A strategic approach to reducing mycoplasma testing costs

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ABSTRACT

Mycoplasma; it is not a household name for many Americans or people around the world, but for those in the livestock industry, it has been a major concern. *Mycoplasma*, a member of the class Mollicutes, has had and continues to have a major impact on the cattle, swine and poultry industry, causing conditions such as arthritis, otitis media, reduced growth rate and reduced egg production (Journal of Veterinary Internal Medicine 2011) (Okwara 2016). This class of bacteria is unlike other classes, as defined by the lack of a cell wall, and is considered by many to be the smallest self-replicating prokaryote (Jack Maniloff 1992). Due to its small size, it can reside within cells and even pass through some of the currently used sterilizing filters in the biological/pharmaceutical industry today (Pall Corporation n.d.). This creates a risk for *Mycoplasma* contamination for those facilities/research centers that use materials of animal origin, as *Mycoplasma* organisms have historically been a common contaminate of cell lines and laboratory cultures, affecting roughly 15-35% of cell cultures (Cara N. Wilder 2015). An added concern is the difficulty in treatment of infected animals once an infection is established. The Mollicutes class has been considered innately resistant to the antibiotic penicillin and other cephalosporins due to the lack of the cell wall (Jack Maniloff 1992).

Due to the clinical significance and risk factors surrounding the Mollicutes class, it is a current regulatory requirement to test materials of animal origin for the presence or absence of *Mycoplasma*. The specific criteria for the presence or absence of Mycoplasma test is dependent upon the country in which the product is intended to be sold. For the purposes of this study, the required method and products will be for those intended for sale

domestically in the United States, or countries accepting US methodologies. To test a material or product for the presence or absence of Mycoplasma according to the current USDA code of federal regulations (CFR), the method is not a rapid procedure or a simple traditional broth inoculation. The domestic method is a minimum 24 day test that requires complex broth and agar media for Mycoplasma recovery. The complex media requirement is due to the fact that Mycoplasma organisms have stringent nutritional requirements due to their simplified cell structure/genome, which often require materials of animal origin, such as serums for lipid supply/metabolism (Jack Maniloff 1992). The 24 day Mycoplasma test requires an initial inoculation into the aforementioned broth and agar media and then 4 subsequent subcultures from the broth media onto the agar media at specified time intervals. All of the broth and agar media plates are incubated at specific atmospheric conditions and temperature for the duration of the test. The initial inoculation and subcultures are all examined by a trained Microbiologist at specific time intervals to search for evidence of viable Mycoplasma growth. The examination by a trained Microbiologist/technician is a vital step as Mycoplasmas do not produce turbidity in media, such as in traditional bacterial growth, nor are they visible by traditional light microscopy (Farzaneh 2011). If a Mycoplasma contamination is found, a biological/pharmaceutical company can pay huge sums of money to investigate the cause of the contamination, initiate corrective action, decontaminate the facility and destroy impacted batches.

As evidenced by the above description, Mycoplasma testing places a large burden on a biological/pharmaceutical production facility or even research institutions. The complex media and labor cost for the 24 day test is extensive, which must be repeated for

each batch of new material received or produced. The cost skyrockets if any contamination event occurs or even appears to occur, as investigation and decontamination add cost due to delay of release or possible destruction.

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CHAPTER I: INTRODUCTION

Mollicutes, the class name of a unique group of microorganisms. Unique, in that, the bacteria have de-evolved and lost their cell wall and become dependent on other organisms for nutrition as parasitic or commensal organisms. The class of microorganisms has been shown to be one of the causative agents of pneumonia, arthritis, otitis media, reduced growth rate and reduced egg production in the cattle, swine and avian industry (Okwara 2016) (Journal of Veterinary Internal Medicine 2011). Studies have shown that the Mollicutes class, or from here referred to as Mycoplasma, has had a significant impact to the agriculture business in terms of medical cost and health of the animals. Infected animals can develop a variety of maladies leading to lower prices, reduced goods or even loss of animals. In the US, studies have estimated that the economic impact of Mycoplasma infection in the swine industry alone to be between $200 million to $1 billion every year (Boehringer Ingelheim 2017). In the poultry industry, *Mycoplasma* infection can produce on average of 15.7 fewer eggs per hen compared to uninfected (Okwara 2016). This is equivalent to an estimated loss of 127 million eggs and an estimated financial loss of approximately $125 million in 1984 (Okwara 2016). A more recent estimate in 1999 in North Carolina estimated losses between $500,000 and $750,000 over a six month period for that company (Okwara 2016). The list of known Mycoplasma species, and their parasitic impacts, continues to increase as researchers learn more about this class of prokaryotes.

For a better understanding of the organism and the significance to the agriculture industry, a historical review of the Mollicutes class would be beneficial. In the below, the information on the historical perspective on the Mollicutes class was found in Maniloff's

(1992) book on *Mycoplasmas: Molecular Biology and Pathogenesis* (Jack Maniloff 1992). Although Bove (1999) reports that the first successful cultivation of a Mycoplasma was reported by Nocard and Roux in 1898 (Bove 1999) a definition of whether mycoplasma was a bacteria or a virus seems to have not been settled for another half century. For example, they were considered to be viruses in the beginning and by the 1930s, when knowledge about viruses was more clearly defined, it became obvious that they were prokaryotes. Yet, this did not settle the argument about their nature. Between 1930s and 1940s, the definition of Mycoplasmas went back and forth between viruses to stable L forms of common bacteria (Jack Maniloff 1992). The L forms of bacteria are bacteria which have partially or entirely lost their cell walls. The controversy ended with the development of genomic analysis in the 1960s (Jack Maniloff 1992). Since then, many microbiologists have come to believe that Mycoplasmas originated from walled microorganisms and lost their cell wall through degenerative evolution. (Jack Maniloff 1992). These organisms are thought to be the smallest, self-replicating prokaryotes. They typically have strict nutritional requirements due to their simplified cell structure and obligate parasitic life cycle. Mycoplasma organisms infect tissues and cells and can be difficult to cultivate. The organisms can reside within cells and even pass through some of the sterilizing filters in use today, such as the 0.2 μm bacterial retentive filters (Pall Corporation n.d.). This presents a major problem for researchers and biotechnology companies in the field of biologics (vaccines and serums), as materials of animal origin are commonly used for research and the production of vaccines.

1.1 Domestic Requirements for Mycoplasma

Due to its prevalence in nature, within livestock populations, and in laboratory cell lines, it is a regulatory requirement in the U.S. and elsewhere to test materials of animal origin for Mycoplasma. For those biological products produced and sold within the United States, the material and testing requirements fall under the Code of Federal Regulations (CFR), specifically 9 CFR section 113.53, "Requirements for ingredients of animal origins used for production of biologics," and 9 CFR 113.28 "Detection of Mycoplasma contamination." In section 113.53, it states:

> "Samples of each lot of ingredient of animal origin, which is not subjected to heat sterilization, used to prepare a biological product shall be shown free of Mycoplasma by the method prescribed in 113.28"

For live and inactivated products (9 CFR 113.64 and 9 CFR 133.100), the sections refer back to 9 CFR 113.53, indicating that the final products are also shown to be free from Mycoplasma by the method 9 CFR 113.28. What this means for a company in the animal health business is the raw materials through finished products used and intended for sale by the manufacturer, must be shown to be free from Mycoplasma in order to be satisfactory. Another key point from 9 CFR 113.53, it states each lot of ingredient must be shown to be free from Mycoplasma. For a large-scale manufacturer in the pharmaceutical/biological production industry, it means that every lot of material of animal origin received or produced must be tested for the presence of Mycoplasma.

As noted above, the number of materials that require Mycoplasma testing can be quite extensive. This gets to be very costly for a manufacturer, as the materials of animal origin received and used, as well as the products produced, require this costly and time consuming test. However, there is one exception to the rule of 9 CFR 113.53, and that exception is in 9 CFR 113.200, "General requirements for killed virus vaccines." A killed

virus is a virus that is inactivated or made inert/non-infectious by an appropriate agent (9 CFR 113.200). In 9 CFR 113.200 (c)(3), it states "If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for Mycoplasma as prescribed in 9 CFR 113.28." This is further elucidated in Veterinary Services memorandum 800.86, which is intended to clarify interpretation of 9 CFR 113.200, and in which the procedure for obtaining an exemption from Mycoplasma is detailed. This memorandum is specific for the killed virus harvest and serial only and does not include master seed materials and material of animal origin ingredients. This memorandum sets specific requirements needed to submit and receive approval for an exemption from Mycoplasma testing. This approval is contingent upon being able to show that the production process would kill any Mycoplasma present in the virus fluids, as well as the intended virus.

1.2 Research Questions

From the foregoing, Mycoplasma is an important resource in vaccine production and a major risk. While testing is necessary to manage the risk, the large number of materials currently tested for Mycoplasma raises two questions for consideration. First, do vaccine manufacturers in the animal health industry have any killed or inactivated virus products that can be conclusively shown to kill Mycoplasma if they are used to achieve effective results as intended virus vaccines? And second, which products, if they exist, would be most economically beneficial for animal health companies in the vaccine production business? The products selected for this project must be shown to be eligible and to be economically beneficial for the company, as it will not be a value added activity if it will not decrease cost.

1.3 Research Objectives

Given the foregoing research questions, the overall objective for this thesis is to identify solutions that minimize the economic effect of current regulatory requirements on verification of Mycoplasma presence in finished products, inputs and facilities. The specific objectives are:

1. Evaluate the bank of inactivated virus products in the company and assess and rank the effectiveness of potential alternatives for the Mycoplasma exemption initiative
2. Conduct the economic analyses of the top-three alternatives to determine the most economically beneficial alternative in meeting economic and regulatory expectations of the company
3. Develop an implementation plan for the most economically feasible alternative and evaluate the economic implications on the company.

1.4 Thesis Outline

To achieve the foregoing objectives, we started with isolating the top three inactivated viruses that could be assessed for their economic value in securing an exemption under Veterinary Services memorandum 800.86. We then analyzed and compared their economic contributions to the operations costs, making a decision based on the best among the three in facilitating a regulatory exemption. The regulatory and testing processes associated with Mycoplasma are reviewed and discussed the next chapter. Chapter 3 presents the sources of data and the analytical methods used. We discuss the results in Chapter 4 and our recommendations in Chapter 5. Chapter 5 also presents the summary and conclusions from the research.

CHAPTER II: LITERATURE REVIEW

This chapter presents the literature surrounding the Mycoplasma exemption project. The focus will be on three main areas:

1. The available information on a Mycoplasma exemption project as per 9 CFR 113.200, 113.28 and Veterinary Services Memorandum 800.86
2. Common characteristics or methods for virus inactivation
3. Economics of Mycoplasma exemption.

2.1 9 CFR 113.53, 9 CFR 113.200, 9 CFR 113.28 and Veterinary Services Memorandum 800.86

Animal welfare and the manufacture of animal health products are governed by the Animal and Plant Health Inspection Service (APHIS) division of the US Department of Agriculture (USDA). APHIS achieves its regulatory objectives using a set of regulations under Title 9 of the Code of Federal Regulations (9 CFR). The 9 CFR govern everything from stockyard administration to animal vaccine manufacturing. For the purposes of this project, the specific guidance relevant to vaccine production and Mycoplasma testing are found in 9 CFR 113.53 "Requirements for Ingredients of Animal Origins," 9 CFR 113.200 " General Requirements for Killed Virus Vaccines," and 9 CFR 113.28 "Detection of Mycoplasma Contamination." 9 CFR 113.53 states the following:

> "Samples of each lot of ingredient of animal origin which is not subjected to heat sterilization, used to prepare a biological product shall be shown free of Mycoplasma by the method prescribed in 113.28"

In 9 CFR 113.200, the requirements for the production of a killed virus vaccine are established. These requirements can be found in table 2.1 General Requirements for Killed Virus Vaccines. The specific guidance related to this project can be found in 113.200(a)

"Killing agent – the vaccine virus shall be killed by the appropriate agent" and 113.200(c)(3) "Mycoplasma - If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill Mycoplasma, each serial of the vaccine shall be tested for Mycoplasma as prescribed in 9 CFR 113.28.

Table 2.1 General Requirements for Killed Virus Vaccines

9 CFR Section	Requirement
113.200(a) Killing Agent	The vaccine virus shall be killed (inactivated) by the appropriate agent
113.200(b) Cell Culture Requirements	If cell cultures are used in the preparation of the vaccine, primary cells shall meet the requirements in in 113.51 and cell lines shall meet the requirements in 113.52
113.200(c)(1) Purity Tests	Final container samples of completed product of each serial shall be tested as prescribed in 113.26
113.200(c) (2)Avian Origin Vaccine	Bulk pooled material or final container samples from each serial shall also be tested for (i) Salmonella contamination as per 113.30, (ii) Lymphoid leucosis virus contamination as prescribed in 113.31 (iii) and hemagglutinating viruses as prescribed in 113.34
113.200(c)(3) Mycoplasma	If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for Mycoplasma as prescribed in 9 CFR 113.28.
113.200(c)(4) Extraneous Viruses	Each lot of master seed virus used to prepare killed virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in 113.55.
113.200(d) Safety Tests	Final container samples of completed product from each serial shall be tested for safety in guinea pigs as prescribed in 113.38 and for safety in mice as prescribed in 113.33: Provided, that, vaccines recommended for use only in poultry are exempt from this requirement
113.200(e) Viricidal Activity Tests	Only serials tested for viricidal activity in accordance with the test provided in 113.35 and found satisfactory by such test shall be packaged as diluent for desiccated fractions in combination packages.
113.200(f) Formaldehyde Content	If formaldehyde is used as the killing agent, the residual free formaldehyde content must not exceed 0.74 grams per liter as determined using the ferric chloride test.

9 CFR 113.200(c)(3) specifically states testing for Mycoplasma as prescribed in 9 CFR 113.28 "Detection of Mycoplasma Contamination," wherein is the specific method that outlines how to perform an APHIS Mycoplasma test by the cell culture method, which is the method of choice for APHIS. It defines the type of media to use, both a broth and agar, as well as the specific test procedure. The test procedure is separated into the following steps as found in 9 CFR 113.28(d)(1-5) and (e)(1-2)[1]:

1) Preparation of inoculum – preparation of test sample, i.e. thawing frozen liquid vaccine or rehydrating lyophilized vaccine
2) Inoculation of plate – Inoculate 0.1 mL of inoculum on agar plate
3) Inoculation of flask of medium – Transfer 1 mL of the inoculum into 100 mL of mycoplasma broth medium
4) Control tests – Use an uninoculated broth medium as a negative control and inoculate mycoplasma broth medium with selected Mycoplasma cultures
5) Incubation – All plates shall be incubated in a high humidity, 4-6 percent CO_2 atmosphere at 33 to 37 °C for 10-14 days.
6) Interpretation of results – If growth is found on at least one of the positive control plates and no growth is found on the negative controls, the test is valid. If Mycoplasma colonies are found on any of the sample plates, the results are positive for Mycoplasma.

So, 9 CFR 113.28 clearly outlines the method to be followed for performing the "Detection of Mycoplasma Contamination" test and is required as per 9 CFR 113.200. However, as per 113.200(c)(3), the licensee has the right to demonstrate that the agent used to kill the

[1] See 9 CFR 113.28 "Detection of Mycoplasma Contamination" Available at https://www.gpo.gov/fdsys/pkg/CFR-2017-title9-vol1/pdf/CFR-2017-title9-vol1-chapI-subchapE.pdf

virus, also would kill Mycoplasma. 9 CFR 113.200(c)(3) does not discuss how this process may be demonstrated or the requirements surrounding that demonstration of Mycoplasma inactivation. The APHIS policy on that demonstration is found in Veterinary Services Memorandum 800.86.

A Veterinary Services Memorandum is a written notification from the USDA intended to clarify regulation. As of September 17, 2017, there are over 80 Veterinary Service Memorandums listed on the USDA website.[2] These memorandums range in content from distribution permits instructions to avian influenza vaccine requirements. The topic of interest for this thesis was Veterinary Services Memorandum 800.86, "Exemption from Mycoplasma Testing Under 9 CFR 113.200(c)(3)." The purpose of this memorandum was stated as follows:

> This memorandum clarifies Center of Veterinary Biologics (CVB) policy for obtaining an exemption to the requirement for testing inactivated viral vaccines for Mycoplasma contamination.

The requirement that the memorandum seeks to clarify is under 9 CFR 113.200(c)(3), which states, "… the harvest fluids used in the production of killed virus vaccines must be tested for Mycoplasma contamination according to 9 CFR 113.28 prior to adding the killing agent." 9 CFR 113.200(c)(3) also states that:

> If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for Mycoplasma as prescribed in 9 CFR 113.28.

This means that the harvest fluid and final product require testing for Mycoplasma contamination unless the production process can be shown to destroy viable Mycoplasma

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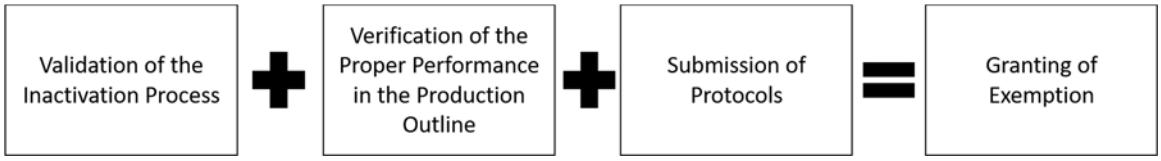
[2] See USDA Animal and Plant Health Inspection Services pages. Available at https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/biologics-regulations-and-guidance/ct_vb_vs_memos.

cells. It is important to note that the Memorandum specifically states that the exemption "only applies to the testing of vaccine virus prior to adding the killing agent and not to the testing of Master Seed, Master Seed Stocks, primary cells or other ingredients of animal origin" (Veterinary Services Memorandum No. 800.86, p. 1). Figure 2.1 provides a summary of the policy clarified in the Memorandum. It shows that to obtain the exemption, the applicant needs to show that the suggested procedure to inactivate vaccine viruses will also inactivate Mycoplasma. This is the validation of the inactivation process. Next, the methods used to verify that that inactivation process is performed properly must also be presented in the production outline submitted with the application for exemption.

Figure 2. 1: Veterinary Services Memorandum 800.86 Process for Obtaining Exemption to Testing Requirements for Inactivated Viruses



The protocols must include the Mycoplasma species that is being targeted for use and the specified species should include those that are recognized as common cell culture contaminants as well those found in the target animals for the product. When all these documents are submitted, the Center for Veterinary Biologic, which is responsible for the exception, reviews them and, if everything is in order, grants the exemption to 9 CFR 113.200.

2.2 What Makes an Inactivated Product?

Viruses can be divided into two groups: (1) Enveloped viruses, which are surrounded by an outer lipid membrane; and (2) Non-enveloped viruses that lack this membrane (Lucas 2010). In general, non-enveloped viruses are more stable, able to

survive longer in the environment and are more virulent (Lucas 2010). Viruses are used in the production of vaccines and either of these viruses may be used. Inactivated vaccines are vaccines produced in a culture of virulent viruses, which are then killed with chemicals, heat or radiation (vaccine.gov 26). The most recognized methods of virus inactivation are pasteurization, dry heating, vapor heat, solvent or detergent, and acid pH and are described in Table 2.2 Inactivation Method Characteristics (World Health Organization 2004).

Table 2.2 Inactivation Method Characteristics

Inactivation Method	Advantages	Disadvantages	Common Usage
Pasteurization	•Relatively simple equipment •Inactivates enveloped and some non-enveloped viruses •Nontoxic	• Does not sterilize material • Many viruses, such as HBV are heat stable • Materials require refrigeration after pasteurization	•Food industry
Dry Heat	• Inactivates enveloped and some non-enveloped viruses •Sterilization on final container •Nontoxic	• Strict control of moisture content • Extensive validation • Heat sensitive materials, such as protein, cannot withstand process • Slow process	•Pharmaceutical industry
Vapor Heat	• Inactivates enveloped and some non-enveloped viruses • Rapid sterilization • Nontoxic • Cycle easy to control/monitor	• Strict control of moisture content • Extensive validation • Complex to implement • Potential for burns • Heat sensitive materials cannot withstand process	• Vaccine industry • Pharmaceutical industry
Solvent/detergent	• Inactivates enveloped viruses • Does not denature protein • High process recovery	• Non-enveloped viruses unaffected • Remove solvent / detergent • Possibly toxic material	• Vaccine industry
Acid pH	• Inactivates enveloped viruses	• Non-enveloped viruses mostly unaffected • Low pH causes damage to most proteins	• Vaccine industry (IgG aggregation)

While pasteurization, terminal dry heat and vapor heat are physical approaches to virus inactivation, solvent or detergent, and acid pH are chemical approaches. The physical approaches use heat to inactivate the virus while the chemical approaches use chemicals. For the solvent/detergent method, an organic solvent and a non-ionic detergent are added to the broth, which inactivates enveloped viruses by disrupting their lipid coat (P. L. Roberts

2008). In the acid pH method, low pH inactivation has been used to inactivate large enveloped viruses by denaturing the proteins in their viral envelope (Joe Makowiecki 2013). There are a number of chemical inactivants that can be used for viral inactivation, but common inactivants found in the biological production industry are listed in Table 2.3 (Selwyn A. Wilson David 2010).

The procedure used for chemical inactivation would be dependent upon the chemical used, but in general, the overall method could be summarized as follows:

1. Production and harvest of live virus
2. Addition of chemical inactivant and hold time
3. Neutralization of chemical inactivant either by further chemical addition or other methods, such as heat
4. Final product preparations for filling/testing/distribution

In the animal health industry, virus inactivation for vaccine production would be dependent upon the nature of the virus. The main distinguishing characteristic would be whether the virus was enveloped or nonenveloped, which would determine the viral resistivity to heat, chemicals and pH.

Table 2.3: Common Chemical Inactivants

Chemical	Inactivation Process	Source
X-Triton 100	A mild nonionic detergent that is often used in biochemical application to solubilize proteins and other macromolecules, disrupting the protein-protein, protein-lipid and lipid-lipid associations. It can be used to inactivate enveloped viruses because of its activity on macromolecules associates, as with the generation of inactivated envelope viruses vaccines	(Francesca Colavita 2016)
Sodium Deoxycholate (DOC)	An anionic detergent which has been used both to destroy infectious virus and to fractionate viral components	(Angela E. Auletta 1968
Cetyltrimethylammonium bromide	A cationic detergent used for DNA removal in viral vaccine manufacturing	Elina Gousseinov 2014
Formaldehyde solution	Solution produced by the oxidation of methanol. It is made of 37% formaldehyde and impurities such as methanol, small amounts of formic acid, aldehydes and ketones, used for cross-linkaging/fixing of cells. In viral inactivation, it is thought that formalin destroys the biological activity of viral RNA	Sigma Aldrich 2017, Thomas Wilton 2014
Binary ethyleneimine (BEI)	An aziridine compound, produced from bromoethylamine hydrobromide (BEA) commonly used as an inactivant during vaccine manufacturing	D. Aarthi 2004
β-Propiolactone (BPL)	Organic compound of the lactone family, commonly used for chemical inactivation of enveloped viruses. BPL was found to inhibit viral membrane fusion in a dose dependent manner that correlates with a loss of infectivity	Pierre bonnafous 2013

2.2 Economics of Mycoplasma Exemption

In terms of economic data on the actual Mycoplasma Exemption project, or cost savings after a project, there is very little information in the literature. The exemption project process would be similar to any facility, but the economic benefit would be dependent upon the inactivation process and inputs/outputs of the individual company. There are numerous laboratories that offer Mycoplasma testing services for the industry. The cost of testing services ranged from $48 (Genetica DNA Laboratories n.d.) to $109 per

sample tested. There is no such data available to those outside these facilities, as production processes and specific costs are closely guarded secrets by all biological manufacturing facilities. Despite the foregoing challenges, there is sample information about Mycoplasma testing in the biopharmaceutical industry. One of the sources projected that the Mycoplasma testing market would be worth $943.3 million (USD) by the year 2022 (Market n.d.). This projection included testing kits, as well as services. This shows how costly the Mycoplasma compendial test can be for manufacturers. There are laboratories that offer Mycoplasma testing for submitted samples, most by PCR methods. The range of pricing for the Mycoplasma test services, started as low as $48 per sample (Genetica DNA Laboratories n.d.) and up to $109 per sample (Clongen Laboratories LLC n.d.). A simple cost estimation for 9 CFR Mycoplasma testing would be to multiply the number of samples tested per year by the cost of analysis per sample. For example, our facility performed approximately 600 9 CFR Mycoplasma tests in 2014. So, if 600 were multiplied by $48, as well as $109, the range for cost of analysis would be $28,800 to $65,400. Unfortunately, the two cost estimates above were for PCR detection methods. As stated earlier, most of these methods were by PCR detection, which has shown difficulty in gaining acceptance by the USDA. Using the PCR method requires qualification and validation testing showing the PCR method was as sensitive as the culture method, or equivalency data, as well as submission to the USDA for approval. From past research, the qualification/validation of this method had challenges, especially with the PCR method being unable to discern between viable and non-viable cell material. The PCR method would require extensive testing and a regulatory submission for approval, which would be labor and cost extensive. The APHIS method of choice at this time is the culture method.

The general cost estimate for undertaking the culture method is about $109 per test. While direct economic data showing the cost benefits or expenses of a Mycoplasma exemption were not available, there was plenty of data available showing the expensive nature of the Mycoplasma test.

CHAPTER III: DATA AND METHODS

Chapter 3 presents the data and methods used for the Mycoplasma exemption thesis project. The chapter is divided into 3 parts. Section 3.1 will describe the data sources, collection methods and verification of data. Section 3.2 will describe the sampling and selection process, as well as the criticality of the sampling/selection, in assessing the inactivation of viruses for vaccines. The final section, Part 3.3, will be a discussion of the methods used to achieve the research objectives and answers to those questions.

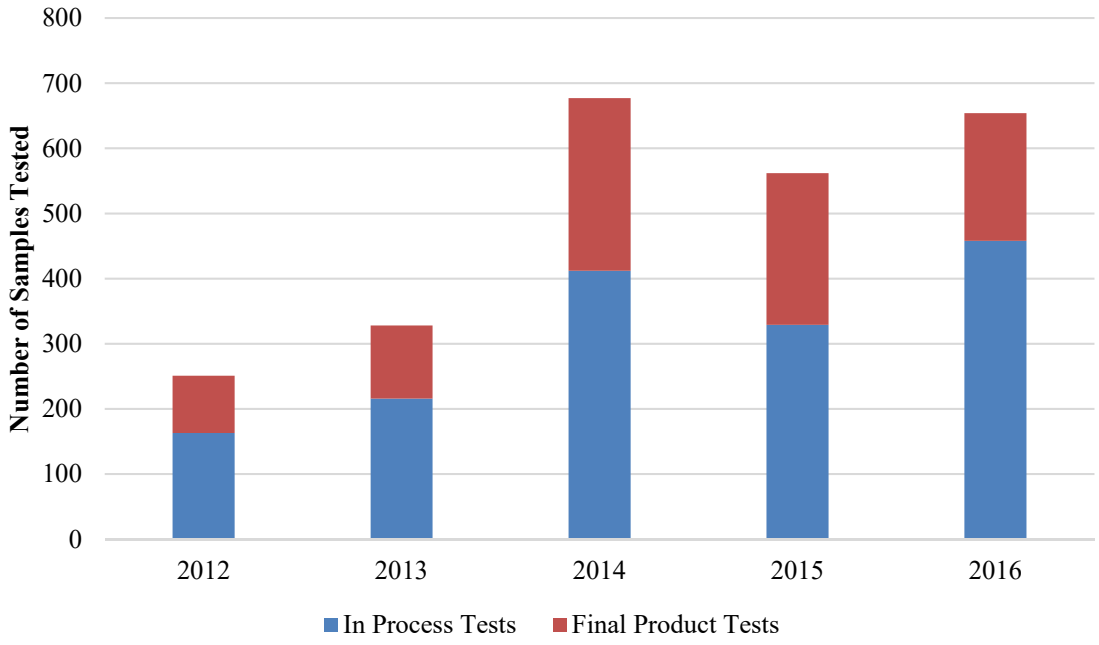
3.1 Data Sources, Collection of Data and Verification of Data

The research data came from the laboratory tracking system for receipt, testing and release of samples. For the Mycoplasma test, our site has two different methods based upon which country the lot is to be distributed. The two methods are denoted as the 9 CFR method (9 CFR 113.28) and the European Pharmacopeia method (EP) (Chapter 2.6.7 *Mycoplasmas*). The 9 CFR Method is used for those products that are marketed and distributed in the U.S. and the EP method is used for those products sold to countries within the European Union and the rest of the world (those outside of the European Union but not domestic). While these tests are looking for the same result, i.e., no Mycoplasma present, the methods are different. The regulatory agencies also have differing acceptance criteria and expectations. The 9 CFR method is governed by the USDA/APHIS and has a provision for the submission and acceptance of the Mycoplasma Exemption project (see Figure 2. 1). The EP Mycoplasma test is governed by the European Pharmacopeia and has no provision for a Mycoplasma exemption from testing.

This thesis focuses only on sales in the United States, and therefore, the relevant test is the 9 CFR Method. We used test data over the past five years and sorted them by 9

CFR 113.28 Mycoplasma tests. The numbers of samples tested for Mycoplasma by year are presented in Figure 3. 1. It shows that over the past five years, 2,472 in process and final product tests for Mycoplasma were conducted. The final products accounted for 36.2% of the total tests for Mycoplasma conducted between 2012 and 2016. The reason why more than 63% of the tests occurred during in process is because all ingredients going to products have to be tested but only the final product is tested. On average, about 179 final product tests using the 9 CFR 113.28 Method were performed per annum. This compares to an In Process tests' average of about 316 tests for the same period.

Figure 3. 1: In Process and Final Tested Mycoplasma Samples Using the 9 CFR 113.28 Method

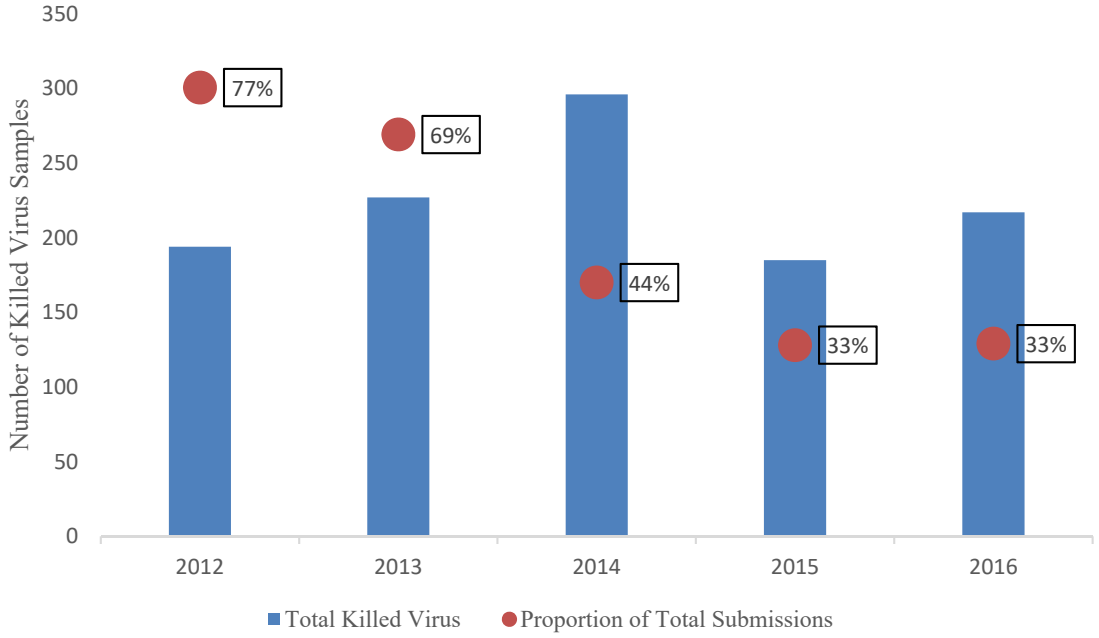


From the foregoing samples tested using the 9 CFR 113.28, we selected the relevant killed virus samples for the study. We did this by sorting by killed virus samples in our laboratory tracking system and searching through registered Outlines of Production (OOPs) to verify that the samples were indeed killed virus materials. An OOP is a

registered production record submitted and filed with the USDA. It is the legal method used for production of a particular product. The manufacturer can only produce the product only after USDA's approval.

Table 3.2 presents the Mycoplasma killed virus samples extracted from the total sample of tests between 2012 and 2016 and their contribution to the total sample. Overall, the average number of Mycoplasma killed virus samples per year was 224 samples with a standard deviation of 44 samples and their share of total sample tested averaged about 45.3%, with a standard deviation of about 20.7%. This provides a large enough sample for use in this project.

Figure 3. 2: Killed Virus Samples Used in the Mycoplasma Exemption Project



3.2 Sampling, Sampling Criticality and Selection

Recall that this research was motivated by the search for killed viruses that could qualify for exemption under the 9 CFR 113.28 method. Selection of a product that is economically beneficial for the facility was the main driver for this thesis. The samples

identified in the preceding subsection were those that had been subjected to the 9 CFR 113.28 method for Mycoplasma testing. Having completed their selection, the next step involved identifying the most common submissions to the laboratory for 9 CFR Mycoplasma testing. Again, the laboratory tracking system was used to examine the list of the most common submissions. As shown in figure 3.2, the number of killed virus submissions in the past five years average approximately 224 per year, with a standard deviation of 44 samples. The laboratory tracking system was used to sort all of the killed virus submissions individually. The sample submissions were then calculated as a percentage based on the number of individual virus samples against the total number of samples tested each year for the previous five years. Table 3.1 shows the viruses most commonly submitted for 9 CFR Mycoplasma testing.

Table 3.1 Percentage of Individual Virus Tests per Year

	Year				
Fraction	2012	2013	2014	2015	2016
Virus 1	45.40%	41.28%	28.92%	29.21%	26.19%
Virus 2	25.77%	30.28%	21.60%	25.28%	37.14%
Virus 3	28.83%	27.52%	27.18%	16.85%	20.00%
Virus 4	0.00%	0.00%	4.18%	11.24%	6.67%
Virus 5	0.00%	0.00%	8.01%	5.62%	5.24%
Virus 6	0.00%	0.92%	2.09%	5.62%	0.95%
Virus 7	0.00%	0.00%	4.88%	3.93%	3.81%
Virus 8	0.00%	0.00%	3.14%	1.69%	0.00%
Virus 9	0.00%	0.00%	0.00%	0.56%	0.00%
Virus 10	3.55%	0.02%	2.05%	3.26%	3.33%

The table shows that while Virus 1 accounted for more than 45% of the samples submitted in 2012, its share had declined to less than 30% by 2015. Likewise, Virus 3 has also experienced a decline in its share of submissions. On the other hand, new viruses have been introduced into the process. For example, Virus 6 was not used in 2012 but accounted

for more than 5.6% of total submissions in 2015. As indicated earlier, more tests are performed in the In Process phase than at during the Final Product phase because during production, each virus type is submitted for vaccine testing. The final product often contains more than one virus, reducing the number of Final Product tests that are done compared to the In Process tests.

In identifying the virus types or fractions submitted for testing, we had to review each OOP and their associated final product format. After reviewing each OOP, the virus fractions were combined to show their final product correspondences. The summary of this exercise is presented in Table 3.2. The table shows that the most commonly submitted and tested virus fractions to the QC laboratory actually belonged in two product lines – Viruses 1 through 9. It would seem that Virus 10 is not used in combination with other killed viruses in the production of final products.

Table 3.2 Virus Fractions as per Final Product by Year

	Year				
Killed Virus Product	2012	2013	2014	2015	2016
Product 1 (Virus 1, 2,3, 9)	84.02%	95.15%	75.34%	69.19%	83.33%
Product 2 (Virus 4, 5,6,7,8	0.00%	0.88%	21.62%	27.03%	16.67%
Product 3 (Virus 10)	15.98%	3.96%	3.04%	3.78%	3.33%

With the knowledge of the most common inactivated virus submissions, the next step was reviewing the OOP of each product to verify that the inactivation method was similar to the findings in the literature review, and to determine if the laboratory could reproduce the inactivation steps. The review of each OOP found that the solvent/detergent method was used for each of the three products presented in Table 3.2, and that they could be replicated in the laboratory setting. The laboratory had access to the appropriate equipment and reagents necessary for a small scale virus inactivation study. With the

verification of the inactivation method and capability of the small scale study, the Mycoplasma exemption could then be pursued. This process would be the first of its kind performed at this company and could be basis for future project decisions about exemptions.

The top-10 killed virus submissions to the Quality Control laboratory for 9 CFR Mycoplasma testing were used in only three final product presentations (Table 3.2). To be the most economically beneficial for the company, the selected product would either need to be the most commonly submitted material or the highest cost savings based on production. From Table 3.2, then, the choice products to assess for economics of the tests are Product 1 and Product 2. Based on this assumption, the average number of tests of Product 1 and Product 2 were calculated and found to be 211 samples per year; 208 in process samples and 3 finished product samples.

3.3 Assessing Exemption Value

Let us assume that the cost of testing for Mycoplasma using the 9 CFR 113.28 methods comprises two principal components: (1) Reagents and other consumables; and (2) Time (scientists and lab technicians). If that is true, then the cost of testing each of the viruses In Process, C_i , may be presented as follows:

$$C_i = \sum_{j=1}^{J} (1+\varepsilon_i)(w_{ij}R_{ij} + y_i T_i) \tag{1}$$

Where ε_i is the estimated risk of re-work due to accidental errors that occur during in process testing, w_{ij} is the price for reagent j, and R_{ij} is the quantity of reagent *j* used in

testing Virus *i*. y_i is the price of time, *T*, used in testing viruses and T_i is the amount of time used to test Virus *i*.

The final product test follows a similar logic of looking at reagents and time, except it is now focused on products instead of viruses. This is presented for clarity in Equation (2) thus:

$$C_P = \sum_{j=1}^{J} (1+\varepsilon_p)(w_{Pj}R_{Pj} + y_P T_P) \tag{2}$$

where C_P is the cost of doing the 9 CFR 113.28 Mycoplasma test for Product *P*, ε_p is the final product rework risk, and the remaining variables are as defined except that instead of *i* they are focused on products. The total cost for each final product, C_P^*, is, then defined as the cost of testing the viruses in the In Process stage and the cost of testing the products in the final product stage. This is summarized in Equation (3) as follows:

$$C_P^* = \sum_{i=1}^{I} C_{Pi} + C_P \tag{3}$$

where C_{Pi} is the cost of testing each virus, *i*, used in the final product and C_P is the cost of testing the final product.

Once the costs of performing the 9 CFR 113.28 Mycoplasma test are known, in terms of reagents/consumables and time, then simulations will be performed to project the cost savings over the next 10 years. The first simulation to be performed will be to estimate the change in cost in performing the test over the next 10 years. It is assumed that the cost of both reagents/consumables and time (wage of scientists and technicians) will increase over the next 10 years. These simulations will be used to estimate those increases in cost. For reagents/consumables, the cost of materials will be compared from 2016 to

2017 and used to estimate the percent increase over that time frame. Then that percent increase will be used to calculate the increase in cost on a yearly basis for 10 years. For the time variable (wages), a cost of living increase of 2.5% will be used to estimate the increase in time on a yearly basis over the next 10 years. The increasing cost in our variables will be defined as the future cost of testing, or FV, and is represented in equation (4) as follows:

$$NPV = \left[\sum_{n=1}^{N} \frac{R_n}{(1+d)^n}\right] + \left[\sum_{n=1}^{N} \frac{T_n}{(1+r)^n}\right]$$

where R is the reagent cost, n is the number of years, *d* is the discount rate increase for reagent s, T is the time cost, and *r* is the discount rate for time. The Mycoplasma growth rate will be calculated based on the previous five years of data. Once the growth rate is calculated, two simulations will be performed to assess the impact of changing growth rates over the next 10 years. Using these equations, the increase in cost of testing can be estimated as well as the overall net savings associated with receiving an exemption from the Mycoplasma testing requirement.

CHAPTER IV: COST ANALYSIS

The objective of this project was to identify the potential cost savings that may be achieved by developing processes that eliminated the need to test for Mycoplasma in a vaccine production facility. The cost savings would be dependent upon several factors. However, the principal sources of savings would be reductions in test material usage and accompanying reductions in cost of labor associated with the testing. The reduction in test materials is defined as the cost savings based upon the fewer laboratory reagents and consumables used. The reduction in labor hours will be defined as the time saved for Quality Control analysts, specifically, the cost of labor for a Scientist or Technician testing for Mycoplasma. Any savings will be calculated based on the available data and then extrapolated forward for the next ten years. The chapter is divided into four subsections. The first and second subsections address laboratory material and labor costs respectively. The final two subsections present the estimated results and analyses for the cost savings in the laboratory and for the products over a ten-year span.

4.1 Laboratory Material Cost Saving Calculation

Testing for Mycoplasma using the 9 CFR 113.28 is a very specific process, requiring specific materials as discussed in Chapter 2. Material savings would emanate from the Mycoplasma agar and broth media, both of which are made in-house by our media preparation group. To estimate the savings associated with the laboratory material, we reviewed the media formulation records for each media and the associated pricing information for the each material in the formulation record for 2016. Table 4.1 and Table 4.2 present the pricing information for the Mycoplasma agar media and the Mycoplasma broth media. The pricing information in these two tables was collected to aid in calculating

the cost of a single broth bottle and a single agar plate. In order to find the cost per bottle or plate, the cost of one gram or milliliter of each component was estimated. The cost of the container was divided by the amount of grams or milliliters in that container. The prices of materials going into the making of the agar and the broth are is presented in Tables 4.3 and 4.4, respectively.

Upon estimating the price per gram of agar or price per milliliter of broth, the next step involved scaling the cost per batch of media. This involved multiplying the unit price of agar and broth by the number units of agar and broth in a one liter batch of media. The sum of each material in the broth and agar provided an estimate of the total cost per liter of each media. Finally, the total cost per liter of each media was divided by the number of media units that were obtainable from a liter of media to estimate the cost per unit of media used in the testing. The information on cost per liter and the cost per unit is presented in Tables 4.5 and Table 4.6 for the agar and broth medias, respectively.

As per Table 4.5 and Table 4.6, the cost of a single agar plate is estimated at $5.52 and the cost of a single broth bottle at $2.23. Each 9 CFR Mycoplasma test requires one product broth bottle and two positive control broth bottles, one negative control broth bottle and five agar plates per broth bottle, i.e., 20 agar plates. This would mean that each test requires four broth bottles, at $2.23 per bottle, and 20 agar plates, at $5.52 per plate. Thus, the material cost per test is estimated at $119.32.

Table 4.1: Mycoplasma Agar Material List and Pricing

9 CFR Mycoplasma Agar Component	Price/container	Container
Component A	$134.06	500 g
Component B	$129.10	500 g
Component C	$116.62	500 g
Component D	$435.50	100 g
Component E	$31.79	100 mL
Component F	$75.35	5 g
Component G	$64.35	100 g
Component H	$59.75	500 mL

Table 4.2: Mycoplasma Broth Material List and Pricing

9 CFR Mycoplasma Broth Component	Price/container	Container amount
Component 1	$129.10	500 g
Component 2	$116.62	500 g
Component 3	$435.50	100 g
Component 4	$31.79	100 mL
Component 5	$75.35	5 g
Component 6	$59.75	500 mL
Component 7	$84.37	25 g

Table 4.3: Mycoplasma Agar Cost per Gram/ Milliliter of Material

9 CFR Mycoplasma Agar Component	Price/g (mL)
Component A	$0.27
Component B	$0.26
Component C	$0.23
Component D	$4.36
Component E	$0.32
Component F	$15.07
Component G	$0.64
Component H	$0.12

Table 4.4: Mycoplasma Broth Cost per Gram/Milliliter of Material

9 CFR Mycoplasma Broth Component	Price/g (mL)
Component 1	$0.26
Component 2	$0.23
Component 3	$4.36
Component 4	$0.32
Component 5	$15.07
Component 6	$0.12
Component 7	$3.37

Table 4.5: Mycoplasma Agar Cost per Liter and per Single Agar Plate

9 CFR Mycoplasma Agar Component	Price/g or (mL)	Grams(mL)/L	Price/L
Component A	$0.27	21	$5.67
Component B	$0.26	8.5	$2.21
Component C	$0.23	8.5	$1.96
Component D	$4.36	Solution	$0.91
Component E	$0.32	85	$27.20
Component F	$15.07	Solution	$4.31
Component G	$0.64	Solution	$0.11
Component H	$0.12	107	$12.79
	Cost per liter (Sum of Price/L)		$55.21
	Cost per plate (Cost per Liter / 15 mL)		$0.82

Table 4.6: Mycoplasma Broth Cost per Liter and per Single Bottle

9 CFR Mycoplasma Broth Component	Price/g (mL)	Grams(mL)/L	Price/L
Component 1	$0.26	23	$5.98
Component 2	$0.23	8.5	$1.96
Component 3	$4.36	Solution	$1.00
Component 4	$0.32	4.5	$1.44
Component 5	$15.07	Solution	$1.05
Component 6	$0.12	90.1	$10.81
Component 7	$3.37	Solution	$0.02
Cost per Liter (Sum Price/L)			$22.26
Cost per bottle (Cost per Liter/100 mL)			$2.23

4.2 Laboratory Labor Cost

We focus on two areas in calculating the labor cost of Mycoplasma testing: Time spent on the 9 CFR Mycoplasma testing itself and the wages/remunerations of the scientists doing the 9 CFR Mycoplasma test. We estimate the time it takes to test a sample of 9 CFR Mycoplasma killed viruses and multiply it by the wage payed to the scientists performing the test.

The time to perform a 9 CFR Mycoplasma test includes preparation, set-up and actual test time. The first step in any test is to start the biosafety cabinet. The biosafety cabinet is a piece of laboratory equipment that is used for controlling the test environment. It provides an aseptic area to perform microbiological work. This unit requires a minimum of 30 minutes to warm up prior to use. The medias and test materials can be gathered during 30 minute warm up time. After the 30 minutes, the entire work surface must be disinfected, which consists of wiping down the interior surfaces of the cabinet with approved disinfectants and allowing a 10 minute contact time with the disinfectant to allow for full disinfection. Then all the materials that will be used for testing would be wiped down and disinfected and placed in the biosafety cabinet, which will also require a 10 minute contact time. The total disinfection time that will include wiping down all materials and the two 10 minute contact time windows, will require a total of approximately 30 minutes. Once the disinfection stage is completed, the testing phase will begin. This includes inoculation into a broth bottle and subculture onto a plate, which depending on the sample, will take 10-15 minutes. For the purpose of this study, we will take the worst case of 15 minutes. This would bring the total time to 1 hour and 15 minutes. This set of samples is referred to as the plant culture. Once the testing is complete, all samples are

placed in a specific incubator to allow for proliferation of any Mycoplasma organisms. The 9 CFR Mycoplasma test requires a subculture out of the broth bottle onto agar media on four more occasions. These are a subculture from the broth bottle onto an agar plate on Day 3, Day 7, Day 10 and Day 14 of incubation. These subcultures require the same process as the initial, except the subculture from the broth to agar will take only 5 minutes. So, each subculture date (day 3, 7, 10 and 14) would require 1 hour and 5 minutes per test. This would bring the total testing time to 5 hours and 35 minutes. This, however, does not include the total test time yet. Each of the five inoculations (plant, Day 3, Day 7, etc.) requires an examination by the scientist 10-14 days after each inoculation. The examination will require removal from the incubator, examination by microscopy, documentation on test data sheets and placement back in the incubator. This step would be a further 20 minutes per each viewing. In reality, the plant and Day 1 can be examined together, as well as Day 3 and Day 7 and finally Day 10 and Day 14 read together. This would mean that there would be only three examinations instead of five. The total time for the examinations would then be a minimum of one hour (if three readings are done and 100 minutes if all five readings are done independently. The total test time including inoculation, subculture and examination would then be a total of 6 hours and 35 minutes at the minimum or 6 hours and 55 minutes. These estimates do no account for any errors on the part of technicians and scientists undertaking the tests.

The second area of focus for the Mycoplasma test labor cost was the technician wage cost. The data for this metric was technicians' compensation related to performing the test. It is standard in the industry to use cross-training and rotations to enhance performance of technicians and fluid operations with little or no disruptions arising from

human factors. The rotation usually lasts for approximately six months and comprises two different pay grades of employees: Technician Level IV and Scientist Level I. The average pay (estimated on an hourly basis) is $21.05 and $22.83 per hour respectively.

4.3 Laboratory Cost Saving Calculation.

We assumed in Subsection 3.3, that the cost of 9 CFR 113.28 Mycoplasma testing would be comprised of two components: (1) Reagents and consumables; and (2) Time (wage paid to scientists and technicians and time of assay). With that assumption, the following equations, for in process and finished product testing respectively, were developed to calculate those costs;

$$C_i = \sum_{j=1}^{J}(1 + E_i)(W_{ij}R_{ij} + y_i T_i) \quad (4)$$

$$C_P = \sum_{j=1}^{J}(1+\varepsilon_p)(w_{Pj}R_{Pj} + y_P T_P) \quad (5)$$

Where, ε_i in Equation 4, is the estimated risk of re-work due to accidental errors that occur during testing, w_{ij} is the price for reagent *j*, and R_{ij} is the quantity of reagent *j* used in testing Virus *i*. y_i is the price of time, *T*, used in testing viruses and T_i is the amount of time used to test Virus *i*. For Equation 5, C_P is the cost of doing the 9 CFR 113.28 Mycoplasma test for Product *P*, ε_p is the final product rework risk, and the remaining variables are as defined except they are focused on products. Recall that in Subsection 4.1, the cost of each agar plate was calculated as $0.82 and the cost of each broth bottle was calculated as $2.23. Also, recall that in Subsection 4.2, the time necessary to perform a Mycoplasma test was calculated as 6 hours and 35 minutes, and the wages paid to technicians and scientists were $21.05 and $22.83 respectively. To estimate this risk, the

laboratory data were analyzed to search for any "No Tests," which are defined as non-product related errors that will cause rework or a retest. The data from this analysis is found in table 4.7 found below:

Table 4.7 Laboratory No Tests (Rework)

	2012	2013	2014	2015	2016	Total
Finished Product	0	0	1	1	0	2
In Process	7	5	1	0	6	19
Total	7	5	2	1	6	21

Recall from Subsection 3.1, that the total number of 9 CFR 113.28 tests conducted over the past five years was 2,472 tests; of which 894 were finished products and 1,578 were in process tests. In those five years, Table 4.7 indicates that there were two occurrences of a No Test in the finished product and 19 occurrences in the in process. This would make the error rate of 0.22% for finished products and an error rate of 1.20% for in process testing.

With all variables now given value, the cost of performing the 9 CFR 113.28 Mycoplasma test would be as follows:

In process (IP)

(1.012)($25.32+$133.67) = $160.90 per Technician IP Process Test

(1.012)($25.32+$144.97) = $172.33 per Scientist IP Test

Finished Product (FP)

(1.0022)($25.32+$133.67) = $159.34 per Technician FP Test

(1.0022)($25.32+$144.97) = $170.67 per Scientist FP

Recall from Subsection 3.3 that the total cost was defined as per the following equation:

$$C_P^* = \sum_{i=1}^{I} C_{Pi} + C_P \tag{6}$$

where C_{Pi} is the cost of testing each virus, *i*, used in the final product and C_P is the cost of testing the final product. Recall from Subsection 3.2 that in process viruses are tested individually, but are later combined to produce finished products. Also, recall from the same Subsection 3.2 that the most economically beneficial products selected are Product 1 and Product 2. In Table 4.8, the virus fractions were combined to show the number of in process and finished product tests for each product per year.

Table 4.8 Breakdown of Product Submissions of Product 1 and Product 2

Sample	2012		2013		2014		2015		2016	
	IP	FP	IP	FP	IP	FP	IP	FP	IP	FP
Product 1	163	2	215	1	223	9	128	3	175	0
Product 2	0	o	1	0	55	0	47	0	35	0

Table 4.9 shows the in process and product testing costs by scientist and technician working on each product. The numbers are estimated using Equation 3. The results show that show that total cost for testing Product 1 ranged from almost $69,000 in 2015 to nearly $122,000 in 2014. The average labor cost for Product 1 over the five years was $96,202.25, with a standard deviation of $$21,221.65. For Product 2, the average labor cost over the five years was approximately $18,000.00 with a standard deviation of $13,465.98. The coefficient of variation for Product 1 was about 22.5%, compared with 93.4% for Product 2. This implies that the variability from year to year in the cost of labor for Product 1 was much smaller than for Product 2. Total labor cost of testing for Product 1 was $316,308.09 over the five years compared to $47,512.79 for Product 2, yielding a total labor cost for both products at $363,820.87 over the five years.

Table 4.9 Labor Cost for Conducting Mycoplasma Tests for Product 1 and Product 2 for 2012-2016

Category	2012	2013	2014	2015	2016
Product 1 Scientist	$ 29,370.91	$ 38,454.74	$ 41,276.04	$23,313.88	$ 31,158.24
Product 1 Technician	$ 27,424.59	$ 35,906.46	$ 38,540.81	$ 21,768.94	$ 29,093.48
Total Product 1	$ 56,795.50	$ 74,361.21	$ 79,816.85	$ 45,082.81	$ 60,251.72
Product 2 Scientist	$0.00	$ 178.05	$ 9,792.59	$ 8,368.21	$ 6,231.65
Product 2 Technician	$0.00	$ 166.25	$ 9,143.67	$ 7,813.68	$ 5,818.70
Total Product 2	$0.00	$ 344.30	$ 18,936.26	$ 16,181.89	$12,050.34
Total Product 1 and Product 2	$ 56,795.50	$ 74,705.50	$ 98,753.10	$ 61,264.70	$ 72,302.07

4.4 10-Year Cost Savings Projection

Suppose firms with operations and costs similar to what have been described here were successful in procuring an exemption from USDA to not conduct Mycoplasma tests from the current year, what would be the value of such exemption to the company? And, would it be worth it to pursue the mycoplasma test exemption? These are the two questions tackled in this subsection.

With the cost of testing calculated, the cost savings for the next 10 years was estimated by assuming an approval of all exemption submissions and no killed virus testing. The Net Present Value (NPV) for the future cash flow from the exemption was estimated using two discount rates – material and labor – as defined in Subsection 3.2. Recall from Subsection 3.2 that the average number of killed viruses from all killed viruses tested over the past 5 years was 224 samples. We, therefore, assume that over the next decade, the laboratory will conduct 224 tests per year in our base scenario. Recall also that

the analysis cost was based on two variables, reagent/consumable cost and time (labor) cost. These costs are not static because of inflation and other external circumstances beyond the control of the company. To account for this, it was assumed that both material and labor costs will increase annually over the decade. The change in material cost was estimated using the difference in material prices in 2016 and 2017 and assume that this will be the annual percentage change over the decade. We used the two near years because there was no credible information on material costs in prior years due to changes in vendors and types of material. Table 4.10 and Table 4.11 present the percentage change in prices for each component of agar and broth. We show that that average change in price of agar price between 2016 and 2017 was 7.2% per plate against 10.8% per 100 ml for the broth.

Table 4.10 Agar Media Price Comparison

9 CFR Mycoplasma Agar Component	2016Price/container	2017 Price/Container	Change in Price (%)
Component A	$134.06	$165.76	23.6%
Component B	$129.10	$129.10	0.0%
Component C	$116.62	$248.72	113.3%
Component D	$435.50	$435.50	0.0%
Component E	$31.79	$31.79	0.0%
Component F	$75.35	$75.60	0.3%
Component G	$64.35	$108.00	67.8%
Component H	$59.75	$60.84	1.8%
Cost Per Liter	$54.95	$58.85	7.1%
Cost Per Plate	$0.82	$0.88	7.1%

Table 4.11 Broth Media Price Comparison

9 CFR Mycoplasma Broth Component	2016 Price/container	2017 Price/Container	Change in Price (%)
Component 1	$129.10	$129.10	0.0%
Component 2	$116.62	$248.72	113.3%
Component 3	$435.50	$435.50	0.0%
Component 4	$31.79	$31.79	0.0%
Component 5	$75.35	$75.60	0.3%
Component 6	$59.75	$60.84	1.8%
Component 7	$84.37	$84.37	0.0%
Cost Per Liter	$22.19	$24.63	11.0%
Cost Per 100 mL bottle	$2.23	$2.46	10.8%

Recall from Subsection 4.2 that two labor grades were used to calculate the labor cost. For the purposes of this projection, we used an average of the two wage rates for the unit broth and agar plate and used the industry's average wage growth rate of 2.5% to make the necessary adjustments over time.

The growth-adjusted costs for materials and labor per test are presented in Table 4.12. The total cost increases from $164.61 per test to $228.69 over the ten years, which is an annual average growth rate of 3.34%. The share of total cost of materials in total cost, which cannot be altered because of the protocols defined by the 9 CFR 113 process, increases from 54.5% in the first year to 64.5% in the tenth year.

Table 4.12 Variable Cost Projection Per Test

Period	Agar	Broth	Labor	Total Cost Per Test
Growth Rate	7.81%	10.81%	2.5%	
Year 1	$ 16.40	$ 8.92	$ 139.29	$ 164.61
Year 2	$ 17.68	$ 9.88	$ 142.77	$ 170.33
Year 3	$ 19.06	$ 10.95	$ 146.34	$ 176.35
Year 4	$ 20.55	$ 12.14	$ 150.00	$ 182.68
Year 5	$ 22.16	$ 13.45	$ 153.75	$ 189.35
Year 6	$ 23.89	$ 14.90	$ 157.59	$ 196.38
Year 7	$ 25.75	$ 16.51	$ 161.53	$ 203.80
Year 8	$ 27.76	$ 18.30	$ 165.57	$ 211.63
Year 9	$ 29.93	$ 20.28	$ 169.71	$ 219.92
Year 10	$ 32.27	$ 22.47	$ 173.95	$ 228.69

The application for the exemption in time and effort is assumed to cost about $35,000 in terms of training, documentation, enhanced awareness of technicians and scientists to be extra careful about processes. Using a discount rate of 5.2%, the Net Present Value (NPV) of the exemption over a decade is estimated at $327,921.92. An 11.2% discount rate was also used as a hurdle rate to calculate the NPV of the exemption over a decade and was estimated at $246,732.60. Given that the exemption has a de facto savings outcome for the firm, the decision to pursue the exemption is straightforward as long as the NPV is greater than zero. Since the required investment for preparing for the exemption is relatively small, and given the benefits from the exemption, pursuing the exemption makes excellent economic sense.

We may consider the number of tests from a fixed instead of a stochastic perspective. Under this perspective, we assume the average number of tests remains unchanged but grows under two different scenarios by 10% and 15% respectively. These are within the growth rate experienced over the past five years' data used for the analyses, which was 12.9%. The number of tests for each scenario is shown in table 4.13. Table

4.16 shows that with a 5.2% discount rate, the NPV under the base test numbers scenario is $327,921.92. Similarly, table 4.17 shows that with a discount rate of 11.2%, the NPV under the base test numbers scenario is $246,372.60. In contrast, the NPV under the 10% and 15% growth rates in the base test numbers using discount rates of 5.2% and 11.2% are $431,954.27 and $540,591.38 at the 5.2%, and $315,935.63 and $387,508.93 at the 11.2% rate. The NPV savings were then annualized using the 5.2% and 11.2% rates to estimate the yearly savings associated with each scenario, which are shown in Table 4.14. The trend in the undiscounted savings is presented for the three scenarios in Figure 4.2.

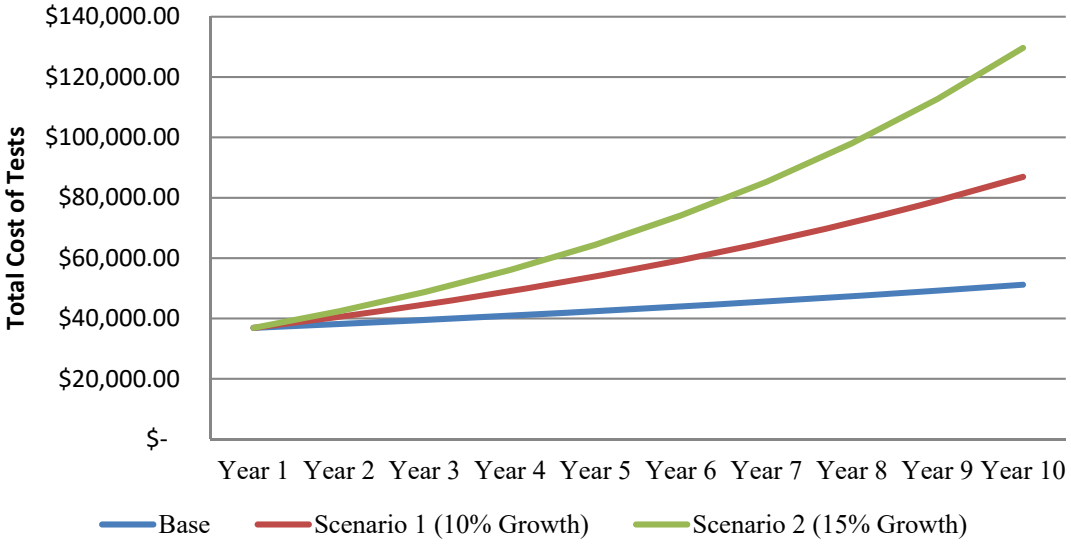
Table 4.13 Number of Test Scenarios

Year	Base	Scenario 1	Scenario 2
1	224	224	224
2	224	238	249
3	224	253	277
4	224	269	307
5	224	285	341
6	224	302	378
7	224	321	418
8	224	340	463
9	224	359	513
10	224	380	567

Table 4.14 10 Year Savings and NPV Calculation

	Base Scenario	Growth Scenario 1	Growth Scenario 2
Year	224	10%	15%
Year 1	$ 36,872.02	$ 36,872.02	$ 36,872.02
Year 2	$ 38,154.93	$ 40,559.23	$ 42,402.83
Year 3	$ 39,503.10	$ 44,615.15	$ 48,763.25
Year 4	$ 40,921.28	$ 49,076.66	$ 56,077.74
Year 5	$ 42,414.67	$ 53,984.33	$ 64,489.40
Year 6	$ 43,988.90	$ 59,382.76	$ 74,162.81
Year 7	$ 45,650.13	$ 65,321.04	$ 85,287.23
Year 8	$ 47,405.07	$ 71,853.14	$ 98,080.32
Year 9	$ 49,261.03	$ 79,038.46	$ 112,792.36
Year 10	$ 51,226.00	$ 86,942.30	$ 129,711.22
NPV (discount rate = 5.2%)	$ 327,921.92	$ 431,954.27	$ 540,591.38
NPV (discount rate =11.2%)	$ 246,372.60	$ 315,935.63	$ 387,508.93
Annualized NPV (5.2%)	$ 17,090.92	$ 22,512.97	$ 28,175.02
Annualized NPV (11.2%)	$ 27,593.81	$ 35,384.89	$ 43,401.13

Figure 4.2 Trending of Cost Scenarios



Once aspect that could change the savings and NPV calculations in the foregoing estimations would be differing rate increases in the consumable cost variables. Recall that the rate increases for the broth and agar variables were estimated based on material price

changes between the years of 2016 and 2017. This was a spot check based on available data. To overcome this weakness in our analysis, we conducted two more simulations on the cost savings data to estimate the impact of consumable price rate changes. Recall that the original price rate increases were agar 7.16% and broth 10.81%. The first simulation was performed using lower price rate increases; agar 5.0% and broth 7.5%. The second simulation was performed using higher price rate increases; agar 10.0% and broth 15.0%. The impact on the cost savings, NPV and base estimation are shown in tables 4.15 and 4.16.

4.15 Low Price Rate Increase Impact; Agar 5.0% and Broth 7.5%

	Base Scenario1	Growth Scenario 1	Growth Scenario 2
Scenario	224	10%	15%
Year 1	$ 36,872.02	$ 36,872.02	$ 36,872.02
Year 2	$ 37,985.57	$ 40,559.23	$ 42,402.83
Year 3	$ 39,139.04	$ 44,615.15	$ 48,763.25
Year 4	$ 40,334.22	$ 49,076.66	$ 56,077.74
Year 5	$ 41,573.00	$ 53,984.33	$ 64,489.40
Year 6	$ 42,857.38	$ 59,382.76	$ 74,162.81
Year 7	$ 44,189.45	$ 65,321.04	$ 85,287.23
Year 8	$ 45,571.44	$ 71,853.14	$ 98,080.32
Year 9	$ 47,005.70	$ 79,038.46	$ 112,792.36
Year 10	$ 48,494.71	$ 86,942.30	$ 129,711.22
sum	$ 424,022.52	$ 587,645.10	$ 748,639.19
NPV (Discount Rate=5.2%)	$ 320,167.92	$ 431,954.27	$ 540,591.38
NPV (Discount Rate=11.2%)	$ 241,202.18	$ 315,935.63	$ 387,508.93
Annualized Savings (5.2%)	$ 16,686.79	$ 22,512.97	$ 28,175.02
Annualized Savings (11.2%)	$ 27,014.72	$ 35,384.89	$ 43,401.13

4.16 High Price Rate Increase Impact; Agar 10.0% and Broth 15.0%

	Base Scenario	Growth Scenario 1	Growth Scenario 2
Scenario	224	10%	15%
Year 1	$ 36,872.02	$ 36,872.02	$ 36,872.02
Year 2	$ 38,319.10	$ 40,559.23	$ 42,402.83
Year 3	$ 39,867.38	$ 44,615.15	$ 48,763.25
Year 4	$ 41,527.75	$ 49,076.66	$ 56,077.74
Year 5	$ 43,312.51	$ 53,984.33	$ 64,489.40
Year 6	$ 45,235.55	$ 59,382.76	$ 74,162.81
Year 7	$ 47,312.52	$ 65,321.04	$ 85,287.23
Year 8	$ 49,561.14	$ 71,853.14	$ 98,080.32
Year 9	$ 52,001.45	$ 79,038.46	$ 112,792.36
Year 10	$ 54,656.11	$ 86,942.30	$ 129,711.22
NPV (Discount Rate=5.2%)	$ 336,900.50	$ 431,954.27	$ 540,591.38
NPV (Discount Rate=11.2%	$ 252,307.77	$ 315,935.63	$ 387,508.93
Annualized Savings (5.2%)	$ 17,558.88	$ 22,512.97	$ 28,175.02
Annualized Savings (11.2%)	$ 28,258.55	$ 35,384.89	$ 43,401.13

The above simulations were performed to estimate the cost savings for the company by pursuing the Mycoplasma Exemption submission projects. The cost of testing was estimated for the past 5 years as well as the current cost of testing based on the average number of samples tested. Using that data, the cost of testing was projected over the next 10 years and estimated using the Net Present Value equation at a 5.2% and 11.2% discount rate. The estimations were assumed to be a cost savings based on no killed virus testing being performed. The NPV results ranged from $320,000 to $540,000 depending on the discount rate and scenario. The calculated NPV's for all scenarios were all positive, which indicates that each scenario was viable and beneficial to the company. Sample growth scenarios of 10% and 15% were used to estimate the impact of changing sample submissions to the baseline calculation of the average 224 samples per year. Pricing

changes in the consumable variables, based on available data, were also used to estimate the impact of differing price rate decreases or increases on the cost savings. As stated earlier, all simulations were performed to estimate any cost savings associated with the project and the benefit to the company. In each scenario, the cost savings as estimated by the NPV equation, appear significant as well as positive. This indicates a beneficial project that should be pursued by the company. Not only will the project produce a cost saving, but in all likelihood will produce an increase in efficiency for the laboratory in general, as any time that was spent on killed virus testing will be spent on other projects/tests.

CHAPTER V: CONCLUSION

The Mollicutes class of organisms, throughout this discussion referred to as Mycoplasma, has been a significant pathogen in the animal health industry. The organisms cause such maladies as otitis media, pneumonia, arthritis, and reduced growth rate in the cattle, swine and avian industry. Not only are the organisms common pathogens to the health industry, but the organisms are common laboratory contaminants of cell lines and materials of animal origin.

It is a requirement of most pharmaceutical/biological regulatory authorities to test materials of animal origin for the presence or absence of Mycoplasma. This is true of the USDA, FDA and European Regulatory Authority. The focus of this research topic was for materials that are under the authority of the USDA. Specific regulatory guidance regarding Mycoplasma testing under the USDA is found in 9 CFR 113.53, which states:

> "Samples of each lot of ingredient of animal origin, which is not subjected to heat sterilization, used to prepare a biological product shall be shown free of Mycoplasma by the method prescribed in 113.28."

9 CFR 113.28 is the specific Mycoplasma test method, which outlines the process for testing a material for the presence or absence of Mycoplasma. As per 113.53, any material of animal origin that is not heat sterilized, is required to be tested for Mycoplasma. However, there is one exception to this requirement, which is found in 9 CFR 113.200(c)(3). 9 CFR 113.200(c)(3) states the following:

> "If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for Mycoplasma as prescribed in 9 CFR 113.28."

The statement in 9 CFR 113.200 is in regards to killed virus production. This statement offers a possibility to be free from Mycoplasma testing for a killed virus material. This

opportunity is further clarified in Veterinary Services memorandum 800.86, which is intended to clarify 9 CFR 113.200. The memorandum is specific for the killed virus harvest and serial, and outlines the requirements necessary to submit a Mycoplasma Exemption request to the USDA. This request is contingent upon the manufacturer proving that the inactivation process for the killed virus would kill any viable Mycoplasma as well.

As a company that manufactures killed viruses, this regulatory guidance is of particular interest, since the Mycoplasma test is a costly and labor intensive assay. This lead to several research questions:

1. Does the facility have any killed virus products that can be conclusively shown to kill Mycoplasma as well as the virus.
2. If multiple products exist, which would be the most economically beneficial for the company?

Given the foregoing research questions, three specific objectives were developed to identify the economic effect of current regulatory requirements for Mycoplasma testing. These three objectives were:

1. Evaluate the bank of inactivated virus products in the company and assess and rank the effectiveness of potential alternatives for the Mycoplasma exemption initiative
2. Conduct the economic analyses of the top-three alternatives to determine the most economically beneficial alternative in meeting economic and regulatory expectations of the company
3. Develop an implementation plan for the most economically feasible alternative and evaluate the economic implications on the company.

Based on those objectives, the common virus inactivation methods were researched and compared to the inactivation methods used at the company. A chemical inactivant was found that was used for killed virus manufacturing at the facility. Knowing the inactivation method was present, the next step was to then analyze the laboratory data to analyze the economics of the Mycoplamsa exemption project.

The previous 5 years of laboratory sample data was analyzed to determine the average number of total killed virus samples tested per year, as well as the number of individual product samples tested per year. Based on this analysis, the top 10 killed virus fractions were found that were present in the top 3 killed virus finished products. It was found that multiple virus fractions went into two of the three final products. Based on the analysis of the laboratory data, it was determined that the average number of killed virus tests performed per year was 224. The bulk of that testing was performed for two products, labeled Product 1 and Product 2. The top 2 products accounted for 211 of those 224 samples. Based on this data, it was found that Product 1 or Product 2 would be the most economically beneficial for this project.

In order to assess the value of the Mycoplasma Exemption project, the cost of the project needed calculated. In order to evaluate this cost, two variables were assumed to be critical. These variables were: (1) Reagents and other consumables; and (2) Time/Labor of analysts. Three equations were developed to calculate the cost of testing based on in-process and final product tests. Once the cost of testing was known, then simulations were performed to project the cost savings over the next 10 years, assuming that all 3 products were approved for a Mycoplasma exemption.

The simulations to project the cost savings were performed based on the consumables variable and the time/labor variable. The increasing costs in the variables were defined as the future cost of testing, or FV, and estimated in the NPV equation. Different scenarios were used to project the cost savings to assess the impact to the company. One scenario used was to assess the impact of increasing sample load. Over the past 5 years, the number of samples tested per year had grown by 12.86%, so sample growth rates of 10% and 15% were calculated to project for growth in the number of tests. Finally, changes in the cost of consumables were used to assess the impact or cost savings to the company. The percent changes in the cost of consumables were calculated based on 2016 and 2017 values, which were agar 7.16% and broth 10.81%. Using these values, a low and high scenario were calculated, in which the following percentages were used; low – agar 5%, broth 7.5% and high – agar 10%, broth 15%.

In all scenarios, the NPV of the project was positive, indicating a viable and beneficial project. The total estimated savings over the next 10 years, based on the NPV equations, were estimated at in the range of $246,000 to $328,000 based on discount rates of 5.2% and 11.2%. The annualized savings were between $17,000 and $43,000. Given the small initial investment and the significant savings of the project, pursuing an exemption makes economic sense, but also presents an opportunity for greater efficiency in laboratory testing.

5.1 Opportunities for further study

The focus of this project was to provide an economic benefit to the company, in the form of reduced Mycoplasma testing, by pursuing a Mycoplasma exemption from the USDA. Not only will this decrease the cost of 9 CFR 113.28 Mycoplasma testing performed in the Quality Control laboratory, it will increase laboratory efficiency. By

spending less time on Mycoplasma testing, analysts can spend more time on other projects. In light of this project, the opportunities for further study lie in future submissions for Mycoplasma exemptions. If all killed virus samples can be submitted and granted, this will reduce the number of tests by the Quality Control laboratory significantly; by 224 samples based on the past 5 years of data.

Another opportunity for further study will be further research USDA guidance looking for similar exemptions or projects. The guidance for a Mycoplasma exemption project was clearly outlined by 9 CFR 113.200 and Veterinary Services Memorandum 800.86. There may be similar guidance or exemptions from other products or methods in the guidance as well. The opportunity will be to find those projects and to continue to decrease cost and increase efficiency.

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