond regulated 24 h CIP treatments (Food and Drug Administration, 2013). Manufacturers typically work with chemical companies to design a sanitation regime that meet their quality, cost and efficiency goals. This flexibility allows for plant to plant variability in cleaning efficacy which can impact day to day sanitation within tanker trucks that deliver to multiple facilities.

Although the CIP process is regulated, the chemicals used, temperatures met and pressures achieved vary from plant to plant. To begin the clean, the truck pulls into the receiving bay and the receiving hose is connected to the plant water supply. During

this set up, flow diversions are created so that the water and chemicals will utilize the

perforated CIP pipe (Figure 2.3). This pipe runs the length of the tank and is designed

similarly to a sprinkler system to create pressurized spray reaching all areas of the

tanker. Using a power take off (PTO) to power the pump from the motor of the truck,

water and chemical are pumped through the receiving hose into the first tanker,

washing both the milk transfer pipe as well as the internal surface of the tank. The same

solution travels through the transfer pump hose into the back compartment

simultaneously cleaning both the front and back compartments at the same pressure.

Although a specific CIP procedure is not detailed for tanker trucks in the PMO, it is a

process which is documented and evaluated during inspections from state regulators.

Typical CIP processes involve a water rinse, detergent, and water rinse followed by a

sanitizer treatment. Although tanks and trucks are typically cleaned as a unit it is

important to note that they are three independent pieces of equipment and thus may

have differing conditions based on previous use. CIP temperatures reach upward of

170°F, making cleaning a very resource intensive step of the manufacturing process. CIP

treatments within a facility can make up half of a dairy plants' energy usage (DMI, 2010)

so it is important for companies to find a balance between cleaning frequently enough

to maintain milk quality while also managing resource usage.

2.3.4 Tanker Design Summary

Evaluation of the design and industry use of the tanker trucks is critical to

understanding the results of our study. The cold conditions maintained by the insulated

tanker helps to substantially slow the bacterial growth in the milk maintaining quality

during transportation. This is further aided by the small amount of residual milk left in

the tanker following load out reducing impact on future loads in between cleans. Impact

of residual milk is also reduced through conducting a CIP wash following every 24 h of

use, although variability in CIP practices can create sanitation issues that vary based on

how an individual facility uses and maintains their equipment.

2.4 Appendix

Figure 2.1 Heat transfer based on a cold tanker of milk at moving and stationary conditions in warm weather. Calculations provided as a worst case scenario to demonstrate the efficacy of the insulating effect of the tanker truck over a 24 h period. Based on properties of dry air chart- Table A.4.A (Singh et al., 2008)

Key assumptions:

1. Front tanker is full of cold milk (5°C) and outside temperature is 35°C
2. Tanker is either stationary for 24 hrs or at steady rate (60 mph) for 24 hrs.

Insulating Air Temperature Calculation:

$$T_{air} = \frac{T_i - T_\infty}{2} \qquad\qquad 15°C = \frac{5°C - 35°C}{2}$$

Grashof formula: Used to determine insulating film layer:

$$Gr = \frac{d^3 \rho^2 g \beta \Delta T}{\mu^2} \qquad 2.28 *10^{10} = \frac{1.73^3 * 1.185^2 * 9.81 * 3.47 * 10^{-3} * (35-5)}{(18.044 * 10^{-6})^2}$$

d= Outside tanker diameter (m)   g= Acceleration due to gravity (m/s^2)
ρ= Density of air at 15°C (kg/m^3)   β = Co-efficient of volumetric expansion (K^{-1})
μ =Viscosity of air at 15°C (Pa s)
ΔT = Temperature difference between wall & milk (°C)

Churchill-Bernstein Equation for Forced Convection around a cylinder (MOVING TANKER)

$$Nu = .3 + \frac{.62\,(Re)^{1/2} * Pr^{1/3}}{[1+(0.4\,Pr)^{\,2/3}]^{1/4}} + \left[1 + \left(\frac{Re}{282,000} \right)^{5/8} \right]^{4/5}$$

$$838.61 = .3 + \frac{.62\,(2.833 * 10^6)^{1/2} * .71^{1/3}}{[1+(0.4 * .71)^{\,2/3}]^{1/4}} \left[1 + \left(\frac{2.72 * 10^6}{282,000} \right)^{5/8} \right]^{4/5}$$

$$Re = \frac{\rho U_{bD}}{\mu} \qquad\qquad 2.72 * 10^6 = \frac{1.1095 * 26.83 * 1.73}{(18.90 * 10^{-6})}$$

$$h = \frac{Nuk}{L} \qquad\qquad 2.71 = \frac{838.61 * .02615}{8.08}$$

Re= Renyold's number   U$_b$= Velocity (m/s)
D= External diameter of tanker (m)   μ = Viscosity of air at 35°C (Pa s)
ρ= Density of air at 35°C (kg/m^3)   k = Thermal conductivity of air at 35°C (W/m K)
L = Length of tanker
h = Convective heat transfer coefficient at 35°C (W/[m^2 K])

Figure 2.1 (Continued) Heat transfer based on a cold tanker of milk at moving and stationary conditions in warm weather.

Heat transfer formula

$$\dot{q} = \frac{T_i - T_\infty}{\frac{\ln(\frac{r_o}{r_i})}{2\pi L k} + \frac{1}{h_o A_0}}$$

$$759.45 = \frac{35-5}{\frac{\ln(\frac{.865}{.83})}{2*\pi*8.08*.025} + \frac{1}{2.71*(2*\pi*.865*8.08)}}$$

$\dot{q}$ = Joule heat per time   (J/s)
T_i -Temperature outside (°C)
T_∞- Temperature Milk (°C)
K= thermal conductivity of Styrofoam (W/m K)
Ho = convective heat transfer coefficient at 35°C (W/[m² K])

Ro= External radius of tanker (m)
Ri= Internal radius of tanker (m)
L= External length
Ao = External area = 2πrL

Natural convection around a cylinder (Stationary Tanker)

$$Nu = \left\{ .6 + \frac{.387\,(Pr*Gr)^{1/6}}{\left(1+\left[\frac{.559}{Pr}\right]^{\frac{9}{16}}\right)^{8/27}} \right\}^2$$

$$261.73 = \left\{ .6 + \frac{.387\,(.71*2.28*10^{10})^{1/6}}{\left(1+\left[\frac{.559}{.71}\right]^{\frac{9}{16}}\right)^{8/27}} \right\}^2$$

$$h = \frac{Nuk}{L}$$

$$.85 = \frac{261.73*.02615}{8.08}$$

Heat transfer formula

$$\dot{q} = \frac{T_i - T_\infty}{\frac{\ln(\frac{r_o}{r_i})}{2\pi L k} + \frac{1}{h_o A_0}}$$

$$517.29 = \frac{35-5}{\frac{\ln(\frac{.865}{.83})}{2*\pi*8.08*.025} + \frac{1}{.85*(2*\pi*.865*8.08)}}$$

$\dot{q}$ = Joule heat per time   (J/s)
T_i -Temperature outside (°C)
T_∞- Temperature Milk (°C)
K= thermal conductivity of Styrofoam (W/m K)
ho = convective heat transfer coefficient at 35°C (W/[m² K])

Ro= External radius of tanker (m)
Ri= Internal radius of tanker (m)
L= External length
Ao = External area = 2πrL

Temperature change calculations

$$\frac{q}{mcp} = \Delta T$$

Moving Tanker:

$$\frac{65616756.4}{17986*3852} = .947\,°C$$ .947*1.8 = 1.7 °F

Standing Tanker:

$$\frac{44693453.5}{17986*3852} = .645\,°C$$ .645*1.8= 1.161 °F

q = Joule heat per time (J/24 hr)
m= mass of milk (kg)

cp= Specific heat of milk (J/Kg°C)
ΔT = temperature change (°C)

Figure 2.1 (Continued) *Heat transfer based on a cold tanker of milk at moving and stationary conditions in warm weather.*
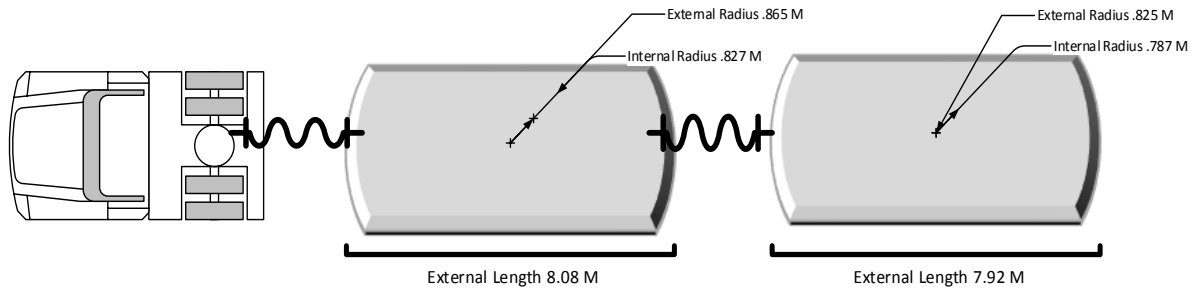
Internal Surface Area of Tanker:

$$A = 2\pi rh + 2\pi r^2$$

Front = 46.28 M ^{2} = 2*π*.83M*8.08M+2*π*.83M^2

Back = 43.05 M ^{2} = 2*π*.79M*7.92M+2*π*.79M^2

Volume of Tanker:

$$V = \pi r^2 h$$

Front Volume: π*.83M^2 *8.08M=17.36 M^3

Front Volume (US gal) : 17.36 M^3 * $\frac{264.172\ gallons}{1\ M^3}$ = 4585

Front tanker weight *of* milk (lbs): 4585 gallons * $\frac{8.64\ lbs/gallon}{1\ gallon}$ = 39616

Back tanker weight of milk (kg): 39616 lbs * $\frac{.454\ kg}{1\ lb}$ = 17986

Back Volume: 15.41 M^3 = π*.79M^2 *7.92M

Back Volume (US gal): 15.41 M^3 * $\frac{264.172\ gallons}{1\ M^3}$ = 4070

Back tanker weight *of* milk (lbs) : 4070 gallons * 8.64 $lbs/gallon$ = 35165

Back tanker weight *of* milk (kg) : 35165 lbs* $\frac{.454\ kg}{1\ lb}$ = 15965

Total Volume per tanker truck (kg of front and back trailer) = 17986+15965= 33951

Figure 2.2 Dimensions of a tanker truck and calculations of internal surface area and tanker load out efficiency
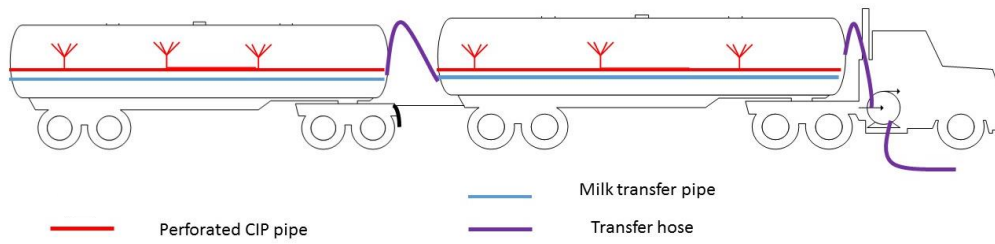
Figure 2.3 Diagram of a Tanker Truck; although all pipes are cleaned during a CIP wash the red pipes are specific to the CIP cycle, blue pipes are used for milk loading and the purple lines are shared and consist of the flexible transfer hoses.

3   Chapter 3- Short Distance and High Frequency Tanker Use Study

Interpretive Summary

This study focused on the impact of frequent tanker use between cleaning treatments on hauled raw milk quality at manufacturing facilities. Three cleaning treatments were evaluated to understand if their addition could mitigate any potential negative impact. Based on this study, current hauling practices do not have any measurable impact on raw milk quality although further investigation is needed prior to making industry wide recommendations.

OUR INDSTRY TODAY

Impact of commercial hauling practices and tanker cleaning treatments on raw milk microbiological quality

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3.1 Abstract

Consolidation of the American milk industry has led to use of tankers for up to 24h in-between thorough cleanings. As the heavy use of tankers has not been previously studied, the impact of this form of hauling on raw milk quality is unknown.

This study focused on the impact of frequent tanker use during hauling on raw milk quality at a commercial facility. Standard tanker use, [cleaned-in-place (CIP) once per 24 h] served as our control and incremental cleaning treatments (water rinse after each load, water rinse after each load with a sanitizer treatment after 12 h, and 12 h sanitizer treatment) were added to the study to understand if any impact could be mitigated by more frequent cleaning. Producer samples were collected from the farm prior to loading milk into the tanker as well as sampling the same milk directly out of the tanker truck prior to unloading at the manufacturer. The study was repeated at two different dairy manufacturing facilities, once during the summer and once during the winter. Milk quality was quantified through industry relevant microbiological tests: individual bacteria count (IBC), thermophillic spore count (TSC), and preliminary incubation count (PI).

Within the study we defined a negative impact on milk quality as a statistically significant difference between the tanker and producer samples in any of the three microbial tests conducted between treatments. Results from the study showed no clear impact due to hauling in IBC, TSC, or PI counts. There was also no difference in milk quality between the two plants suggesting that neither season nor location impacted our results in the standard use variable. As we did not see a negative impact on milk

quality in the standard use variable, the addition of cleaning treatments did not appear

to provide any clear benefit.

Tanker surface swabs and ATP swabs were also used to monitor tanker

sanitation and the efficacy of cleaning treatments. Both surface and ATP swabs

revealed differences between cleaning efficacy at the two facilities. Although the

differences in efficacy did not influence tanker milk quality within our study, variability

in sanitation may provide a source of contamination that could negatively impact raw

milk quality in other areas.

Based on this study, current hauling practices appear to be effective in

mitigating any measurable impact on raw milk quality however, further investigation is

needed prior to making industry wide recommendations.

Key words: Hauling, Milk Tanker, Bacteria, Cleaning

3.2   Introduction

Due largely to the demand of the export market, domestic dairy volumes have continued to increase (USDA, 2012, 2014b) . Raw milk quality is of increasing importance to US dairy producers due to tighter quality specifications demanded by the global market.

Specification limits have historically been difficult for domestic producers to meet partly due to the added complexity that has occurred with consolidation of the domestic dairy industry (Ollinger et al., 2005; MacDonald et al., 2007; Watterson et al., 2014). The focus on consolidation has pushed the industry to improve efficiency and reduce resource usage throughout the supply chain. An example of this is milk hauling, where historically tankers were cleaned after every load, but the consolidation of the industry has led to longer routes and more frequent use of tankers between cleans.

Within the United States, all Grade A dairy products are regulated by the Pasteurized Milk Ordinance (**PMO**). As stated in the PMO, milk tanker trucks can be used repeatedly for a full 24 hours in-between mandated clean-in-place (**CIP**) treatments (Food and Drug Administration, 2013). While individual truck utilization varies, routes can involve frequent tanker use, during which each load of milk has a short duration within a tanker, but the truck transports multiple loads per day. Following each load hauled, any residual milk remaining in the truck may impact the microbiological quality of subsequent loads.

Although many studies have helped the industry improve milk quality at the farm and production level, few studies have investigated the impact of hauling practices.

Of previous studies, many were conducted outside of the United States and were unable to capture the impact of domestic milk hauling practices in situ.

Previous studies suggest that the potential impact of hauling on milk quality is dependent on the conditions of the tanker and quality metrics used. A few studies have shown that multiple loads can be hauled between CIP treatments without a measurable increase in the total bacteria count of raw milk (Dommett et al., 1980; Stewart, 1985). Whereas other studies have identified areas within a tanker truck that can remain soiled after cleaning (Luck and Lategan, 1979; Gerlach and Sabolic, 1980; Bell et al., 1994; Paez et al., 2013) which provide sources of contamination as well as opportunistic environments for biofilms to form (Donlan, 2002; Teh et al., 2012).

The objective of this study was to understand the impact of frequent use hauling practices on milk microbiological quality within domestic industrial settings. The study was outlined to measure i) impact of operating trucks for extended duration between CIP treatments ii) impact of incremental between load cleaning procedures beyond the standard 24 h CIP and iii) differences between manufacturing facilities.

3.3   Material and Methods

Study Overview. This study was conducted through a partnership with a large commercial dairy in the Pacific Northwest to ensure that the hauling conditions were representative of domestic dairy practices. Samples were collected within the standard operations of two manufacturing plants (plant A and plant B) and analyzed using common quality metrics.

Commercial Facilities. The first study was conducted in mid-November at a mid-sized manufacturing plant (Plant A). The second study was conducted in late August at a

large manufacturing plant (Plant B). Both studies were conducted in the Pacific Northwest with the facilities located 280 miles apart.

Tanker Trucks. Two trucks and four trailers were isolated for the duration of the study at each location. Trucks and trailers remained as one unit for the entire study. All equipment was of similar age and considered to be in good condition based on regulatory inspection. All milk was hauled within double trailer tanker trucks with a flexible transfer hose to connect the two compartments. These trailers were transported by a truck that carried the transfer pump and hose which loaded the milk from the farm bulk tank into the trailer compartment. Milk was always first loaded into the front trailer and when filled was diverted to the back trailer. Both trailers were simultaneously emptied at the plant.

Farm Routes. Each truck was assigned to a route which determined what farm milk each truck would pick up within a 24 h period. Routes were selected based on their ability to be repeated daily and were specific to each study location. Within each route there were up to 9 loads scheduled. Each load was either filled from a single farm or was commingled and contained multiple farms within the same truck. A load was completed when a full truck delivered milk to the manufacturing plant.

Cleaning Treatments. The study investigated the addition of cleaning treatments incremental to the standard operating procedure of a 24 h CIP which served as a standard use variable (control) (Table 1). Two trucks underwent different cleaning treatments each day, creating four replicated days for each of the four cleaning treatments over the eight day study (Table 2). Cleaning treatments were partial stages of the full CIP cycle and utilized existing chemicals and equipment. All water rinses conducted were 2-3 minutes in duration and utilized ambient temperature water. All sanitizer rinses at both plant A

and B were conducted using a spray of nonanoic and decanoic acid (Mandate Plus- Ecolab US, St. Paul, MN).Water samples were analyzed from the CIP system daily to ensure that rinse water was not a source of contamination. All cleaning treatments, including CIP, were conducted in the receiving bay of the plant immediately after unloading milk and prior to continuing on to the next load.

Samples

Over 100 samples were collected each day (Table 3), and were identified by a sample location and unique bill of lading (**BOL**) number which was traceable to a specific farm, truck, treatment and load. Training of receivers and haulers was conducted to ensure that sampling and cleaning procedures were consistent throughout the study.

Milk Samples. Producer samples were collected by the hauler from every raw milk bulk tank after agitation using a sanitized stainless steel dipper or sample port (Food and Drug Administration, 2013). Producer samples were stored in temperature-monitored (< 7°C) coolers during transport.

Receivers collected the tanker samples once the truck entered the receiving bay of the plant. At plant A, samples were taken from the back outlet of the front and back trailer during pumping. At plant B, samples were taken using a sanitized stainless steel dipper from the top hatch of the milk tank. A different dipper was used for front and back trailer of the truck to avoid cross contamination. All liquid samples were collected in sterile containers and stored in a temperature monitored refrigerator (< 7 °C) following collection.

Surface Swabs. Sponge-stick swabs moistened with Letheen broth (3M US, St. Paul, MN) were used after unloading milk to measure residual bacteria on the internal

surface of tank. For every load, a 900 cm^2 area (30 cm x 30 cm) was swabbed per manufacturer's instructions. Following treatment, sponge and ATP swabs (3M US) were used to measure the efficacy of the clean. Receivers were trained to rotate the area swabbed with each incoming load and to conduct the ATP swab prior to the sponge swab to avoid false positives. ATP swabs were read and recorded immediately after sampling utilizing a Luminometer (3M US). Sponge swabs were stored in a temperature monitored refrigerator (<7 °C) following collection.

3.3.1 Sample Analysis

All samples were analyzed at the same corporate laboratory for both studies. All samples were transported (< 7°C) to the laboratory via daily courier service. Samples were received within 48 hours of sampling and were tested upon arrival.

Microbiological Analysis. All milk samples were analyzed for individual bacteria count (**IBC**), thermophilic spores count (**TSC**), and preliminary incubation (**PI**) most probable number (**MPN**). Individual bacterial counts of all milk samples were conducted using a Bactoscan FC (FOSS, Hillerød, Denmark). Conversion of IBC to cfu was calculated using Bactoscan software. The method described by Wehr and Frank (2004) was used to quantify thermophilic spores. A 5 mL sample of milk was heated in a sterile test tube to 80°C and held for 12 minutes. Following heat treatment, the tubes were chilled in an ice water bath for 10 minutes prior to pour plating using Standard Method Agar (Neogen, Lansing, MI) and incubated for 48 h at 55 ± 1°. Preliminary incubation was conducted by adding a 0.1 mL diluted sample of milk (Butterfield's Buffer, 3M US) to 3.9 mL sterile water into a TEMPO Total Viable Count (**TVC**) vial (bioMérieux; Marcy l'Etoile, France). The TVC vials were incubated at 13° ± 1°C for 18 h followed by 32° ± 1°C for 48 h. Following

incubation, MPN was determined using a TEMPO reader following manufacturer instructions.

Both the rinse water and sponge swabs were evaluated for aerobic plate count (**APC**) using Petrifilm (3M US) incubated at 32° ± 1°C for 48 h. Petrifilms were enumerated using an automated counter (3M Petrifilm reader).

3.3.2 Statistical Modeling

The study was outlined using a mixed model with repeated measures design. Possible confounding factors identified were the equipment (truck A vs B), location of sampling (front vs back trailer), variability in farm milk quality, and day to day operational variability. The model includes four cleaning treatments with sampling repeated after every delivery.

Statistical Analysis. Impact on milk quality was defined as a significant change ($P < 0.05$) in the tanker microbiological count as compared to the same load producer microbiological count across load number and between cleaning treatments. For the sake of comparison all samples below the limit of detection for PI (< 1,000 cfu/mL) and TSC (< 10 cfu/mL) were scored as 500 cfu/mL and 5 cfu/mL respectively. For commingled loads, the weighted average of the producer microbial counts were calculated for the front and back trailer.

Linear mixed effect analysis was conducted on the log transformed producer and tanker data for the three microbial tests from both studies. Statistical analysis on the data was conducted using R software (R Development Core Team, 2013) and the nlme package (Pinheiro et al., 2015). Fixed effects were identified as the truck and cleaning treatment with the producer milk quality considered a covariate. Sample location (front or back

tank) was found to be a non-significant fixed effect and was dropped from the model. A correlation structure to account for the potential dependence between loads within the same truck was not found to contribute positively to the model based on Akaike information criteria (Akaike, 1973); therefore, it was removed as an effect for model parsimony.

A comparison of the two studies was conducted through a Welch's t-test. This test was conducted by comparing the average difference between the tanker and producer microbial count in the standard use milk samples from both plant A and plant B for IBC, TSC and PI. A Pearson's chi-squared test (χ^2) was conducted to determine the significance of ATP swab data between plant A and plant B for each cleaning treatment.

3.4   Results

Milk Samples.   Temperature during the study at plant A averaged 3°C with a daytime high of 11°C and nighttime low of -5°C. Farm routes at plant A were consistently repeated, with up to 9 loads per truck within a 24 h period. Of these loads, most were commingled and all contained between 1 and 5 producers per tanker truck.

Temperature during the study at plant B averaged 23°C with a daytime high of 36°C and a nighttime low of 12°C. Routes were not as consistent as plant A and consisted of fewer commingled loads. Trucks at plant B averaged 7 deliveries per 24 h of use and contained between 1 and 3 farms loads per tanker truck. Due to concerns around sample temperature abuse, day one of the plant B study was removed from analysis reducing the total repetition of treatments to three days for both the water rinse (**WR**) and the 12 hour

sanitizer treatment (**SO**). Milk quality was overall very good at both facilities with over 80% of all producer samples testing below 10,000 cfu/mL (Figure 1).

The measured cfu change during the hauling process was determined by averaging data from both trucks and sampling locations (front and back) at each plant. When looking at the data (Figure 2 and 3) for the overall trend across cleaning treatments and number of loads hauled, all data was clustered around the center line showing little difference between the tanker and producer samples regardless of treatment or duration of use at both facilities.

Statistical results showed that when keeping everything else constant there was a significant impact between trucks in IBC (P = 0.006), TSC (P = 0.012), and PI counts (P < 0.001) at plant A. Although this was a possible confounding influence, any impact due to the truck was balanced with two replicates of every cleaning treatment occurring within each truck (Table 2). There was no significant impact due to the truck found at plant B.

At plant A, there was no significant impact due to the cleaning treatments in IBC nor PI. Slight evidence (p = 0.043) of a statistical impact due to treatment was found for TSC. Keeping everything else constant, the rinse treatment showed a 40% reduction in the median TSC as compared to the median standard use TSC (6.3 cfu/mL). Although this was found to be significant within the model, the reduction in spore counts would not be practically significant for industry as the difference is below the commonly used detection limit for the test (< 10 cfu/mL). Within this study, 36% of all spore samples were below the limit of detection from plant A and 15% from plant B. Plant B's statistical results showed no significant effect in the IBC, TSC, nor PI for any of treatments as compared to standard tanker use. When comparing the standard use variable at plant A and plant B,

there was no significant difference found between the results in any of the microbiological tests.

Swabs. Following CIP treatment, the average surface bacteria count of a clean truck prior to starting a route was 1.89 cfu/900cm^2 at plant A and 1.50 cfu/900cm^2 at plant B. Tankers showed a similar pre-treatment average surface bacteria count between plant A (3.36 log cfu/900cm^2) and plant B (3.32 log cfu/900cm^2) (Figure 4). The water rinse (WR) treatment was more effective in reducing surface bacteria at plant B with an average post treatment count of 1.24 log cfu/900cm^2 as compared to 2.60 log cfu/900cm^2 at Plant A.

ATP swabs (Table 4) also showed a plant to plant difference in cleaning efficacy, with 90% of tankers at Plant B and 23% of the tankers at plant A meeting the industry partner's standard for a clean tanker [<150 Relative Light Units (**RLU**)] following a water rinse treatment (P < 0.001). Following CIP, 100% of tankers at plant B and 86% of the tankers at plant A met ATP cleanliness standards (P = 0.045).

3.5   Discussion

Heavy and extended use of tankers. Residual bacterial counts between the producer and tanker samples remained consistent, providing no evidence that the extended use of tanker trucks have an impact on raw milk quality as measured by IBC, PI or TSC. Even with variable producer milk quality, the impact of a preceding load on the subsequent load of tanker milk was negligible. The results of our study align with previous in situ work, proposing that any bacterial differences seen between producer and tanker

samples are not operationally significant as compared to the variability typically seen in milk quality across producers (Dommett et al., 1980; Stewart, 1985).

The lack of measureable impact from hauling is largely due to the low levels of residual milk remaining in the tank after pumping and the limited sensitivity of test methods used within industry. Typical shrinkage allowance within industry is 0.02% of a load, allowing for less than 10 kg of milk remaining in a tanker after load out. The bacteria within this residual milk is diluted by next load (34,000 kg) of milk making it difficult to measure significant changes using typical industry milk quality tests. The accuracy of the Bactoscan FC is reported to be +/- 0.25 Log units across the measuring range (FOSS). Within this study all averaged differences between the producer and tanker results fell within the margin of error, further suggesting that any differences found could be due to sampling and testing variability and not the impacts due solely to hauling. This variability also created occasions where the tanker data averaged lower than its producer sample even though counts should not be reduced due to hauling. A similar study in Ireland, found only marginal increases (0.14 Log cfu/mL) in total bacteria counts could be attributed to the hauling process and accounted for situations in which the tanker counts differed from the producer counts due to sampling variability (Stewart, 1985).

When investigating industry practices, finding only a marginal increase in bacteria counts during the hauling process is expected. As milk remains at temperatures below 7°C during transportation, only psychrotrophic bacteria may grow. Even for psychrotrophic bacteria, generation times are significantly longer than the duration of time the milk was held within a tanker truck under frequent use situations. Within this study, each load remained in the tanker truck for less than 4 h, limiting potential bacterial

growth. Of psychrotrophic bacteria found in raw milk, *Pseudomonas* spp. has one of the shortest generation times of 5.5-14.7 h at 4-6°C (Cousin, 1982; Sørhaug and Stepaniak, 1997) and has been found to make up only 8% of the bacteria isolated from the surface of a milk tanker (Teh et al., 2011).

Theoretical calculations investigating the worst-case scenario suggest that even a highly contaminated preceding load of milk will have negligible impact on a low microbial load of milk subsequently loaded into the tanker prior to cleaning treatment. This impact is insignificant due both to the slow rate of bacteria growth and dilution effect. Based on the maximum shrinkage allowance, highly contaminated milk (150,000 cfu/mL) remaining in a truck following pump out would contribute less than 0.003 Log cfu/mL of bacteria to the next load of milk (10,000 cfu/mL), making its bacterial contribution impossible to accurately measure with the microbiological tests typically used in industry.

Location and seasonal variability. The replication of the study in multiple locations provided a broader picture into the operation of trucks with different systems. As milk quality results were similar in both the studies, location did not appear to have an impact on the results of our study. Even though location did not impact the milk quality results, we found differences in cleaning efficacy between plant A and B, suggesting operational differences could cause impacts that were not captured within this study.

Impacts of seasonality were predicted to cause changes in farm milk quality between studies and increased milk receiving temperatures due to temperature extremes during the summer study (Plant B). During the study, all tanker loads were received under < 7°C and milk quality was consistent across both studies. As results were similar in both studies, seasonality did not appear to have an impact on the results.

To avoid confounding operational impacts from conducting the studies at different locations, cleaning treatments were not compared across seasons in statistical analysis. When comparing standard tanker use across the winter (Plant A) and summer (Plant B) study, results show that differences in average microbiological counts between the tanker and producer samples were not significant. This suggests that regardless of season, the extended use of tanker trucks does not negatively impact milk quality in the metrics measured.

The lack of impact due to season is reasonable when investigating how trucks were utilized. Due to industry pressure to maximize use of equipment, short duration hauls typically occur consecutively, leaving little time for the tanker to sit empty before picking up the next load. When a tanker is full of cold milk, the insulation within the compartments is effective at maintaining temperature regardless of external temperatures. The cold temperature of the milk tanker prevents significant microbiological growth from occurring during short duration hauls, thus minimizing any seasonal impact due to increased environmental temperatures. When tankers are in motion, even at very warm temperatures (35°C), there is very little change in temperature. Based on the Churchill-Bernstein Equation, a tanker at constant motion (60 mph) filled with cold milk (5°C) will gain less than 2°C over a 24 h period (Perry et al., 1997) allowing for tanker trucks to be used for extended periods without issue.

Tanker Trucks. There was no significant impact attributed to the sampling location within trailers. Within this study we compared the milk in the front versus the back trailer to understand if there was any contamination due to the transfer hose. As transfer hoses are uninsulated, this area can see elevated temperatures in warm weather.

Although this is an area of potential microbial growth, the risk is mitigated through purging the hose with air after pumping to reduce the amount of residual milk remaining in the line.

Truck to truck variability was investigated as a potential confounding influence as tankers are often cleaned at multiple locations leading to variability in tanker maintenance. The statistical difference in tanker milk quality between trucks at plant A suggests that regular maintenance of both the trucks and the CIP system is important. Plant A conducted treatments in multiple bays of the receiving facility which could explain the variability in cleaning efficacy. Results showing no impact due to the truck or across treatments at Plant B suggests that when properly maintained, current hauling equipment is effective at preventing growth during 24 h of continuous use.

Cleaning treatments. There is no compelling evidence that the addition of cleaning treatments could provide a positive impact on milk quality as compared to standard use. As there is not an increase in microbiological counts over time in the standard use variable, the addition of a cleaning treatments provided no measurable benefit to milk quality. The only statistical difference found between treatments in our study (TSC at Plant A) would not provide a practical significance to industry as the reduction in spore count was only estimated to be 2.5 cfu/mL, which would be below the limit of detection of typical test methods. The slight evidence of significance was likely created due to skewed data from the large percentage of counts that were below the limit of detection.

Cleaning treatments were outlined in partnership with our industry sponsor with the goal of identifying treatments that were robust enough to remove residual milk yet

rapid enough to minimize impact to operational efficiency. The cleaning treatments that are outlined are partial stages of the full CIP cycle and utilized existing chemicals and equipment. These treatments were all in addition to the mandated CIP treatment once every 24 h which occurred on every truck regardless of assigned cleaning treatment. Within this study, swab data shows that water rinses reduced residual surface bacteria counts to levels similar to post-CIP treatment levels. These results align with a previous study which found that a water rinse in-between loads was as effective in preventing significant bacterial growth in hauled milk as a full CIP treatment (Dommett et al., 1980). Although we believe a water rinse can be a tool to remove surface bacteria, we suggest their use in addition to current sanitation practices, not as a replacement for full CIP treatments.

As our study only investigated short term microbiological growth, not biofilm formation within tanker trucks, hauling could still have a negative impact on raw milk quality that is not detectable with the test methods used within this study. A study by Teh (2011) isolated biofilm forming bacteria from the internal surface of milk tankers following use and before cleaning. Although Teh's study found biofilm forming milk bacteria within tankers, the formation (2.7- 7.6 log cfu cm^{-2}) was documented at in-vitro conditions more extreme (25°C for 24 h) than a frequent use tanker would typically experience in the United States. It is important to note that the isolated tanker bacteria was also found to produce enzymes which negatively impacted milk quality (Teh et al., 2011). Our swab data suggests that differences found in plant to plant cleaning efficacy could create situations where sporadic tanker sanitation issues may impact downstream milk quality for manufacturers.

As the addition of cleaning treatments consumes plant resources (receiving bay space, water, chemicals, employee time) there is not sufficient evidence to suggest the addition of incremental cleans would provide any operational benefit in terms of improved quality. Alternatively, incremental cleans did not show any evidence of negatively impacting milk quality so their use could be adopted by industry as a preventive measure in extreme situations, such as occasions where a truck will be empty but soiled for an extended period of time .

3.6   Conclusion

Within this study, extended use of tanker trucks (24 h CIP) did not appear to have a negative impact on raw milk quality. Our results align with the findings of previous in situ studies as well as theoretical calculations. We caution that although we saw no benefit to adding cleaning treatments, this sole study should not be used as a justification to reduce cleaning treatments at any individual facility. As this study focused on frequent use of tankers, further studies should investigate the impact of extended duration hauling on milk quality. Continued investigation utilizing more sensitive test methods may also find impacts that we were unable to detect. For improved milk quality, industry should focus on producing high quality milk at the farm, loading milk as cold as possible and creating consistency in current hauling practices through maintenance of equipment and conducting regular CIP treatments.

3.7   Acknowledgements

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3.8   Appendix

Table 3.1: Overview of cleaning treatment variables utilized in the mixed model design

Cleaning Treatment [a]	Procedure
Standard Use (SU)	Standard operational use (control). CIP after 24 h.
Water Rinse (WR) [b]	2-3 minute water rinse following every load.
Water Rinse with Sanitizer Treatment (WS)[bc]	2-3 minute water rinse following every load. One 2-3 minute water rinse followed by sanitizer spray after approximately 12 hours of use.
Sanitizer Treatment (SO) [bc]	One 2-3 minute water rinse followed by sanitizer spray after approximately 12 hours of use.

[a] All cleaning treatments were in addition to the standard operational CIP following 24 h of use per the PMO.
[b] The water rinse used non thermally controlled water
[c] Sanitizer used was Mandate Plus (Ecolab)

Table 3.2 Mixed model with repeated measure study design for both studies. Outline of the scheduling of trucks and routes with assigned cleaning treatments

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8	
Cleaning Treatment [a]	WR	SO	WS	WR	WR	SU	WS	WR	SU	SO	SO	WS	SU	WS	SO	SU
Truck [b]	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2

Treatment Key: SU: Standard use (control) WR: Water rinse after each load WS: Water rinse after each load and sanitizer treatment after 12 hours of use SO: Sanitizer treatment after 12 hours of use

[a.] The same cleaning treatments were assigned at both facilities but operational differences prevented complete replication of treatments across both studies
[b.] Trucks remained consistent across a given study but different equipment was used at plant A and B

Table 3.3 Outline of milk and swab sample location, collection frequency, and analysis method for both manufacturing locations

Sample Location	Frequency	Analysis
Producer Milk	Samples were collected from every bulk tank that was loaded into tanker (n=446)	IBC[1], TSC[2], PI[3]
Tanker Milk	Samples were collected from the front and back trailer of every tanker load that was delivered to plant (n= 617)	IBC, TSC, PI
Tanker Surface Swab	Swabs were taken from the front and back trailer ceiling[4] following every load delivered to the plant. When a cleaning treatment occurred a second set of swabs were taken before a truck continued on route (n=721)	APC[5]
Tanker ATP Swab	Swabs were taken from the ceiling of the front and back trailer following every cleaning treatment conducted and prior to sponge swab sampling. (n= 261)	Luminometer
Rinse Water	Samples were taken at least once daily from CIP tank or outlet pipe (n=19)	APC

[1]   Individual Bacteria Count : Bactoscan FC
[2]   Thermophilic Spore Count
[3]   Preliminary Incubation Count: TEMPO
[4]   Location of swab sample rotated with every load
[5]   Aerobic Plate Count : Petrifilm

Table 3.4 ATP swab data from post cleaning treatments. Percent pass of treatments based on cleanliness threshold of <150 RLU[2].

	CIP n= 54	Water Rinse n=97	Rinse and Sanitizer Treatment n=96	Sanitizer Treatment n= 14
Plant A	86%[a]*	23%[a]***	45%[a]***	38%[a]
Plant B	100%[b]	90%[b]	81%[b]	50%[a]

[a-b] % pass between plants with different superscripts differ ($P < 0.05$)

[1] *$P < 0.05$, ***$P < 0.001$.

[2] Relative light unit (RLU)
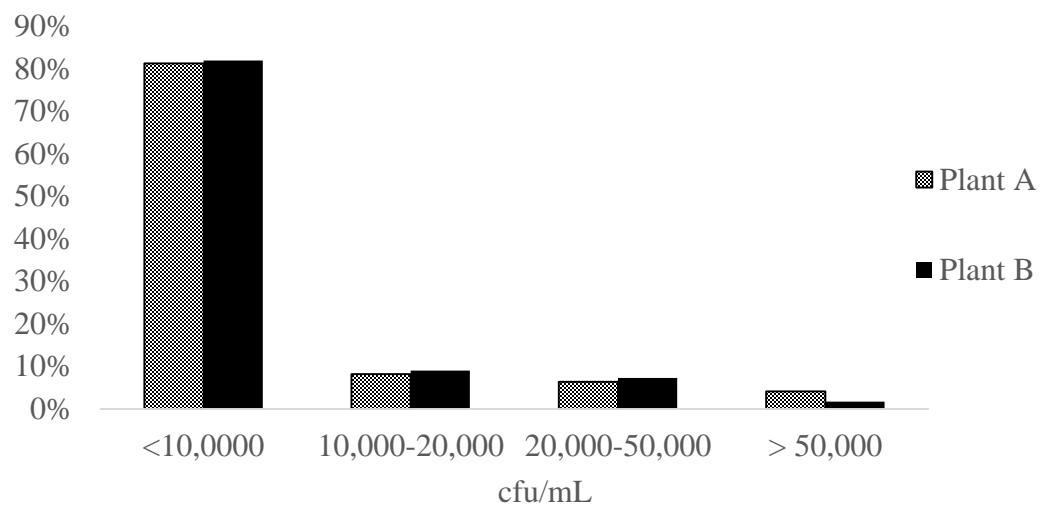
Figure 3.1 Producer milk quality for both study locations: plant A (n= 270) and plant B (n=178). Categorized as percentage of samples within individual bacterial count (IBC) quality categories.

a.   Plant A
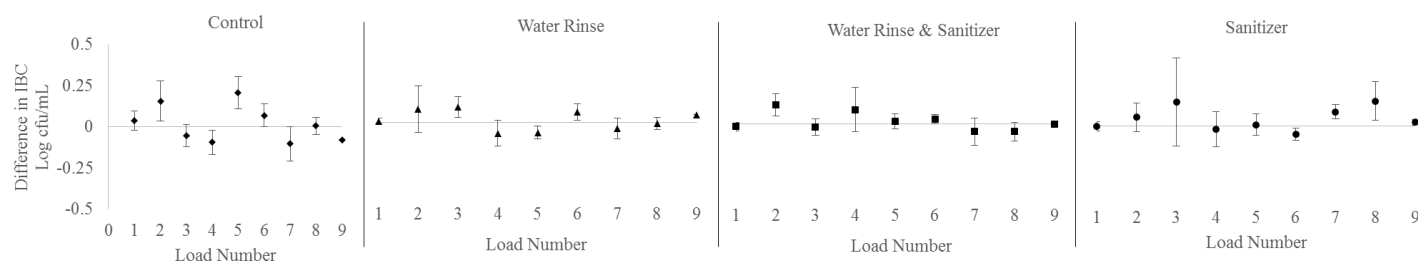
b.

Plant B
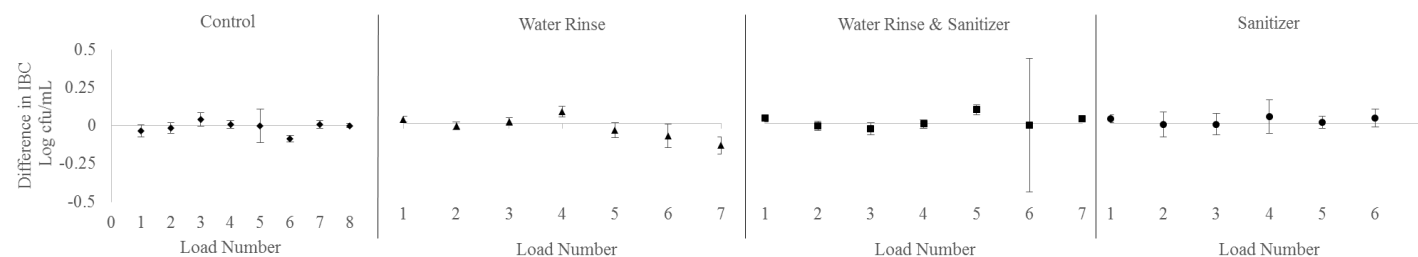
Figure 3.2 Impact of cleaning treatments on tanker bacteria growth. Raw milk individual bacterial count (IBC) results displayed as the average difference between the tanker and corresponding producer milk across treatments and each load within 24 h cycle at Plant A (2.a) and Plant B (2.b). Each cleaning treatment was replicated 4 times at Plant A and 3-4 times at Plant B and contained up to 9 loads per 24 h use period at plant A and up to 8 loads at plant B.

Figure Key: ◆: Standard use, ▲: Water Rinse, ■: Water Rinse and Sanitizer, ●: Sanitizer

a.

b.

c.

d.
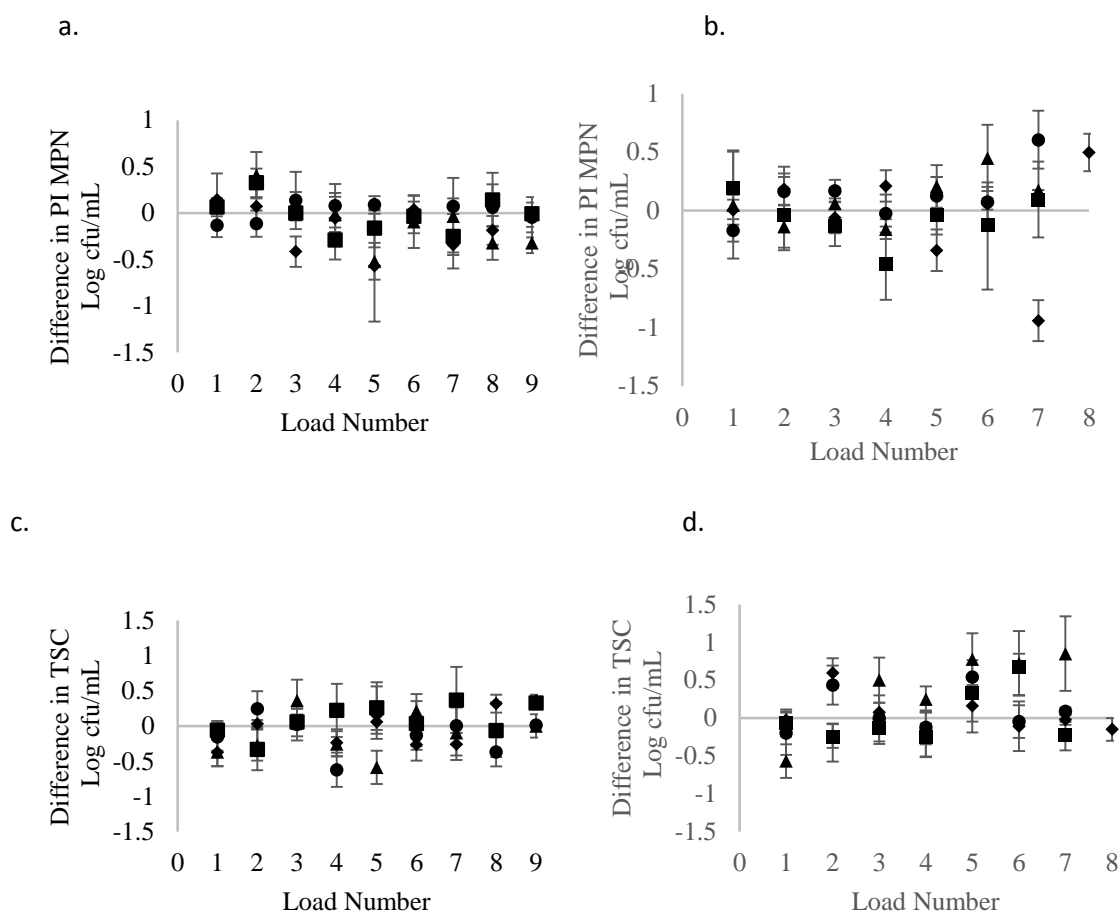
Figure 3.3 Impact of cleaning treatments on tanker PI and TSC growth. Raw milk preliminary incubation (PI) (3.a, 3.b) and thermophilic spore count (TSC) (3.c, 3.d) results are displayed as the average difference between the tanker and corresponding producer milk across treatments and each load within 24 h cycle at Plant A (3.a, 3.c) and Plant B (3.b, 3.d). At plant A, each cleaning treatment was replicated 4 times and contained up to 9 loads per 24 h use period. At plant B, each cleaning treatment was replicated 3-4 times and contained up to 8 loads per 24 h use period.

Figure Key: ♦: Standard use, ▲: Water Rinse, ■: Water Rinse and Sanitizer, ●: Sanitizer
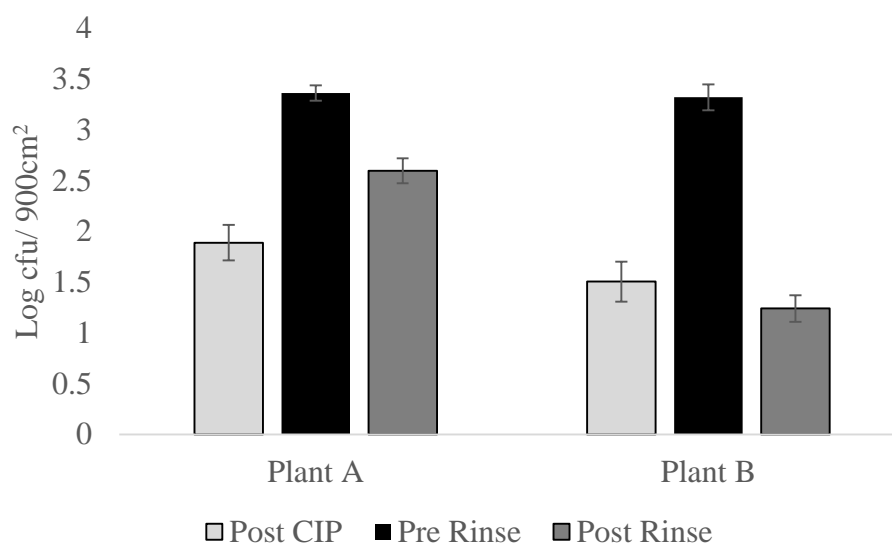
Figure 3.4 Tanker surface sponge swab results from Plant A and Plant B to measure cleaning efficacy. Data reported is the average aerobic plate count per swabbed surface area (900 cm^2) of a soiled tanker before and after a water rinse treatment. Averaged post clean-in-place (CIP) treatment data was included as a benchmark for starting tanker cleanliness across the duration of the study at both plants. Plant A: CIP n= 26, Rinse Pre n= 60, Rinse Post n= 60, Plant B: CIP n= 24 Rinse Pre n= 32, Rinse Post n= 32

4 Chapter 4-Long Distance and Low Frequency Tanker Use Study

Interpretive Summary

This study focused on the impact of long distance raw milk hauling at a manufacturing facility. Raw milk quality was investigated before and after an extended duration haul during summer conditions. Based on this study, long distance milk hauling practices do not appear to have a measurable impact on raw milk quality.

SHORT COMMUNICATION: **Microbial quality of raw milk following commercial long distance hauling. Darchuk**

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4.1   Abstract

Hauling is a critical part of the commercial milk supply chain, yet very few studies have aimed to understand its impact on raw milk quality. This study focused on the impact of extended duration tanker use during hauling on raw milk quality at a commercial facility. Standard tanker use, (cleaned-in-place **(CIP)** once per 24 h) served as a control and an incremental between load water rinse with sanitizer treatment (**RS**) was evaluated to mitigate any impact from extended duration hauling. During this study, one commercial truck with two trailers was monitored for 10 days. The truck collected milk at a large dairy farm, transported the milk to a manufacturing facility, and then returned to the same farm for a second load. Each round trip journey took between 10-12 h allowing for two loads per 24 h use period. Following the second delivery, the truck was cleaned by CIP treatment starting a new treatment day. Producer samples were collected from the farm prior to loading milk into the tanker as well as sampling the same milk directly out of the tanker truck prior to unloading at the manufacturer. Milk quality was quantified through common industry tests: individual bacteria count (IBC), thermophillic spore count (TSC), and preliminary incubation count (PI). Surface sponge swabs were also used to monitor tanker sanitation and the efficacy of cleaning treatments. Results did not identify a negative impact on raw milk quality due to extended duration hauling. Therefore, the addition of a rinse with sanitizing treatment RS did not provide any measurable quality benefit. Swabs results demonstrated that the RS treatment was able to reduce surface bacteria on the tanker, although it was not as effective as the full CIP treatment. Based on this study, current long haul practices appear to be effective in mitigating any measurable impact on raw milk quality.

Key words: Hauling, Milk Tanker, Bacteria, Cleaning

4.2   Introduction

Within the United States, all Grade A dairy products are regulated by the Pasteurized Milk Ordinance (**PMO**). As stated in the PMO, milk tanker trucks can be used repeatedly for a full 24 h between Clean in Place (**CIP**) treatments (Food and Drug Administration, 2013). Although individual truck utilization varies, routes can involve extended duration hauls during which, each load of milk has a long interval within a truck but only a few loads can occur per each 24 h period. Extended duration hauls may also include situations where a truck remains soiled and empty for extended periods of time between loads. Residual milk remaining in the truck may lead to microbial growth as well as the formation of biofilms that could negatively impact the microbiological quality of subsequent loads, making extended duration hauling an industry practice that poses potential risk to raw milk quality.

The objective of this study was to understand the impact of extended duration hauling practices on raw milk microbiological quality within an industrial setting. The study was outlined to measure i) the impact of operating trucks for an extended duration between CIP treatments, and ii) the impact of an incremental between-load water rinse with sanitizer treatment to remove milk residue.

4.3   Material and Methods

Study overview

This study was performed within the standard operations of a commercial dairy manufacturing plant. Samples were analyzed using common quality metrics to ensure that the study was representative of industry practices.

Tanker Trucks. Milk was hauled within one double trailer tanker truck with a flexible transfer hose to connect the two compartments. These trailers were transported by a truck that carried the transfer pump and hose which loaded the milk from the farm bulk tank into the trailer compartment. Prior to the study, all equipment had passed regulatory inspection.

Tanker Routes. To reduce variability in producer milk quality, one route was repeated for the duration of this study. This route consisted of milk from one farm which was collected twice daily. Prior to the first load, the truck underwent a CIP treatment at the manufacturing plant. Following CIP, the tanker would travel to the farm which was located approximately five hours away. All milk was loaded from a single bulk tank, filling both trailer compartments of the truck. Once loaded, the truck would return to deliver the milk to the same manufacturing plant. Following delivery of the first load, the truck would either return to the same farm without any cleaning treatment (standard use -**SU**) or a water rinse followed by a sanitizing spray (**RS**) would occur prior to the second farm pick up. Regardless of treatment, following the delivery of the second load the truck would undergo a CIP treatment and a new treatment day would begin. All cleaning treatments, including CIP, were conducted in the receiving bay of the plant immediately after unloading milk and prior to continuing on to the next load.

Cleaning Treatments. This study investigated the addition of a between load water rinse and sanitizer treatment (RS) which was incremental to the standard operating procedure of a 24 h CIP (SU). The truck underwent each treatment for multiple days, creating seven replicated days for the RS treatment followed by three replicated days of the SU treatment over the ten day study.

Incremental treatments were partial stages of the full CIP cycle which utilized existing chemicals, receiving bays, and equipment. The RS treatment consisted of 2-3 minute ambient water rinse followed by a sanitizing spray containing a blend of peroxyacetic acid and hydrogen

peroxide (Oxonia Active – Ecolab US, St. Paul, MN). Water samples were taken from the CIP system throughout the study to ensure no contamination of the tanker occurred due to the RS treatment.

Sampling

Samples were collected daily (Table 1) at both the farm and plant. Prior to the study, training of both the receivers and haulers was conducted to ensure that sampling and cleaning procedures were consistent throughout the study. All samples were kept below 7°C during storage and transport and were tested within 36 h of sampling at a corporate laboratory.

Milk Sampling. Haulers followed PMO regulations when collecting producer samples from the farm bulk tank (Food and Drug Administration, 2013). Receivers took tanker samples using a sanitized stainless steel dipper from the top hatch of the front and back tanker trailer. A different dipper was used for each compartment of the truck to avoid cross contamination.

Surface Swabs. Sponge-stick swabs moistened with Letheen broth (3M US, St. Paul, MN) were used after unloading milk to measure residual bacteria left on the internal surface of tank. For every load, a 900 cm^2 area (30 cm x 30 cm) was swabbed per manufacturer's instructions. Following CIP or RS treatment, a second swab was taken to measure the efficacy of the clean. Receivers were trained to rotate the area of the ceiling swabbed with each incoming load and before and after cleaning treatments.

Microbiological Analysis. Milk samples were evaluated at the same location using the same microbiological techniques as was described in detail within Darchuk et al. (In Review). Briefly, all milk samples were analyzed for individual bacteria count (**IBC**), thermophilic spore count (**TSC**), and preliminary incubation (**PI**) most probable number (**MPN**). Individual bacteria counts of all milk samples were conducted using a Bactoscan FC (FOSS, Hillerød, Denmark).

Thermophilic spores were quantified using the method described by Wehr and Frank (2004). Preliminary incubation was conducted by adding a diluted samples to a TEMPO Total Viable Count (**TVC**) vial (bioMérieux; Marcy l'Etoile, France). The TVC vials were incubated at 13° ± 1°C for 18 h followed by 32° ± 1°C for 48 h and enumerated using TEMPO reader following manufacturer instructions.

Both the rinse water and sponge swabs were evaluated for aerobic plate count (**APC**) using Petrifilm (3M US) incubated at 32° ± 1°C for 48 h. Petrifilms were enumerated using an automated counter (3M Petrifilm reader).

Statistical Analysis. The impact on milk quality due to extended duration hauling was defined as a significant change ($P < 0.05$) in the microbiological count (difference between the producer and tanker milk) between load one and load two within a treatment (RS or SU). Impact on milk quality due to the cleaning treatment was defined as a significant change ($P < 0.05$) in the microbiological count (difference between the producer and tanker milk) between RS and SU treatments. All samples below the limit of detection for PI (<1,000 cfu/mL) and TSC (< 10 cfu/mL) were scored as 500 cfu/mL and 5 cfu/mL respectively.

Statistical analysis on the data was conducted using R software (R Development Core Team, 2013). The difference in tanker milk quality due to extended tanker use was determined through a paired t-test comparing the difference between the tanker and producer milk of the first and second load of milk for both the SU and RS treatment. The impact of cleaning treatment on tanker milk quality was determined through a Welch's t-test comparing the difference between the tanker and producer milk between treatments. Prior to conducting statistical analysis, all samples were averaged so one tanker output value could be compared to the single producer input value for every load.

4.4 Results

Producer milk quality during this study was good with over 95% and 68% of the loads testing below 10,000 cfu/mL in IBC and PI, respectively (Figure 1).

Statistical analysis found no significant difference between the average difference between producer and tanker IBC (P = 0.228), TSC (P = 0.7071) nor PI (P = 0.7923) counts for the rinse and sanitize (RS) treatment as compared to standard tanker use (SU) (Figure 2). There was also no significant difference between the average difference in the producer and tanker IBC, TSC, or PI count between load one and load two in either SU or RS treatments, demonstrating no difference between the pre- and post-hauled milk quality over the course of a 24 h period.

Following CIP treatment, the average surface bacteria count of a clean truck prior to starting a route was 1.23 log cfu/900cm^2. A tanker after delivering one load of milk had an average surface bacteria count of 4.00 log cfu/900cm^2. The use of a water rinse with a sanitizer treatment reduced the surface bacteria count to 2.68 log cfu/900cm^2 (Figure 3).

4.5 Discussion

The difference in microbiological counts between the producer and tanker samples did not increase significantly between loads, suggesting that the extended use of a tanker truck has no impact on raw milk quality as measured by common industry test methods. As there was not an increase in microbiological counts over time in the standard use variable, the addition of a RS treatment provided no measurable benefit to raw milk quality. As the data provided no convincing evidence that hauling has a significant impact on milk quality in any of the microbiological parameters measured (IBC, TSC, PI), current industry practices based on PMO regulations appear to be effective (Food and Drug Administration, 2013).

A similar outcome was found when investigating frequent use hauling, during which tanker trucks were used for the same period of time (up to 24 h between CIP), but each load of milk had a short duration within a tanker and more loads were hauled per day (Darchuk et al , 2015 – In review). It is reasonable to find similar results between frequent use and extended duration studies, as the lack of measureable impact from hauling is largely due to the low levels of residual milk remaining in the tank after pumping and the limited sensitivity of test methods used within industry; suggesting that regardless of the type of hauling, 24 h continual use of tanker trucks can occur without negatively impacting raw milk quality based on the metrics we investigated.

Within our study, swab data showed that RS treatments could reduce residual surface bacteria counts, but it was not as effective as a full CIP treatment. Although the RS treatment was able to reduce surface bacteria, there is not enough evidence to suggest that its use would provide a quality benefit to industry that would be worth the resource usage required to implement it after every load.

Our study only investigated short term surface bacteria growth, not biofilm formation, hauling could still have a negative impact on raw milk quality that was not detectable with the test methods used within this study. Although biofilm formation within a tanker is a concern, the risk of its development within a truck is less than other areas of a dairy plant. Biofilm formation within a tanker is expected to be less likely due to the cold temperature, low shear, and smooth surface area that the raw milk is exposed to within a stainless steel tanker truck (Marchand et al., 2012). The risk of biofilm development is also managed through regular CIP treatments, but overall tanker sanitation is only as good as the efficacy of any given facility's regular practices. A previous study investigating plant to plant differences in tanker sanitation (Darchuk et al., In

Review), found efficacy differences in CIP and incremental cleaning treatments between facilities. As biofilm forming bacteria isolated from tanker trucks have been found to have negative impacts on raw milk quality (Teh et al., 2011, 2012, 2013), variability in truck sanitation could lead to sporadic downstream milk quality issues that would be difficult for a company to trace.

4.6 Study Summary

Within this study, extended use of tanker trucks (24 h CIP) did not appear to have a negative impact on raw milk quality. The results of this study along with a previous study investigating high frequency hauling, suggests that regardless of how a tanker is used, 24 h of continuous use can occur without a negative impact on milk quality. Based on the results of our study, there is not sufficient evidence to suggest the addition of a between load RS treatment could provide any operational benefit in terms of improved quality. We suggest that industry instead focus on effective CIP treatments at all manufacturing facilities.

As this study was an initial investigation, its goal was to provide a preliminary look into the impact of extended duration hauling. Continued research into the impact of commercial hauling practices is recommended as further investigation utilizing more sensitive test methods may find relevant impacts that we were unable to detect. Based on the limited scope of our study and the variability in plant to plant operations, this sole study should not be used as a justification to reduce cleaning treatments at any individual facility.

4.7 Acknowledgements

everyone who supported this trial at the manufacturing plant, hauling company, corporate lab,

and corporate office.

4.8   Appendix

Table 4.1 Outline of milk and swab sample location, collection frequency, and analysis method

Sample Location	Frequency	Analysis
Producer Milk	Duplicate samples were collected from every bulk tank that was loaded into tanker (n=40)	IBC[1], TSC[2], PI[3]
Tanker Milk	Duplicate samples were collected from the front and back trailer of every tanker load that was delivered to plant (n= 80)	IBC, TSC, PI
Tanker Surface Swab	Swabs were taken from the front and back trailer ceiling[4] following every load delivered to the plant. When a cleaning treatment occurred a second set of swabs were taken before a truck continued on route (n=74)	APC[5]
Rinse Water	Samples were taken throughout the study from the CIP tank or outlet pipe (n=6)	APC

[6]   Individual Bacteria Count : Bactoscan FC
[7]   Thermophilic Spore Count
[8]   Preliminary Incubation Count: TEMPO
[9]   Location of swab sample rotated with every load and before and cleaning treatment
[10]   Aerobic Plate Count : Petrifilm
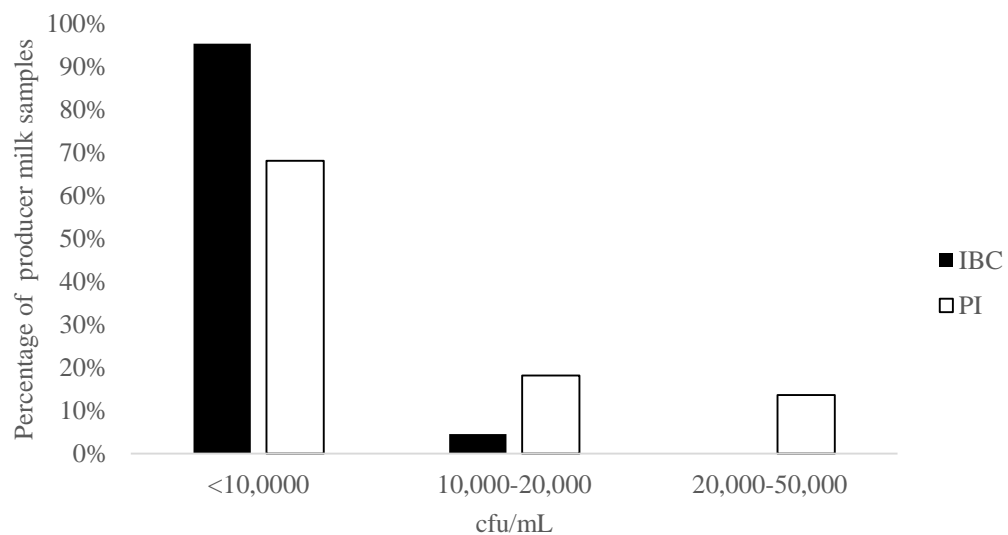
Figure 4.1 Producer milk quality. Categorized as percentage of producer milk samples within individual bacterial count (IBC) and preliminary incubation (PI) most probable number quality categories (n=20).

a. IBC
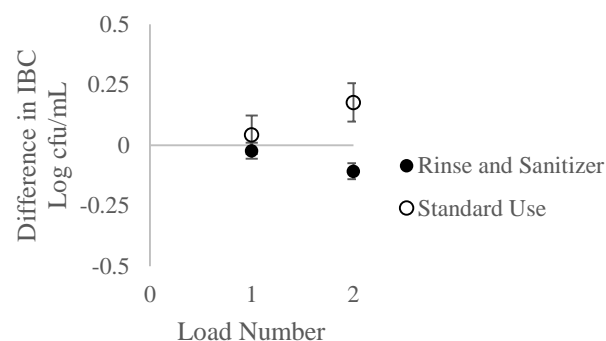
b. PI MPN
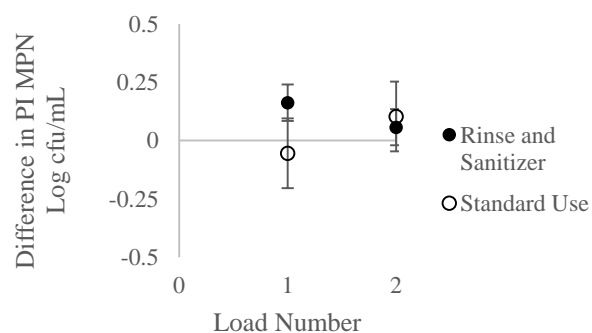
c) TSC
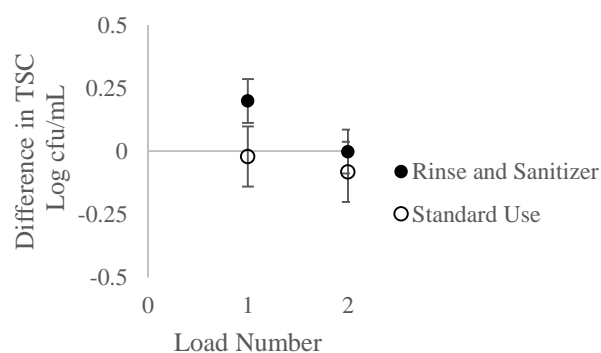
Figure 4.2 Impact of cleaning treatments on tanker microbiological growth. Raw milk individual bacterial count (IBC) (a), preliminary incubation (PI) most probable number (MPN) (b), and thermophilic spore count (TSC) (c) results displayed as the average difference between the tanker and corresponding producer milk across treatments and each load within 24 h cycle. Rinse and sanitize (RS) treatment was replicated 7 times and the standard use (SU) treatment was replicated 3 times. Both treatments consisted of 2 loads per 24 h use period with all milk sourced from the same farm.
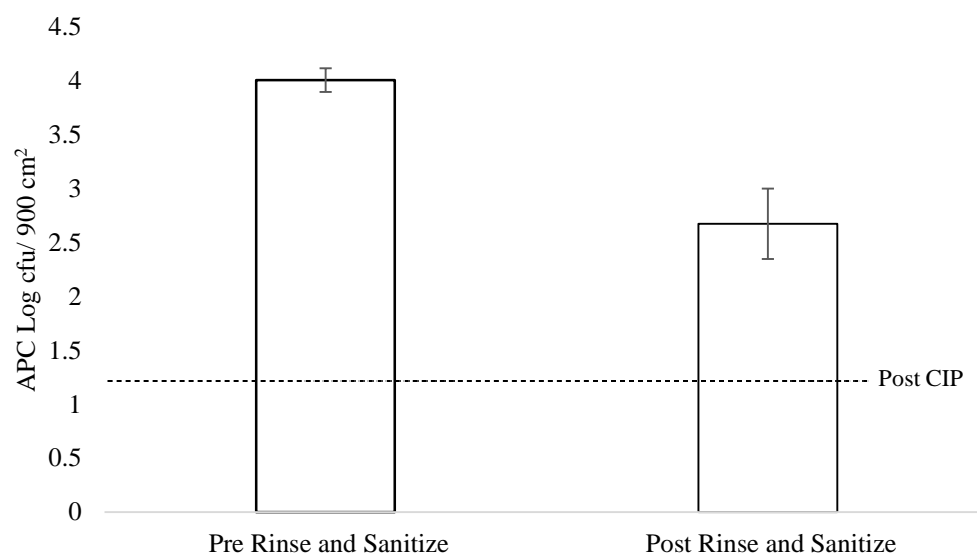
Figure 4.3 Before and after average surface bacteria count from the rinse and sanitize (RS) cleaning treatment. Data reported is the average aerobic plate count (APC) of a soiled tanker before (n=14) and after (n=14) a water rinse and sanitizer treatment as measured by sponge swab sampling. The dotted line represents the average starting APC count of a clean tanker post CIP treatment (n= 20).

5   Chapter 5- Conclusion

5.1   Research Summary

Controlling milk quality upstream is very important as the value and quantity of milk increases with each step of the pooling chain (Harding, 1995). Raw milk quality is critical in producing a high quality finished product as the effects of abuse cannot be reversed downstream.

Based on this study, both short and long duration hauling practices do not appear to have a negative impact on raw milk quality based on commonly used microbial test methods. Thus, our recommendation is to continue current hauling practices (24 h CIP) as our data does not suggest the addition of incremental cleaning treatments can improve raw milk quality.

It is important to note that although the incremental treatments did not significantly improve raw milk quality, we saw no negative impact from their use, and as it does reduce tanker surface bacteria they could be used as a preventative measure in situations when a truck will remain empty for an extended period. Although we are not recommending additional treatments we think focusing on proper cleaning of tanker trucks and lines should be important for all facilities and location specific investigation should be done prior to reducing any facilities' current cleaning practices. We also suggest focusing on high quality producer milk as the lower bacterial count milk will further prevent significant growth downstream.

5.2 Future Work and Opportunities

Although we are confident in our results, there are limitations to this study which could have missed quality impacts due to hauling. Impacts could have been missed due to the limited sensitivity of common microbiological tests or by not capturing hauling situations that occur outside of the scope of our design. Based on this we suggest continued hauling research focused on long distance hauling. It is our hypothesis that long distance hauling is most likely to see issues due to extended periods of time that a truck could remain empty and soiled. Any future studies investigating long distance hauling should contain a larger sample size and use more sensitive test methods focused on detecting downstream milk quality defects. Further investigation into tanker sanitation, especially focused on the formation of biofilms within tanks, could provide additional context to the impact of extended use of tanker trucks between cleaning treatments.

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Appendices

Appendix 1: Neutralization capacity of letheen broth on Mandate Plus Sanitizer which was used in both short haul studies as described in Chapter 3. Table taken from a study conducted by Ward (2013).

Table 8. Neutralization activity profiles of 4 collection broths with Mandate Plus acid sanitizer

Sanitizer Type	Manufacturer	Collection Broth	Dilution of Sanitizer Used in Assay					
			1:100	1:200	1:400	1:800	1:1600	1:3200
Mandate Plus (Nonanoic (Pelargonic) Acid 6.30%; Decanoic (Capric) Acid 1.09%) - Use dilution is 1:427 for coarse spray application	Ecolabs	D/E Neutralizing Broth	-	+	+	+	+	+
		Letheen Broth	-	-	+	+	+	+
		Neutralizing Buffer	-	-	-	-	+	+
		HiCap Neutralizing Broth	-	+	+	+	+	+

Note 1. The blue highlighted area gives an approximation of the highest concentration of sanitizer (lowest use dilution) recommended by the manufacturer.

Note 2. + is positive for neutralization in the bioassay. Without neutralization, this concentration is lethal to the test organism.

Note 3. HiCap is a trademark of World Bioproducts

Winter Study: 1344-2016PPM

Appendix 2 Product information for Sanitizer used in short duration study (chapter 3)

ECOLAB®

42503

Net Contents: 55 U.S. gal/208.2 L

MANDATE™ PLUS

CIP Acid Sanitizer
For Dairy & Food Processing Equipment

Mandate Plus is a non-iodine acid sanitizer for CIP and COP systems, tanks and back-flush systems.

ACTIVE INGREDIENTS:
Nonanoic Acid 6.30%
Decanoic Acid 1.09%
INERT INGREDIENTS:..92.61%
TOTAL:..100.00%

KEEP OUT OF REACH OF CHILDREN
DANGER
PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS
DANGER: CORROSIVE: Causes irreversible eye damage or skin burns. May be fatal if absorbed through skin. Harmful if swallowed or inhaled. Do not get in eyes, on skin or on clothing. Do not breathe vapor or spray mist. Wear goggles or face shield (safety glasses). Wear protective clothing (long-sleeve shirt and long pants, socks plus shoes and chemical resistant gloves, such as water proof gloves). Wash thoroughly with soap and water after handling and before eating, drinking, using tobacco, or using the toilet. Remove contaminated clothing and wash before reuse.

U.S. Patent No. 6,472,358

FIRST AID
IF IN EYES: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment advice.
IF SWALLOWED: Call a poison control center or doctor for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control center or doctor. Do not give anything by mouth to an unconscious person.
IF INHALED: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.
FOR EMERGENCY MEDICAL INFORMATION IN USA OR CANADA, CALL: 1-800-328-0026.
FOR EMERGENCY MEDICAL INFORMATION WORLDWIDE, CALL: 1-651-222-6352 (IN THE USA).
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.
NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage.
ENVIRONMENTAL HAZARDS: This product is toxic to fish. Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the U.S. Environmental Protection Agency.
PHYSICAL AND CHEMICAL HAZARDS: Mix only with water following label directions. Do not mix with chlorinated cleaners or sanitizers. Toxic chlorine gas will be formed.

EPA Reg. No. 1677-194
EPA Est. No. 1677-IL-2 (J), 1677-TX-1 (D), 1677-GA-1 (M), 1677-CA-1 (S), 1677-MN-1 (P), 70271-CA-2 (A), 1677-CA-2 (R), 1677-WV-1 (V), 303-IN-1 (L), 66593-PA-1 (FT).

DIRECTIONS FOR USE
It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.
For Dairy and Food Processing Equipment, Tanks, Vats, Pails, Pipelines, and Closed Systems: Remove all gross food particles and soil by a pre-flush or pre-scrape, and when necessary, a prescak treatment. Clean all surfaces thoroughly with proper detergents and rinse with potable water.
Sanitize at a concentration of 0.5 to 1.0 ounces per 3 gallons (1.30 to 2.6 mL per Liter, 1300 to 2600 ppm) of water. At this dilution, *Mandate Plus* is effective against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229). Sanitize CIP or COP equipment by immersion, circulation or coarse spray sanitizing techniques, as appropriate. Expose all surfaces to sanitizing solutions for a period of not less than 2 minutes. Drain thoroughly and then air dry. Regular use of *Mandate Plus* will prevent the formation of milk stone or mineral stone on processing equipment.
Follow state and local Health Department regulations for cleaning and sanitizing food processing and dairy equipment.
NOTE: For mechanical operations repeated use solutions may not be reused for sanitizing, but may be reused for other purposes such as cleaning.

STORAGE & DISPOSAL
DO NOT CONTAMINATE WATER, FOOD, OR FEED BY STORAGE OR DISPOSAL.
PESTICIDE STORAGE: Store in a cool, dark, dry place in original container. Always replace covers. Do not store below 0° F for extended periods.
PESTICIDE DISPOSAL: Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.
CONTAINER DISPOSAL: Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying. Offer for recycling, if available, or discard in trash.

Department of Agriculture
STATE OF HAWAII
LICENSED
PERIOD 2015-2017 LIC. NO.
9203.356

Department of Agriculture
STATE OF HAWAII
LICENSED
PERIOD 2012-2014 LIC. NO.

Corrosive Liquid, N.O.S., (Acetic & Nitric Acid),
UN1760
8

Appendix 3 : PMO Cleaning Regulations (Food and Drug Administration, 2013)

APPENDIX F. CLEANING AND SANITIZATION
I. METHODS OF SANITIZATION
CHEMICAL
Certain chemical compounds are effective for the sanitization of milk containers, utensils and equipment. These are contained in either in 40 CFR 180.940 and shall be used in accordance with label directions, or ECA device manufacturer's instructions if produced onsite in accordance with Section II below.
HOT WATER
Hot water may be used by pumping it through the inlet, if the temperature at the outlet end of the assembly is maintained to at least 77ºC (170ºF) for at least five (5) minutes.

Appendix 4 : Plant Training Materials from Short Distance Study (Chapter 3)

Milk Tanker Quality Investigation:

November 2013 & August 2014

OSU
Oregon State

Study Deliverables

1. Determine the impact of operating trucks for an extended period of time (24 hrs and up to 10 loads)
2. Impact of seasonality/ location
3. Impact of different cleaning procedures

Background

- Two trucks and two routes have been isolated for this study
- All trucks studied will undergo 4 cleaning treatments over an 8 day study
- Producer sample data for each load will serve as the milk quality control
- Consistency in sampling and testing is critical for validity of the study

---

Cleaning Variables to Investigate

1. Standard use (24 hr CIP)
2. Water rinse after each load
3. Water rinse after each load & sanitizer treatment after 12 hrs
4. Sanitizer treatment after 12 hrs

Day	Day 1/ Aug 19		Day 2/ Aug 20		Day 3/ Aug 21		Day 4/ Aug 22		Day 5/ Aug 23		Day 6/ Aug 24		Day 7/ Aug 25		Day 8/ Aug 26	
Cleaning Treatment																
Truck	10	14	14	10	14	10	10	14	10	14	10	14	14	10	10	14
Route	1	2	2	1	2	1	1	2	2	1	2	1	1	2	1	2

Sample Collection Sites

Producer Milk

Provide baseline milk quality data

Frequency:
- Sample from every farm bulk tank loaded into truck

Testing:
- Bactocan (BC)
- Thermophilic Sporeformer (TSC)
- Preliminary Incubation (PI)

Tanker Milk

Show the effect of hauling

Frequency:
- Sample from the front and back of every load

Testing:
- Bactocan (BC)
- Thermophilic Sporeformer (TSC)
- Preliminary Incubation (PI)

Tanker Surface

Efficacy of cleaning

Frequency:
- Before and after each treatment

Testing:
- Sponge swabs (APC)
- ATP Swab (post cleaning treatment only)

Sampling Procedure

- Tanker samples taken from the front and back compartment
- Samples will need to be properly labeled and isolated for shipment
- A copy of the BOL to be included in the sample shipment

Appendix 4 (Continued): Plant Training Materials from Short Distance Study (Chapter 3)

Samples Prior to First Delivery

➤These samples will act as the baseline to determine micro growth over study

➤These areas should be swabbed following COP/CIP
- Tanker Roof
  - Both front and back trailer of both trucks

➤Water sample should be taken from source of rinse water for tanker cleanings

Sample Collection: Impact to Plant

Cleaning Variable 1: Standard Procedure – No rinse and 24 hr CIP
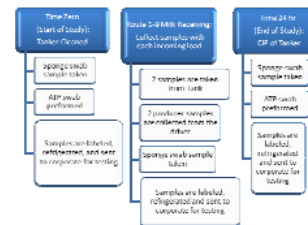
Sample Collection: Impact to Plant

Cleaning Variable 2: Water Rinse
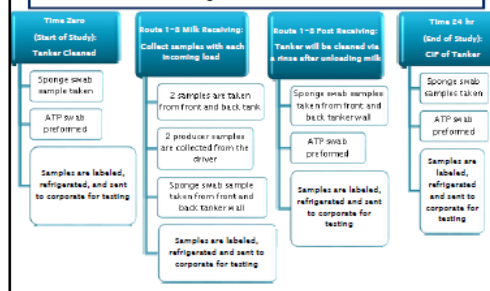
Sample Collection: Impact to Plant

Cleaning Variable 3: Rinse after each load and 12 hr Sanitizer Treatment
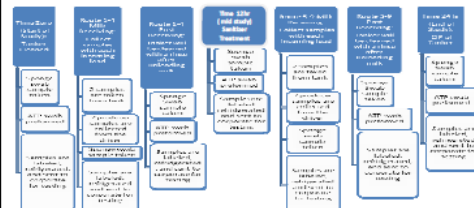
Sample Collection: Impact to Plant

Cleaning Variable 4: No Rinse and 12 hr Sanitizing Treatment
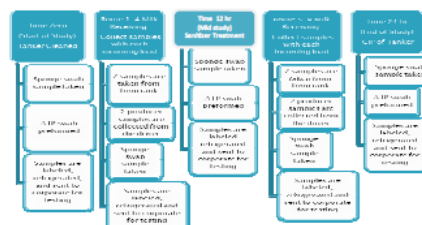
Swabbing Technique

▸ 3M Sponge Swab Stick w/ 10 ml Letheen Broth
http://www.youtube.com/watch?v=KBCD5Heidd0

- Sample should be pre-labeled and gloves put on prior to opening

- Area swabbed should be 12" x 12" and done in both directions and with consistent pressure taking care to avoid contamination of the sample.

- Once collected sponge should be cleanly broken off the stick and sealed for shipment to the lab

Appendix 4 (Continued): Plant Training Materials from Short Distance Study (Chapter 3)

Tanker Swabbing Areas

12 am/ 12 pm Swab Area

9 am/ 9 pm Swab Area

Hatch

3 am/ 3pm Swab Area

6 am/ 6 pm Swab Area
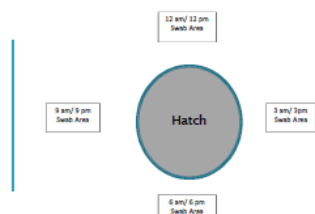
Note: The area of the tanker to be swabbed should be different each time. The area swabbed should relate to the time the sample is taken pretending that the tanker hatch is a clock. Samples before and after clean treatment can be from same area. All tanker samples are swabbed on the ceiling of the tanker.

ATP Swabbing Technique

➢ ATP swabs
http://www.youtube.com/watch?v=ZAkn4q-iFE0
- ATP Swabs are to be used after cleaning treatments only
- ATP Swabs should be taken in different area or before sponge swabs
- Area swabbed should be 4"x 4" and done while rotating with consistent pressure avoiding contamination of the sample
- Insert sample into the luminometer
- Once collected record ATP reading on data sheet– No sample is shipped

Microbiological Test Methods

▸ Testing methodology is designed to provide critical data while minimizing impact at the plant
▸ Microorganisms will not be identified
▸ Finished product shelf life studies will not be conducted

Methods used within this study:

- Bactoscan
- Petrifilm (APC)
- Sporeforming Count
- Preliminary Incubation (PI)

Aerobic Plate Count (APC)/Bactoscan(IBC)

▸ Used to determine the bacterial load of the tanker milk, producer samples, tanker wall, transfer pumps, receiving hose, and rinse water
▸ An increase in the total bacteria count of the tanker sample in comparison to the producer sample will be indicative of dirty equipment or abuse in the transportation of milk
▸ Bactoscan can be used for the raw milk samples
▸ Petrifilm will be used for the water, tanker wall, pump and hose samples

- Supplies: Bactoscan/ 3M petrifilm
- Raw Milk Dilution: N/A
- Environmental Sample Dilution: (1:10/1:100– Clean & 1:100/1:1000– Dirty)
- Water Sample: (Direct)
- Incubation conditions: 32°C for 48 hrs for Petrifilm

Sporeformer Count

▸ Sporeforming bacteria can survive heat treatment and thrive in the processing environment

▸ Sporeforming thermophiles are of concern for powder plants

- Supplies: SMA Pour Plates
- Raw Milk Dilution: 1:10
- Pre–Plating Treatment: Heat sample to 80°C for 12 min in water bath
- Incubation conditions: 55°C for 48 hrs

Preliminary Incubation (PI)

▸ Indicator of contamination or issues with transportation
▸ PI value is compared to the total bacteria count number

- Supplies: Tempo Reader (MPN)
- Incubation conditions: 13°C for 18hr and 32°C for 48 hr

Appendix 4 (Continued): Plant Training Materials from Short Distance Study (Chapter 3)

Sample Collection: Impact on Lab

▸ Each day the following samples and tests will be run at the corporate lab :

- 106-108 samples of milk (with duplicates)
  - 1 water sample
- 56-86 sponge swabs

Sample Labeling: Milk

▸ Producer Samples: barcode on the container and BOL # sticker on bottle
- Producer samples will be correlated to each BOL
- Driver should record BOL# and Time on each sample via a sticker
- Paper BOLs will be included in the shipment after scanning
- Duplicate producer samples should be taken

▸ Tanker Samples: Label on container
- 4 tanker samples per BOL
  - 2 Front & 2 Back (duplicates)

BOL #
FRONT or BACK
Time
Date
Sample 1 or 2
Milk Tank Sample

Sample Labeling

▸ Tanker Swab: Sticker on bag
- 2-4 samples per BOL

BOL #
FRONT or BACK
Time
Date
Pre or Post Clean
Tanker Swab Sample

▸ Water: labeled container
- 1 Sample per day

Time
Date
Water Sample

In Summary....

▸ Partnership is key to ensure a successful study and minimize impact to operations

▸ Results from this study will provide valuable information to optimize cleaning procedures

Thank you !

Appendix 5 : Lab training materials from short and long distance studies (Chapter 3 & 4)

Study Lab and Testing
Procedures
November 2013 & August 2014

Outline of Tasks

1. Sample Sorting
2. Data Entry
3. Sample resorting
4. Test tube/ dilution buffer labeling
5. Sample testing
6. Plate Reading

Sample Sorting

• Samples will arrive ~8 am and all our
  samples should be pulled by lab

• Each shipment should have:
  • Collection Document Outlines (2 sheets)
  • Environmental Collection Sheet (1 sheet)
  • BOLs (16 sheets)
  • Plant data sheet (1 sheet)

• Use documents to help sort and group
  samples ensure all pages are put into data
  folder

BOL

• One BOL for each load of
  milk (16 per shipment)
• Between 3- 6 different
  coded milk samples will be
  for each BOL
• There are duplicates for
  each milk samples
• 2- 4 swabs will be with
  each BOL
• Contains Producer no.,
  time, date, weight,
  location, truck number

Plant Collection Documents

Collection Document Outlines (2 sheets)
• Outlines cleaning treatments on each
  truck
• Outlines number of swab samples per BOL

Environmental Sampling Outlines (1 sheet)
• Outline of all environmental swab samples
  taken
• Guide for times and number of swabs that
  should be in shipment
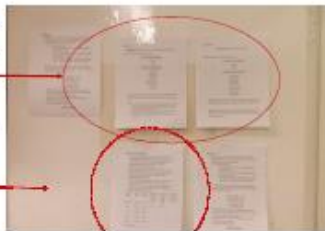
Plant Data Sheet

Appendix 5 (Continued): Lab training materials from short and long distance studies (Chapter 3 & 4)

Instructions for ordering samples: BOL/Milk

- Order paper BOL by increasing numerical order (506602, 506603,554531....)
- Separate all milk samples and sort by BOL number order on racks
  - Group duplicates
  - Sort producers by order on BOL
  - Sort tanker sample by front then back
- Once sorted on racks write increasing numerical code on and lab No on the samples starting with the producer of the lowest number BOL
- Ensure all required data is captured on spreadsheet: (DCL # ,Sample #, Producer #, BOL #, Date , Time, sample type, pre/post treatment, and location)

Producer and Tanker Sample

Instructions for ordering samples: Swabs-Data Entry

- Group by BOL in the following order:
  - front pre (dirty)
  - front post (clean)
  - back pre
  - back post

Instructions for ordering samples: Swabs-Data Entry and Sorting

- Once all swabs are entered write sample number on bag with sharpie keeping same sample entry order
- After labeling the bags and entering all data in spread sheet sort swabs by clean and dirty keeping increasing numerical order within subset
- Separate dirty (pre) and clean (post) swabs into different containers for proper dilution and plating

Swab sample preparation

- Squeeze sponge in bag to release broth, dispose of sponge then pipet required amount for dilution

Appendix 5 (Continued): Lab training materials from short and long distance studies (Chapter 3 & 4)

Swab Analysis

- Each swab has 10 ml of a broth
  - A direct plate of the swab is the same as a 1:10 dilution
  - Plate on petri film labeled with date, sample number, and dilution
  - Separate plates and stack no more than 25 label with date plated and date to pull
- Dilutions with butterfield broth:
  - **1:10 & 1:100- Clean (POST)**
  - **1:100 & 1:1000- Dirty (PRE)**
    - 1:10 – 1 ml direct
    - 1:100 – .1 ml w/ .9 ml buffer
    - 1:1000 – 1 ml w/ 99 ml buffer -> 1 ml plate
- Incubation conditions: 32°C for 48 hrs

Water

- Enter water last on the data sheet
- Plate directly onto petrifilm

Petrifilm results

- Petrifilm is used using reader
- Sample number is entered into system for reading
- Read samples in increasing numerical order and dilution

Milk Analysis

- The following tests need to be run:
  - Thermophilic Sporeformer
    - We conduct preparation and plating
    - We read results and record results
    - Lab provides prepared agar
  - Preliminary Incubation (PI)
    - We conduct preparation. Lab analyzes samples with TEMPO
    - Results after 4 days- print out is given
  - Bactoscan
    - We conduct preparation. lab analyzes
    - Results are instant and a printout is given
- Once results are given place in the folder from the date the samples arrived

Milk Sample Processing

- **Once samples are sorted lay out numbered test tubes for milk :**

- Put 5 ml of each sample (not duplicates) into a test tube for Sporeforming heat treatment (always use sample 1 unless damaged)
- Put make dilution for tempo (PI) and give to lab contact (including duplicates)
- Give remaining sample in sample container to lab contact for bactoscan (including duplicates)

Sporeformer Count

- 1:10 dilution by plating 100 ul
- Label lid with sample number
- No duplicates
- Include one control with each bottle of agar used
- Tape stacked together plates in incubator
- Label tape with OSU date in and date out

- Supplies: SMA Pour Plates
- Raw Milk Dilution: 1:10
- Pre-Plating Treatment: Heat sample to 80°C for 12 min in water bath
- Incubation conditions: 55°C for 48 hrs

Appendix 5 (Continued): Lab training materials from short and long distance studies (Chapter 3 & 4)

Appendix 6 : Short duration hauling (Chapter 3) normality plots and statistical data. Winter Study (Plant A)

a) Bactoscan

Normal Q-Q Plot


```
Fixed effects: log(No.inc) ~ Treatment + Truck + Bacto.pro
                                  Value Std.Error  DF  t-value p-value
(Intercept)                    7.916361 0.1473226 222 53.73488  0.0000
TreatmentSanitize12Hr          0.069246 0.1201320 222  0.57642  0.5649
TreatmentWaterRinse           -0.112091 0.1185226 222 -0.94573  0.3453
TreatmentWaterRinseSanitize12Hr 0.121567 0.1199265 222  1.01368  0.3118
Truck69                        0.420909 0.1331034  15  3.16227  0.0064
Bacto.pro                      0.000027 0.0000024 222 11.39487  0.0000
```

Appendix 6 (Continued): Short duration hauling (Chapter 3) normality plots and statistical data. Winter Study (Plant A)

b) Spore

Normal Q-Q Plot
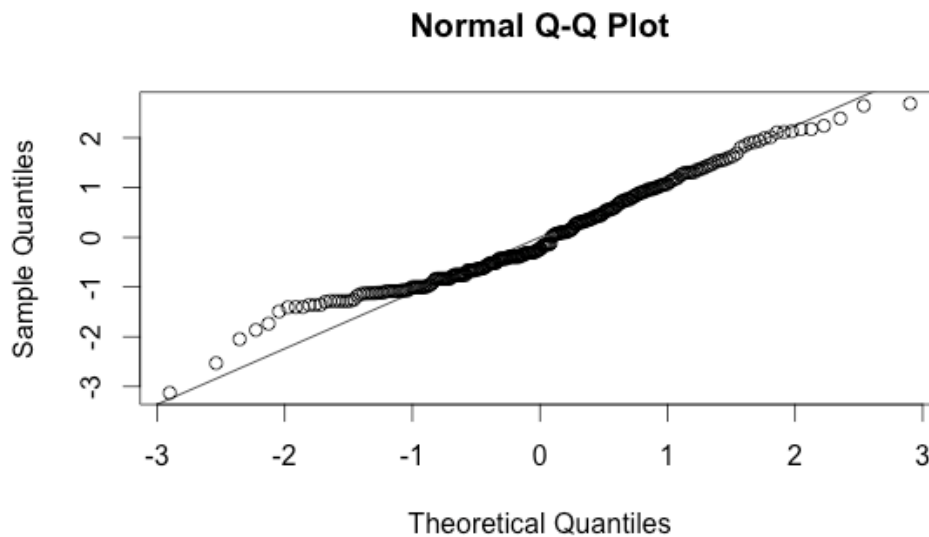
	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.8392609	0.20430495	231	9.002527	0.0000
TreatmentSanitize12Hr	0.3848151	0.25364645	231	1.517132	0.1306
TreatmentWaterRinse	-0.5081353	0.24918077	231	-2.039223	0.0426
TreatmentWaterRinseSanitize12Hr	0.0817376	0.2461679	231	0.332040	0.7402
Truck69	0.5023921	0.17527928	15	2.866238	0.0118
Spore.pro	0.0107760	0.00150324	231	7.168511	0.0000

Appendix 6 (Continued): Short duration hauling (Chapter 3) normality plots and statistical data.
Winter Study (Plant A)

c) PI

Normal Q-Q Plot
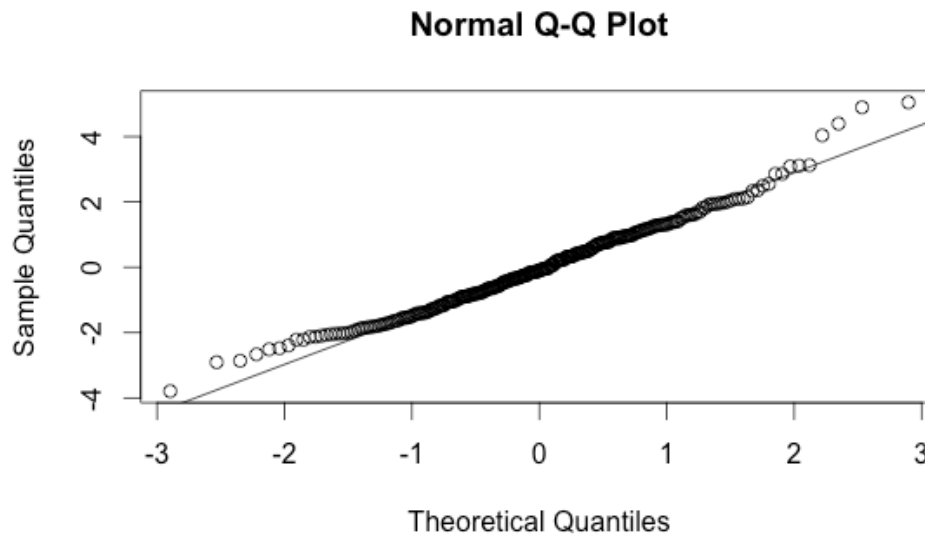
	Value	Std.Error	DF	t-value	p-value
(Intercept)	7.732795	0.3452272	227	22.399146	0.0000
TreatmentSanitize12Hr	0.378229	0.2611312	227	1.448425	0.1489
TreatmentWaterRinse	-0.112709	0.2585698	227	-0.435895	0.6633
TreatmentWaterRinseSanitize12Hr	0.275569	0.2574897	227	1.070213	0.2857
Truck69	0.985073	0.2234377	15	4.408715	0.0005
PI.pro	0.000006	0.0000013	227	4.738891	0.0000

Appendix 7 *: Short duration hauling (Chapter 3) normality plots and statistical data. Summer Study (Plant B)*

    a) Bactoscan

Normal Q-Q Plot
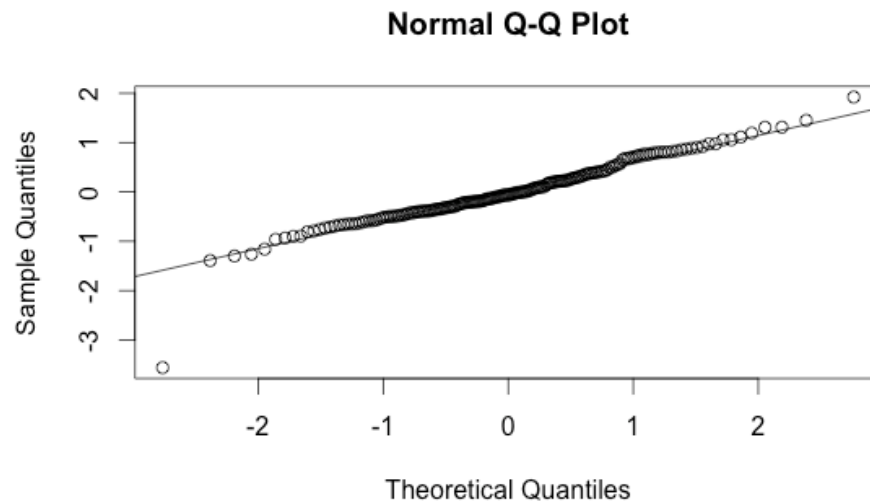

```
Fixed effects: log(BactoInt) ~ Treatment + Truck + Bacto.pre
                           Value   Std.Error  DF   t-value  p-value
(Intercept)             8.197488 0.12403491 141 66.09017   0.0000
TreatmentRinse         -0.086643 0.14089446 141 -0.61495   0.5396
TreatmentRinse/Sanitize -0.160976 0.13280923 141 -1.21209   0.2275
TreatmentSanitize       0.024225 0.13995948 141  0.17308   0.8628
Truck3614              -0.022624 0.10221995  13 -0.22132   0.8283
Bacto.pre               0.000036 0.00000347 141 10.28341   0.0000
```

Appendix 7 (Continued)*: Short duration hauling (Chapter 3) normality plots and statistical data. Summer Study (Plant B)*

b) Spore

Normal Q-Q Plot
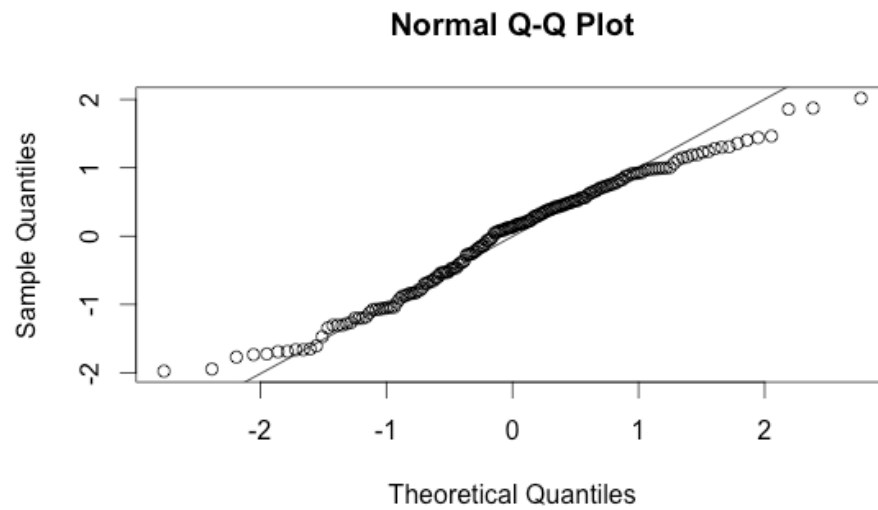
```
Fixed effects: log(Spore) ~ Treatment + Truck + Spore.pre
                           Value    Std.Error   DF    t-value  p-value
(Intercept)              3.0596178 0.15971789 141 19.156387  0.0000
TreatmentRinse           0.1748887 0.19490563 141  0.897299  0.3711
TreatmentRinse/Sanitize  0.0003181 0.18354144 141  0.001733  0.9986
TreatmentSanitize       -0.2629891 0.19533057 141 -1.346380  0.1803
Truck3614                0.0368233 0.14074663  13  0.261628  0.7977
Spore.pre                0.0080823 0.00139385 141  5.798564  0.0000
```

Appendix 7 (Continued)*: Short duration hauling (Chapter 3) normality plots and statistical data. Summer Study (Plant B)*

**c) PI**

Normal Q-Q Plot
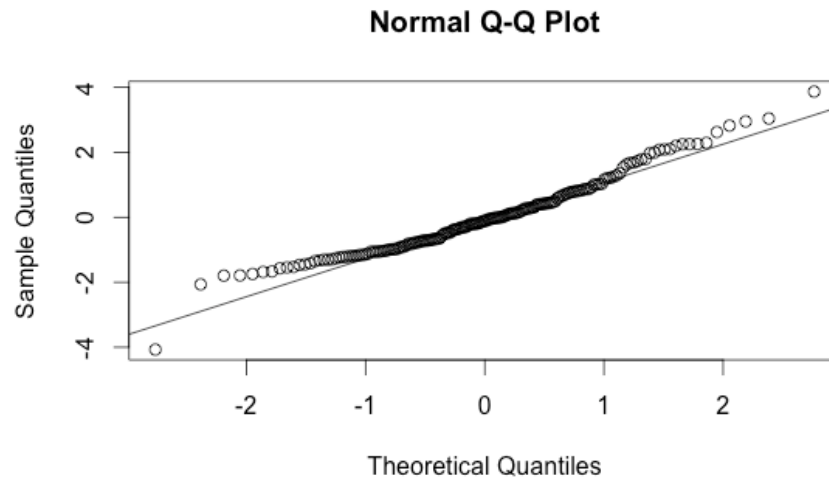

```
Fixed effects: log(PI) ~ Treatment + Truck + PI.Pre
                              Value   Std.Error  DF  t-value p-value
(Intercept)               7.692741 0.21935058 141 35.07053  0.0000
TreatmentRinse            0.359588 0.26084108 141  1.37857  0.1702
TreatmentRinse/Sanitize  -0.057320 0.24542402 141 -0.23355  0.8157
TreatmentSanitize         0.005983 0.26094334 141  0.02293  0.9817
Truck3614                -0.387775 0.19383741  13 -2.00052  0.0668
PI.Pre                    0.000035 0.00000756 141  4.64301  0.0000
```

Appendix 8 : Long duration hauling (Chapter 4) normality plots. Plotted as the difference between tanker and producer samples in both treatments (All Data)

a. Bactoscan
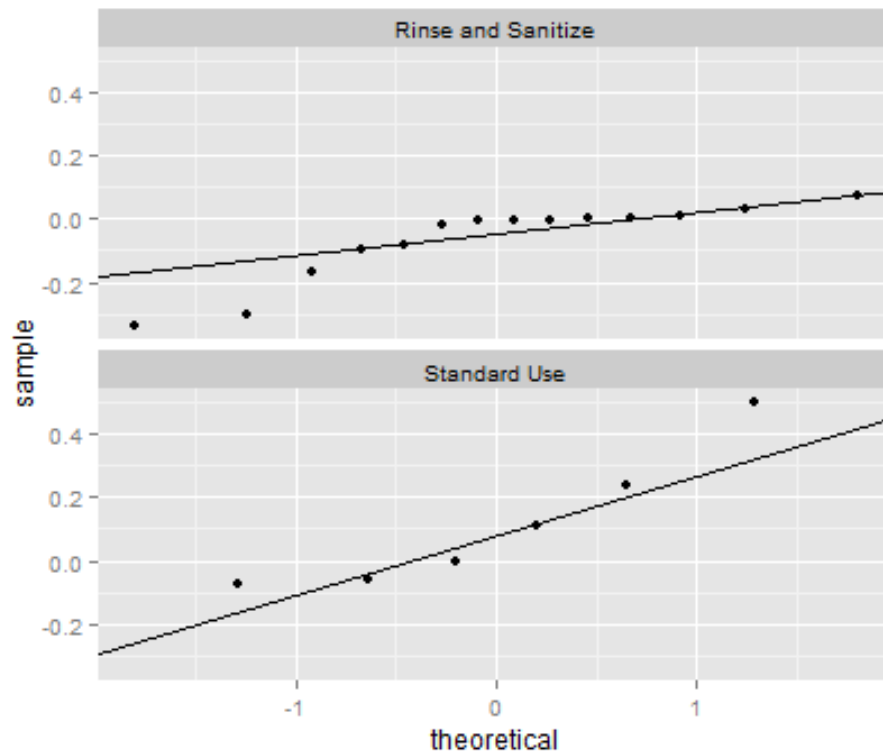
Appendix 8 (Continued): Long duration hauling (Chapter 4) normality plots. Plotted as the difference between tanker and producer samples in both treatments (All Data)

b. Spore

Appendix 8 (Continued): Long duration hauling (Chapter 4) normality plots. Plotted as the difference between tanker and producer samples in both treatments (All Data)

c. PI
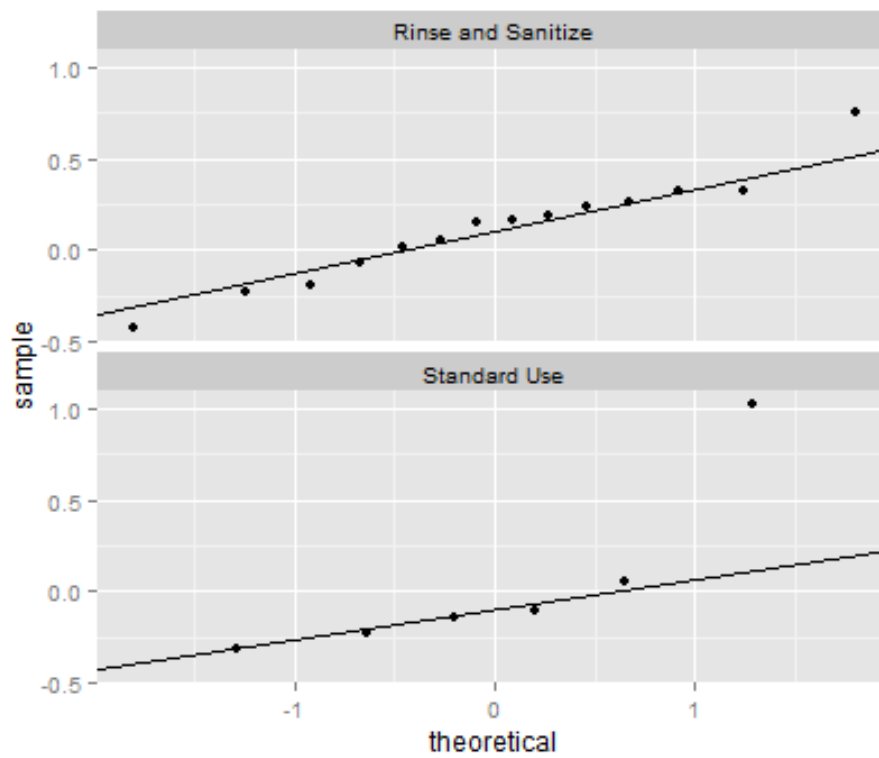
Appendix 9 Statistical data for Long duration hauling practices (Chapter 4) impact on microbial counts (IBC, Spore and PI) over time. Load 1 and Load 2 microbiological comparison. No significant difference was found between load 1 and 2 within a treatment. RS= Rinse and Sanitize, SU= Standard Use

IBC Data

```
Paired t-test

data:  IBC.SU.1 and IBC.SU.2
t = -0.6166, df = 2, p-value = 0.6003
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.9479977  0.7103540
sample estimates:mean of the differences
            -0.1188219



        Paired t-test

data:  IBC.RS.1 and IBC.RS.2
t = 1.4601, df = 6, p-value = 0.1945
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.05782436  0.22894987
sample estimates:mean of the differences
            0.08556275
```
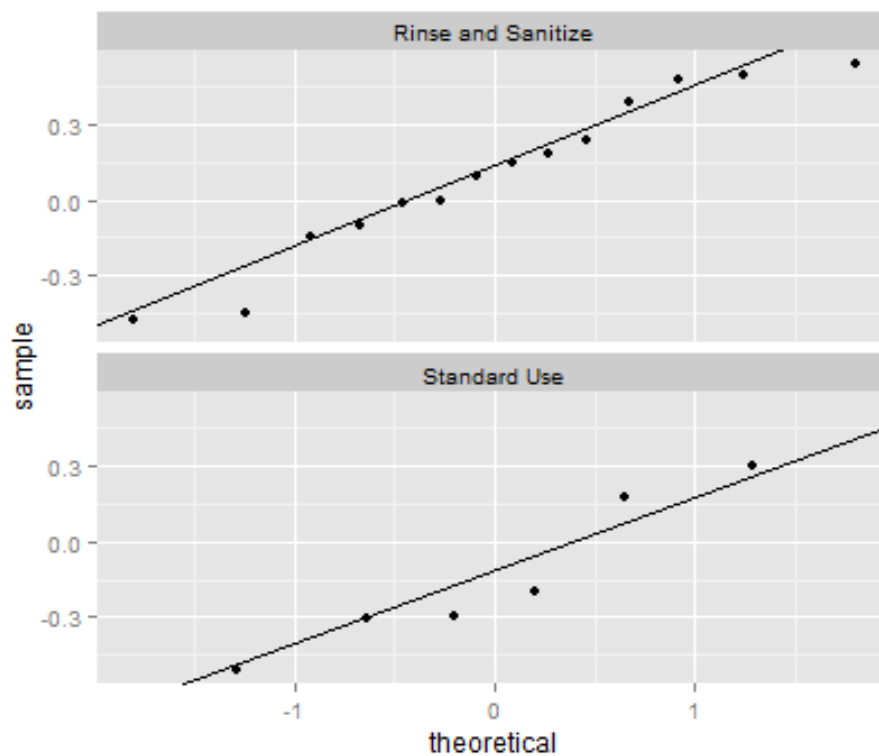
Spore Data

```
Paired t-test

data:  Spore.SU.1 and Spore.SU.2
t = -0.7825, df = 2, p-value = 0.5158
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.4917133  0.3403809
sample estimates:
mean of the differences
            -0.07566624

Paired t-test

        data:  Spore.RS.1 and Spore.RS.2
t = 1.3354, df = 6, p-value = 0.2302
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.1673738  0.5695359
sample estimates:mean of the differences
             0.2010811
```

Appendix 9 (Continued): Statistical data for Long duration hauling practices (Chapter 4) impact on microbial counts (IBC, Spore and PI) over time.

 PI Data

```
Paired t-test

data:  PI.SU.1 and PI.SU.2
t = -0.6314, df = 2, p-value = 0.5923
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -2.188218  1.628160
sample estimates:mean of the differences
             -0.2800289




        Paired t-test

data:  PI.RS.1 and PI.RS.2
t = 0.6306, df = 6, p-value = 0.5516
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.3030890  0.5135379
sample estimates:mean of the differences
              0.1052244
```

Appendix 10: Statistical data for cleaning treatments (Chapter 4) impact on microbial counts (IBC, Spore and PI). Demonstrates if there is a difference in tanker bacteria growth based on cleaning treatment by comparing load 2 across treatments. No significant treatment effect found when comparing averaged RS and SU data. Rinse= Rinse and Sanitize (RS), Control= Standard Use (SU)

IBC

```
        Welch Two Sample t-test

data:  Control.Bacto and Rinse.Bacto
t = 1.5782, df = 2.548, p-value = 0.2283
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.3514449  0.9205803
sample estimates:
 mean of x  mean of y
 0.1770694 -0.1074983
```

Spore

```
        Welch Two Sample t-test

data:  Control.Spore and Rinse.Spore
t = -0.4009, df = 4.413, p-value = 0.7071
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.7776132  0.5750211
sample estimates:
    mean of x     mean of y
-0.103298126 -0.002002052
```

PI

```
        Welch Two Sample t-test

data:  Control.PI and Rinse.PI
t = 0.2923, df = 2.531, p-value = 0.7923
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -1.451732  1.712744
sample estimates:
 mean of x  mean of y
0.18745666 0.05695083
```

Appendix 11 : Statistical data for comparing sampling location's (Chapter 4) impact on microbial counts (IBC, Spore and PI). Demonstrates if there is a difference in tanker bacteria growth based by comparing log transformed results of milk taken the front and back tank. Significant location difference found in RS treatment for IBC and PI. Although statistically significant practical relevance for industry is unknown. Rinse= Rinse and Sanitize (RS), Control= Standard Use (SU) Bacto= IBC

```
Welch Two Sample t-test

data:  Back.Control.Bacto and Front.Control.Bacto
t = 0.3562, df = 8.36, p-value = 0.7305
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.2909884  0.3982653
sample estimates:
mean of x mean of y
 3.712967  3.659329


        Welch Two Sample t-test

data:  Back.RS.Bacto and Front.RS.Bacto
t = 2.6981, df = 21.567, p-value = 0.01328
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 0.06406026 0.49189716
sample estimates:
mean of x mean of y
 3.825204  3.547225


        Welch Two Sample t-test

data:  Back.Control.Spore and Front.Control.Spore
t = -0.287, df = 9.737, p-value = 0.7802
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.5831990  0.4505523
sample estimates:
mean of x mean of y
 1.209212  1.275535

        Welch Two Sample t-test

data:  Back.RS.Spore and Front.RS.Spore
t = -0.3213, df = 20.477, p-value = 0.7512
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.3341031  0.2447911
sample estimates:mean of x mean of y
 1.350468  1.395124
```

Appendix 11 (Continued): Statistical data for comparing sampling location's (Chapter 4) impact on microbial counts (IBC, Spore and PI).

```
Welch Two Sample t-test

data:  Back.Control.PI and Front.Control.PI
t = -0.8362, df = 8.662, p-value = 0.4255
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.6663702  0.3082570
sample estimates:
mean of x mean of y
 3.786275  3.965331



        Welch Two Sample t-test

data:  Back.RS.PI and Front.RS.PI
t = 2.0928, df = 23.053, p-value = 0.04757
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 0.003368085 0.574518286
sample estimates:
mean of x mean of y
 4.177677  3.888734
```

Appendix 12 Statistical data comparing sampling location's (Chapter 4) impact on tanker sanitation and efficacy based on sponge swabs APC results. a) Comparison of pre-treatment APC counts based on sampling location within a treatment and combined location comparison across treatment (log cfu/900 cm^2). No significant difference in pre-treatment tanker sanitation found in either tanker location nor between treatments. b) Comparison of post-treatment APC counts based on sampling location (log cfu/900 cm^2) . Significant difference fount in RS but not SU counts. Although statistically significant practical relevance for industry is unknown. c) Comparison between pre and post treatment APC counts (combined front and back location) for RS treatment. Significant difference found showing treatment was effective. d) Comparison between post RS and CIP swab APC counts. Significant difference showing that RS was not as effective as CIP in reducing surface counts (log cfu/900 cm^2).
Key : Rinse= Rinse and Sanitize (RS), Control= Standard Use (SU) , CIP= Post 24 h Clean in Place treatment

a)

```
        Welch Two Sample t-test

data:  Back.SU.Swab and Front.SU.Swab
t = -1.4963, df = 9.942, p-value = 0.1656
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -1.0223846  0.2012977
sample estimates:mean of x mean of y
 3.533911  3.944455

Welch Two Sample t-test

data:  Back.RS.Swab and Front.RS.Swab
t = -1.5345, df = 19.591, p-value = 0.1409
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.7908250  0.1209674
sample estimates:mean of x mean of y
 3.840846  4.175775


Welch Two Sample t-test

data:  Pre.All.SU.Swab and Pre.All.RS.Swab
t = -1.4715, df = 24.503, p-value = 0.1539
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.6461798  0.1079252
sample estimates:mean of x mean of y
 3.739183  4.008310
```

Appendix 12 (Continued) Statistical data comparing sampling location's (Chapter 4) impact on tanker sanitation and efficacy based on sponge swabs APC results

b)

```
Welch Two Sample t-test

data:  Post.Back.CIP.Swab and Post.Front.CIP.Swab
t = -1.0885, df = 18, p-value = 0.2908
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -1.2583131  0.3994538
sample estimates:
mean of x mean of y
 1.186407  1.615836


Welch Two Sample t-test

data:  Post.Back.RS.Swab and Post.Front.RS.Swab
t = -3.0743, df = 11.789, p-value = 0.009821
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -2.6840396 -0.4549649
sample estimates:
mean of x mean of y
 1.890168  3.459670
```

c)

```
        Welch Two Sample t-test

data:  Post.All.RS.Swab and Pre.All.RS.Swab
t = -3.8488, df = 16.096, p-value = 0.001405
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -2.067467 -0.599315
sample estimates:
mean of x mean of y
 2.674919  4.008310
```

d)

```
        Welch Two Sample t-test

data:  Post.All.RS.Swab and Post.CIP.All.Swab
t = 3.3245, df = 22.209, p-value = 0.00305
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 0.4796113 2.0679841
sample estimates:
mean of x mean of y
 2.674919  1.401121
```

Appendix 13 Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts and b) Thermophilic Spore Counts and c) Bactoscan specification ranges. Procedures taken from corporate laboratory SOP.

a)

Scope

This method is intended for the determination of the Most Probable Number (MPN) in PI samples.

Definition

Most Probable Number (MPN): The MPN method is an enumeration technique which allows any microorganisms present in the sample(s), to grow in suitable conditions in tubes, using a minimum of three dilutions and three tubes per dilution. When the combination of positive results (growth) is known a mathematical formula can be used to estimate the most probable number of microorganisms per milliliter or per gram of sample.

Principles

This method involves the following steps:
1)   Preparation a 1/100 dilution of the sample in phosphate buffer dilution blank (final dilution volume = 100 ml).
2)   Addition of 1/100 dilution into a TEMPO Total Viable Count (TVC) vial for a final dilution of 1/4000 (final dilution volume in vial = 4 ml).
3)   Attachment of a TEMPO TVC card corresponding to the initial sample containing the 1/4000 dilution via TEMPO Filler.
4)   Incubation of cards at 13°C for 18h.
5)   Incubation of cards at 32°C for 48h.
6)   Reading and validation of cards via TEMPO Reader.

Safety Considerations

 A.   MPN is a non-selective enumerative method. As this method is non-selective, one has to assume that pathogens or opportunistic pathogens can be present. All steps in the procedure can be performed in the main microbiology laboratory. Good Laboratory Practice (GLP) must be maintained during all steps in the procedure. The site bio-safety plan should be referred to for appropriate control of microbiological waste.
B.   Appropriate PPE should be worn. For hazards specific to reagents and chemicals refer to the relevant Material Safety Data Sheets (MSDS).

C.   During the use of the TEMPO Filler and TEMPO Reader, all corresponding racks should be placed in the correct orientation as to minimize equipment errors and

Appendix 14 Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts and b) Thermophilic Spore Counts and c) Bactoscan specification ranges.

reduce safety risk. Be sure to keep filled cards vertical and do not drop the cards prior to reading.
D. When autoclaving TEMPO water dispensers/bottles be sure to loosen pump/cap from the bottle so as to release the pressure formed inside of the bottle which may result in equipment malfunction and personal safety risk.

 Chemicals/Media/Reagents

A.   Dilution Blanks – commercially available ready-to-use plastic single service dilution blanks.
B.   TEMPO TVC Kit – commercially available TVC media and card kits from Biomérieux used to conduct MPN method.
a.   Reagents are light and temperature sensitive and should be stored in a cool, dark area; may be stored at room temperature for up to one week.
b.   TEMPO TVC tests should not be performed past the expiration date indicated on the label.
C.   Ethyl alcohol: 70%
D.   Distilled Water

Equipment

A.   Autoclave: Capable of raising to temperature within 15 minutes (preferably within 5 minutes) of starting air exhaust and capable of maintaining 120° ± 1°C.
B.   Balance: Sensitive to at least 0.1 g
C.   Incubators: 32° ± 1°C and 13° ± 1°C
D.   Refrigerator: Range capable from 0° to 4.4°C
E.   TEMPO Preparation Station & TEMPO Reading Station consisting of:
a.   Central processing unit
b.   Monitor
c.   Mini-keyboard
d.   Bar code reader
e.   TEMPO Filler and TEMPO Reader
f.   Vortex
g.   TEMPO Water Dispenser and corresponding 1 L water bottles
h.   Manual or electronic pipettors (1 ml and 100 µl) that conform to APHA standards
i.   Filling and Incubation/Reading racks

Appendix 15 (Continued) Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts b) Thermophilic Spore Counts and c) Bactoscan specification ranges

Method

Preparation of Test Sample
1.   Raw liquid milk products;
a.   Maintain samples and dilutions at 0° to 4.4°C with refrigeration from collection to completion of analysis
b.   Begin analysis within 48 hours of collecting the sample(s).
c.   Unless otherwise specified, mix liquid samples by shaking 25 times in 7 seconds with a 1-foot movement.
d.   The interval between the completion of mixing and removing the test portion must not exceed 3 minutes.
e.   Before opening a sample container, remove all obvious materials that may cause contaminate the sample from the closure. If necessary, wipe the tops of unopened sample containers with a sterile cloth/paper towel saturated with 70% ethyl alcohol.

Preparation of Required Equipment
1.   TEMPO water dispenser
a.   Autoclave water dispensers for every two to three days of use.
i.   Make sure TEMPO water dispensers or caps are loosened from the water bottles and tubes are separated from the pump component.
b.   Control plating – to be done each time a new TEMPO water dispenser is used.
i.   Prepare a Standard Methods Agar plate by dispensing 2-3 ml of water onto the plate.
ii.   Let sit for 5 minutes and drain excess liquid before incubating at 32° ± 1°C for 48 hours. Check for growth after the first 24 hours as well.
c.   Calibrate dispenser by making sure that 5 aliquots of water at 3.9 ml is between 18.6 g and 20.4 g, adjusting to make sure the 5 aliquots fall between this range if necessary.
2.   TEMPO Filler Station
a.   Make sure filler station is empty and the door is closed before powering on. Self-test will be run at power up (approx. 3 minutes) and once the green light is visible, use of the machine may begin.
b.   TEMPO preparation station CPU should always be on (automatic backup happens at 01:00 every day), but should be restarted once a week.
3.   TEMPO Reading Station
a.   Make sure reading station is empty and the door is closed before powering on. Self-test and lamp warm-up will be run at power up (approx. 15 minutes) and once the green light is visible, use of the machine may begin.

b.   TEMPO reading station CPU should always be on (automatic backup happens at 01:00 every day), but should be restarted once a week.

Appendix 13 (Continued) Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts and b) Thermophilic Spore Counts. c) Bactoscan specification ranges

 Procedure
1.   For each required dilution transfer, using both sterile pipettor and tip, pipette 1 ml sample into a 99 ml phosphate buffer dilution blank. If the initial suspension or a dilution (with the exception of oils) has been left stationary for greater than 3 minutes prior to preparation of further dilutions or inoculation of
Petri-dishes, mix well by shaking the container through an approximate 1-foot arc 25 times or equivalent.
2.   Open TEMPO prep program on TEMPO prep computer.
3.   Scan sample barcode into TEMPO preparation station CPU using scanner, making sure the barcode scans in correctly, and confirm sample creation (if needed). If the sample barcode cannot be read, manually enter it by selecting the keyboard icon to the left of the screen (hotkey: F8). If the sample barcode is incorrect, highlight the sample and delete by selecting the trash can icon to the left of the screen (hotkey: F7).
4.   Place TEMPO TVC media vial in the filling rack and dispense 3.9 ml distilled water from the calibrated TEMPO water dispenser into the vial prior to sample inoculation as to remove the risk of contaminating the dispenser and hydrate the media.
5.   Using both sterile pipettor and tip, pipette 0.1 ml of the corresponding sample dilution into the TEMPO TVC media vial with 3.9 ml water, and vortex for 5 seconds to mix.
6.   Attach TEMPO TVC card by scanning barcode, confirm the creation of the card to the corresponding sample, and place filling tube in the appropriate sample vial. Be sure the correct sample is highlighted by the green arrow. If card attachment is incorrect, highlight the card and delete by selecting the trash can (F7).
7.   Place filling rack into TEMPO filler when the light above the start button is green, close door, and press start. Remove once light has turned back to green and is blinking (approx. 3 minutes). After removal of filler rack, be sure to check and make sure any material that may be caught in the filling station is removed prior to the next round.
8.   Double check to make sure TEMPO TVC card is filled and place in a reading rack. (Note: Slot 1 is in the back of the tray while slot 20 is the front.) Be sure cards are upright and barcodes are facing front.
9.   Store at 0° to 4.4°C until ready to incubate at 13° ± 1°C for 18 hours.
10.   Move from 13°C incubator to 32° ± 1°C incubator for 48 hours.
11.   Open TEMPO read program on TEMPO read computer and press F3 to access main page.
12.   Remove reading rack from 32° incubator and place in TEMPO reading station once the green light is solid above the start button, close door, and press start.

Appendix 13 (Continued) Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts and b) Thermophilic Spore Counts. c) Bactoscan specification ranges

13.   Monitor reading progress on computer screen of TEMPO reading station and re-read any cards that could not be read after the first try. Refer to the TEMPO Reading Station manual for trouble-shooting tips and information on any errors that may occur.
14.   Remove cards from the TEMPO reading station and dispose in a nearby hazardous waste container.
15.   To validate cards press F5 and press on the filter button on the right side of the screen. Select a time-frame that encompasses all TEMPO TVC tests to be validated by selecting the appropriate dates and times. Be sure to select the circles next to 'tests with results' and 'tests awaiting validation' and select 'ok' at the bottom of the window. Make sure the box to the left of all the samples is highlighted with a green checkmark (press 'select all') and click on the green checkmark icon on the left side of the screen.

References

1.   TEMPO Preparation Station User's Manual Version B. Biomérieux. Revised 2004.
2.   TEMPO Reading Station User's Manual Version B. Biomérieux. Revised 2004.

Appendix 16 (Continued) Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts b) Thermophilic Spore Counts and c) Bactoscan specification ranges

b)

Scope

This method details the proper procedure for the quantification of spore formers for dairy products.

Definition

Bacteria that create spores are called spore formers. A spore is a refractile, oval body formed within bacteria, and is characterized by its resistance to environmental changes, specifically heat.

II.   **Equipment**
F.   Incubators: 55° ± 1°C
G.   Pipets or pipettors that conform to APHA standards
H.   Water baths: 80°C
I.   Ice Bath

Procedure
- Pipette 5mL of sample into empty, autoclaved 10 mL test tube
- For test code 72 (SMEDP) place test tube rack in an 80°C water bath for 12 minutes
- Place the test tubes in an ice water bath for 10 minutes
- Aseptically pipette sample into sterile petri dish
- Pour 15mL of the appropriate agar into each petri dish and swirl the petri dish until it is uniformly mixed. Use standard method agar. Let media solidify.

References

Wehr M. H., Frank J. F. *Standard Methods For the Examination of Dairy Products 17th edition*. 2004. American Public Health Association. Washington DC pgs 239-242.

Appendix 13 (Continued) Microbiological test procedures for a) Preliminary Incubation Most Probable Number Counts b) Thermophilic Spore Counts and c) Bactoscan specification ranges

Specifications

Performance

Repeatability*:

Range (IBC/µl)	S_r (log-units)	Typical S_r (log units)
10 – 50	0.07	0.06
51-200	0.05	0.04
>200	0.04	0.02
Entire range	0.05	

Reproducibility* (between instruments):

Range (IBC/µl)	S_R (log-units)	Typical S_R (log units)
10 – 50	0.11	0.08
51 –200	0.07	0.06
>200	0.06	0.04

Carry-over effect:	< 0.5 % (uncompensated)
Working factor:	Standard 300, (optionally: 95, 600 and 1200)
Accuracy:	Typical Sy.x < 0.25 log units in the entire measuring range
Reference or anchor method:	Standard Plate Count (SPC) (IDF Standard100B:1991)

*For the performance on sheep and goat milk please refer to the Application Note 3511