Master of Public Health

Integrative Learning Experience Report

Rabies Titer Monitoring and Disease Surveillance

by

Allyson Katrina Monique Barksdale

MPH Candidate

submitted in partial fulfillment of the requirements for the degree

MASTER OF PUBLIC HEALTH

Graduate Committee:
Dr. Justin Kastner
Dr. Yunjeong Kim
Dr. Jeba Chelladurai

Public Health Agency Site:
Kansas State University Rabies Laboratory
August 2024 – April 2025

Site Preceptor:
Janine Seetahal DVM, MPH, PhD

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Copyright

Summary

Rabies is a dangerous disease of importance to public health. Its high case-fatality rate, zoonotic status, and the inability to treat it after symptoms have emerged make it a dangerous disease, but its economic burden makes it a disease of concern across many countries. Globally, dogs are the most common vector of rabies virus, but bats, skunks, and raccoons are the most common vectors of human exposure in the United States. Bats are the focus of this Applied Practical Experience due to their abundance, proximity to human dwellings, and their ability to infect via bite without notice.

This APE involved learning Next Generation Sequencing to detect viral ribonucleic acid (RNA) viruses in bat samples. The future goal of this would be to collect samples from bats that were involved in human exposure cases but tested negative for rabies. Surveillance testing these bats would determine if other common bat diseases are on the rise and is important to maintain proper protocol for human safety. Most bats are brought in for rabies testing and are disposed of afterward. The results of a surveillance study would be useful to determine if this is still the common protocol, or if bats with human exposure should require other diagnostic tests for diseases after rabies testing is completed. The second goal of the APE involved learning the entire process of the titer monitoring side of rabies laboratory work. Guidelines are produced to keep laboratory workers protected when working with infectious pathogens and multiple sections and systems are needed for this to be effective. I worked with Quality Control, our Medical Records Coordinator, and Dr. Seetahal in her capacity as the Rabies Laboratory director, and this culminated in creating a Standard Operating Procedure (SOP) for rabies titer monitoring among the employees of the KSU Rabies Laboratory and the KSU College of Veterinary Medicine and a titer monitoring spreadsheet for tracking going forward.

Subject Keywords: rabies, titer monitoring, laboratory safety, disease surveillance, bats

Table of Contents

Chapter 1 - Introduction

Rabies Overview

Rabies has a reputation as a dangerous, deadly disease, and deservedly so. This disease has a case-fatality rate of nearly 100% once symptoms appear, making it one of the deadliest diseases in the world [1]. Rabies has been described in ancient texts dating back to 1950 BCE [1] Mesopotamian texts from around this time describe a “poison or venom” in the bite of dogs or other animals that lead to death [1]. Even the earliest texts acknowledging this disease recognized its zoonotic nature and its fatality. An early Roman writer, Aelian, also noted that bites from rabid dogs were always fatal and mentioned rabid dogs when describing venoms and poisons for arrows [2]. While not mentioned explicitly as rabies, there were some suggestions of it in Kautilya’s *Arthashastra*, regarding bio-weapons causing “biting madness” among enemy combatants that sound similar to the disease [2]. There was also a strategy of using “hollow spheres with the slobber from rabid dogs [to] cause epidemics” described by a Polish general, Jan Kazimierz Siemienowicz, in 1650 [2, 3].

Rabies is a negative-sense, single-strand RNA virus from the family *Rhabdoviridae* and the genus *Lyssavirus*. It is typically transmitted via saliva through bites or scratches. Symptoms include fever, headache, agitation, hallucinations, seizures, and hydrophobia before death due to brain encephalitis [4]. Rarer routes of transmission include organ transplants and airborne transmission [1]. Airborne transmission of rabies is typically a risk for laboratory workers handling concentrated virus. The incubation time for rabies virus varies from a few weeks to a few months but has been documented at over a year for organ transplant cases (1). Rabies is responsible for around 59,000 deaths per year and with over 40% of these deaths being children under 15 years of age (who.int).

All mammals can be a vector for rabies, but dogs are the primary vector and reservoir for around 99% of rabies deaths worldwide [1]. In addition to rabies vectors, there are different variants of rabies virus that are often identified with a primary host reservoir, due to specific virus adaptations. Skunks typically carry skunk rabies variants and raccoons typically carry raccoon rabies variants, but any mammal can carry any variant of rabies. For example, it is not uncommon for domestic pets to become infected with wildlife variants of rabies due to playing with an infected carcass or getting into a fight with an infected wild animal. An outbreak of rabies in Cape fur seals that has persisted since 2024 shows that even marine mammals are not exempt as rabies vectors [5]. The primary reservoir for rabies in a geographic location often determines the dominant rabies variant there. Since dogs are the most common rabies vector

worldwide, it is also noted that canine rabies is the most common variant found when variant typing.

Rabies in the US

In the United States (US), wildlife is the primary vector for rabies virus and human deaths are much less prevalent at less than 10 per year [1]. Successful public health initiatives, including the registration and vaccination of pet dogs, and the use of animal control to reduce the number of stray dogs, eliminated the dog rabies variant from the US in 2007 [6]. This is a noteworthy achievement, but it is important to remember that dogs can still carry other variants if they are exposed and infected. Over 90% of human rabies infections in the United States are caused by bats, raccoons, skunks, foxes, and mongooses (Fig 1.1). Bats are implicated in only 33% of US rabies infections, but are responsible for roughly 70% of US deaths due to rabies [4]. Most US bat species have teeth small enough to make a bite imperceptible. This means bat bites and scratches may be less recognizable as potential exposures compared to bites and scratches from larger animals.

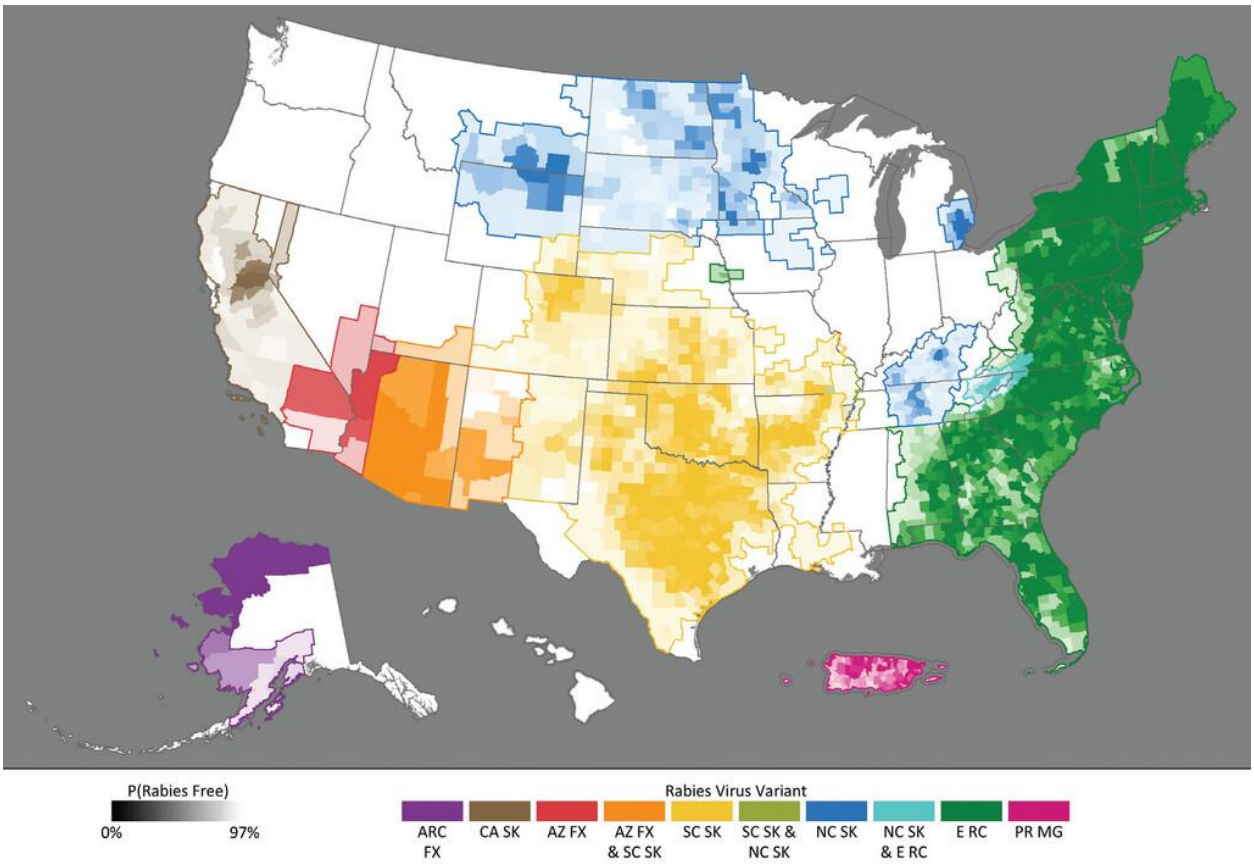


Figure 1.1: Map of Mesocarnivore Rabies Variants in the United States

RVV = Rabies Virus Variant. ARC FX = Arctic Fox RVV. AZ FX = Arizona Gray Fox RVV. CA SK = California Skunk RVV. E RC = Eastern Raccoon RVV. PR MG = Puerto Rico Mongoose RVV. NC SK = North Central Skunk RVV. SC SK = South Central Skunk RVV. [7]

Kansas State University Rabies Lab

The Kansas State University Rabies Laboratory is located in Manhattan, Kansas, and performs serological and diagnostic testing on samples submitted from around the globe (Fig 1.2, 1.3). The primary tests performed are called Fluorescent Antibody Virus Neutralization (FAVN) tests for animal export and Rapid Fluorescent Foci Inhibition Tests (RFFIT) to detect and quantify rabies titers in humans and animals [8, 9]. FAVN tests measure an animal's response to the rabies vaccine whereas the RFFIT measures the patient's ability to neutralize rabies virus [10]. RFFIT assays are also utilized for research studies, vaccine and pharmaceutical testing since the lab can perform testing to Good Manufacturing Practice, Good Clinical Practice, and Good Laboratory Practice guidelines. The Rabies Laboratory also performs diagnostic Direct Fluorescent Antibody Tests (DFAT) on necropsy specimens to determine if the animal was infected with rabies or not [10]. These results are reported, monitored, and used for surveillance of rabies in the state of Kansas in collaboration with the Kansas Department of Health and Environment (KDHE). This data can be used to track changes in rabies case submissions, the type and number of species submitted, and the frequency of rabies cases across these metrics. This type of monitoring is critical to studying the epidemiology of rabies in the US and alerting public health officials if an increase in positive cases is observed.

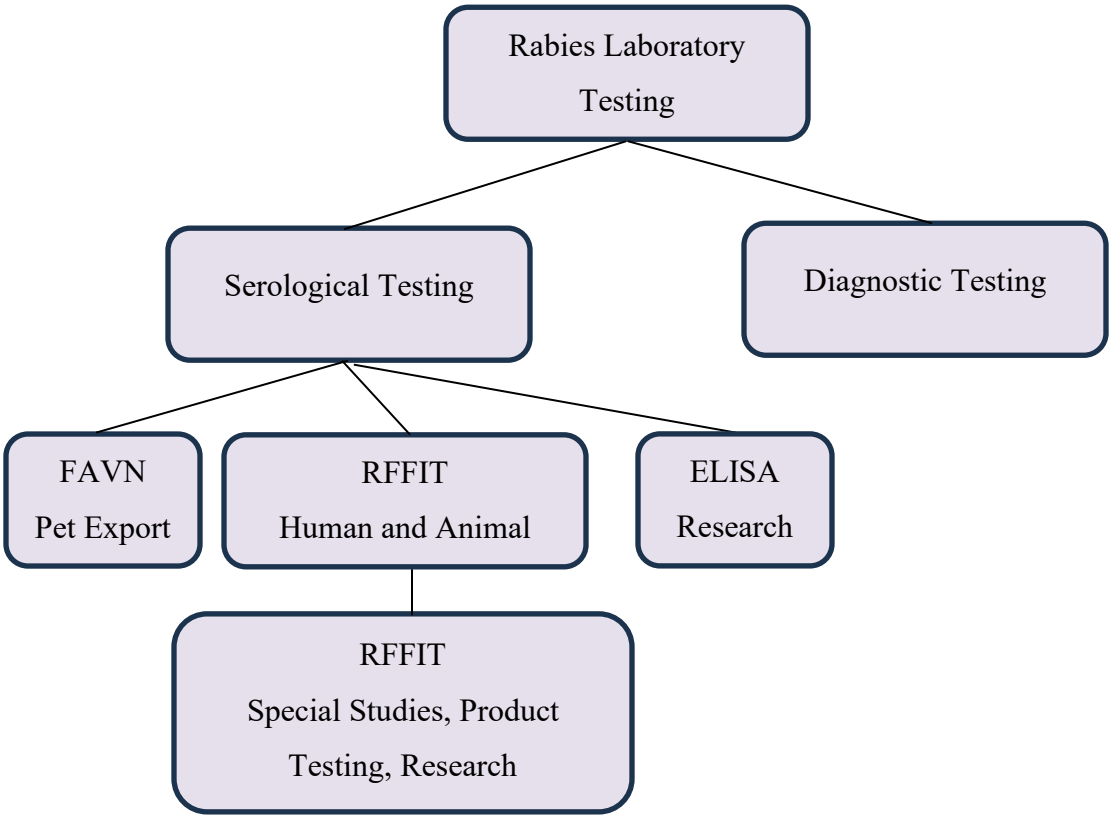


Figure 1.2: The testing sections of the Kansas State University Rabies Laboratory [8-10]

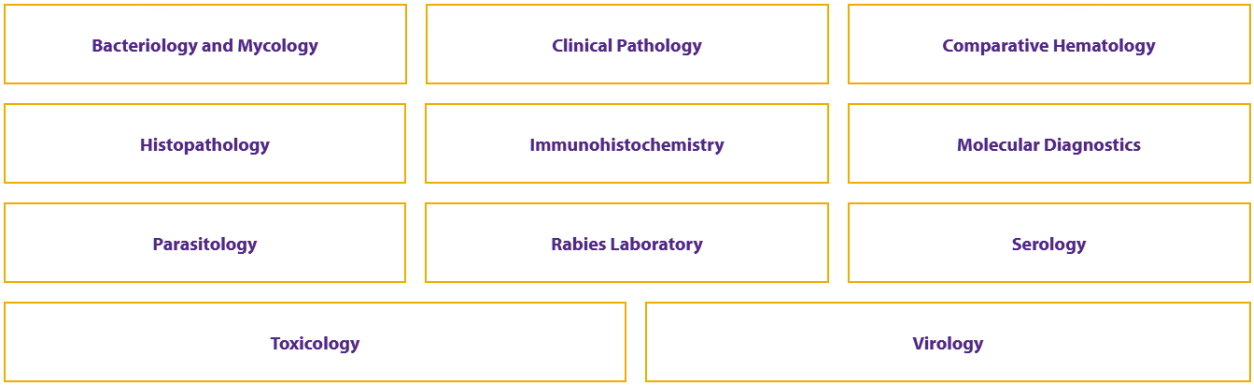


Figure 1.3: The Kansas State University Veterinary Diagnostic Laboratory Sections [11]

Rabies Treatment and Prevention

As deadly as rabies can be, it is not without treatment or prevention. Louis Pasteur first developed a crude rabies vaccine in 1885 by passaging rabies virus in rabbits [12]. This process was improved over time with the use of chemicals to improve virus inactivation [12]. Treatment consists of post-exposure prophylaxis (PEP). When a bite or scratch is observed from a rabies-suspect animal, the wound should be thoroughly washed with soap and water for 15 minutes as soon as possible. If treatment for rabies is indicated, human or equine immunoglobulin (HRIG or ERIG) is injected with dose dependent on bodyweight, and an intramuscular rabies vaccine is applied on days 0, 3, 7, and 14 [12]. This treatment protocol has been effective in nearly 100% of cases when administered as soon as possible after exposure. Pre-exposure prophylaxis (PrEP) is also available to those whose work puts them at a higher risk for rabies infection.

Rabies Safety and Laboratory Work

The World Health Organization (WHO) defines occupational health as “promoting and maintaining the highest degree of physical, mental, and social well-being of workers in all occupations” [13]. This can look quite different depending on the occupation and laboratory work requires special considerations to keep its workers safe while being productive. Laboratory technicians may work with sharps, dangerous chemicals, aerosol-generating equipment, and live animals, in addition to their work with infectious pathogens. This is why multiple safety training sessions are implemented in the form of online training programs, the assigning of Standard Operating Procedures (SOP) related to the job, and the reading of biosafety manuals. All of this is done to educate new workers thoroughly and prevent laboratory acquired infections and other workplace safety incidents. The worry of getting a laboratory acquired infection working with rabies virus is low today, but the consequences if it occurs is extremely high. That is why some laboratories have a titer monitoring program in effect for scientists working with certain agents. The Advisory Committee on Immunization Practices (ACIP) is the advisory board that recommends when to vaccinate workers against what agents [14]. Workers that handle rabies virus are one of the few groups that are recommended to get the vaccine due to the virus’ lethality and the vaccine’s efficacy. This area of occupational health became the main topic of my project.

The dangers of rabies have been well-established, but work with the virus is necessary to perform assays and research to expand current knowledge and make improvements. This means people will be working with rabies-suspect animals or highly concentrated laboratory

strains of rabies virus and need to be safe doing so. Rabies is classified as a Biosafety Level 2 pathogen and requires the appropriate equipment and training to handle this pathogen safely without causing an exposure to oneself or a colleague [15]. Rabies is fragile in the environment and does not last long outside of the body [16], so the biggest risk when handling concentrated virus is accidentally aerosolizing it and inhaling it. There are two separate cases of rabies laboratory workers getting exposed to rabies as a result of their jobs. One was a veterinarian who died after attempting to create an experimental rabies vaccine in 1973 [17]. While appropriately clothed, the protocol required blending the brains of rabid goats and aliquoting the homogenized mixture via mouth pipette. It was believed that he was exposed during this procedure since the mask would have to be removed in order to properly mouth pipette. This person developed symptoms 3 weeks later and passed away despite having had PrEP 13 years earlier.

A second case occurred in 1977 when a laboratory technician came down with symptoms after spraying a modified live virus suspension through a pharmaceutical manufacturing machine for several hours over three days [18]. Apparently, the machine was prone to leakage, and the worker became exposed to aerosolized rabies virus. This person also had PrEP and had been tested for an active titer six months prior to this incident. Despite an active titer, this person contracted rabies and fell ill. After slipping into a coma for over two weeks, this worker awoke and started making some progress toward recovery.

Both of these cases involved equipment that was not appropriately designed to perform the task while keeping the worker safe from aerosols. Use of equipment, such as biosafety cabinets, and sparing use of equipment like vortex mixers and centrifuges helps to minimize the aerosol and droplet creation risk. Certain techniques, such as mouth pipetting, are no longer used due to the safety risk to workers. Manual or electronic pipettes are now the standard. While we look at leaky equipment and mouth pipetting with incredulity today, it is likely that some of today's standard equipment and techniques may be derided in the future as incredibly unsafe. This is why safety equipment and protocols ought to be continuously reevaluated.

One Health and Rabies

Zero by 30 is a global plan created through the cooperation of the WHO, the Food and Agriculture Organization (FAO) of the United Nations (UN), the World Organization for Animal Health (WOAH), and the Global Alliance for Rabies Control; their collaborative initiative seeks to eliminate human deaths due to dog-variant rabies by 2030 [19]. The countries that have already achieved this goal did so through large-scale dog vaccination campaigns that achieved at least

a 70% vaccination rate, through controlling the stray dog population, and through education of pet owners and children [20, 21]. Getting these results from countries with higher incomes and more resources was still a challenge. Looking forward, the harder work now begins; public health leaders must find methods to work with countries that have larger stray dog populations with fewer resources to tackle the problem. This will be a considerable undertaking, but other countries have shown that not only can dog variant rabies be managed, but it can be eliminated.

Chapter 2 - Learning Objectives and Project Description

My APE consisted of two objectives: become familiar with Next Generation Sequencing (NGS) testing to expand my knowledge of viral assays beyond those typically performed in the rabies laboratory, and to become more familiar with the titer monitoring process to create a proper SOP for the task. This first objective came about when Dr. Seetahal and I were discussing a joint interest in wildlife health. My prior experience with rabies serological assays and diagnostics testing on necropsy specimens meant there was more opportunity in me expanding outside of rabies into some surveillance testing. I shadowed a laboratory technician in the NGS section for a week, learning the process of performing the assay and obtaining base pair results for analysis. I also attended a workshop to learn how to operate a new NGS machine ordered for the college.

We created a study to look at bats that were submitted to the KSU Rabies Laboratory for diagnostic testing that had tested negative for rabies but had an exposure history of physical human interaction. This proved difficult since most bats brought in for rabies testing have human interaction via proximity or pet interaction. Bats may live in attics or get into the house by accident, but most do not engage in physical incidents with humans, like bites or scratches, that would indicate rabies exposure. Once this was recognized, we had to reevaluate our approach to exposure history and expand it to include bats that were reported in close proximity to humans. Another issue is that sample quality degrades over time and the bat specimens are often not in great condition when submitted for testing. That further narrowed down potential samples. We also planned to keep track of whether frozen or refrigerated storage could potentially affect results. I was able to narrow down 10 bat samples, 5 frozen and 5 refrigerated, with appropriate histories and serviceable sample status, to collect oral and fecal samples from. The samples were stored in a guanidine buffer solution created with the help of Dr. Lance Noll from the Molecular team featuring guanidine isothiocyanate, Tris-HCl, EDTA, and distilled H_2O. Unfortunately, the NGS team remained steady with large sample testing volumes throughout the end of the APE, and we were unable to run the samples or analyze them. However, the samples may still be run and analyzed in the future. The experience learning the new assay, planning the study, and researching the reagents and materials has still broadened my experience in the research of infectious disease and the process required to conduct surveillance studies on necropsy specimens.

I also needed to become familiar with the rabies titer monitoring process. This included learning the Advisory Committee on Immunization Practices (ACIP) guidelines for employees at

risk of rabies exposure. Rabies laboratory employees working with concentrated rabies virus for serological assays merit the most frequent titer monitoring schedule at every 6 months [14]. Those who handle rabies-suspect animal carcasses, for diagnostic testing purposes, require a schedule of at least every 2 years, but the head staff at the KSU Rabies Laboratory elected to put those individuals on the 6-month schedule instead of the 2-year schedule. It was also decided that staff on the Molecular team that perform typing of rabies positive samples would also be tested every 6 months. While not always necessary from a safety standpoint, more testing is better than less in this case. The institution in charge can always push for more testing to be safe, but it is ill-advised to not meet the minimum recommendations of the ACIP.

Chapter 3 - Description of Products

The products produced for my APE consist of (1) an SOP detailing the process of titer monitoring for employees and (2) a monitoring spreadsheet to keep track of rabies titers for current and past employees.

The SOP includes a short background on the scientific and practical aspects of rabies virus, how it can be transmitted and through what vectors, and an overview of pre- and post-exposure prophylaxis. Risk categories as described by the Advisory Committee on Immunization Practices (ACIP) are defined by risk of recognized or unrecognized exposure to rabies [14]. The ACIP also defines a minimum acceptable titer for working within the rabies laboratory. Obtaining pre-exposure prophylaxis (PrEP) for unvaccinated employees, postexposure prophylaxis (PEP) or a booster for vaccinated employees, and performing the routing titer checks of employees are all detailed in the SOP. This required learning the new system for requesting purchase orders from VDL Supply for supervisors, medical records management, the appointment process at Lafene Health Center to acquire the sample, sample transport back to the rabies laboratory, and the mail room process for accessioning the samples for testing via the Laboratory Information Management System (LIMS).

The monitoring spreadsheet contains a list of KSU Rabies Laboratory and KSU Veterinary Diagnostic Laboratory employees dating back to 2018 that currently, or formerly, needed to maintain an active rabies titer. Records are required to be kept on file for seven years post departure to comply with regulations. A separate spreadsheet allows the laboratory director to view the titer information as a whole and over time, in a centralized location instead of just in part. This presentation also allows the director to observe any outliers and document any incidents to explain them. Titers less than the minimum acceptable value are automatically flagged in red to alert the director as they input values. This makes it easy to start the booster process described in the SOP. The monitoring spreadsheet, while seemingly technical in nature, is truly focused on public health by providing the Rabies Laboratory with a tool for safeguarding occupational health.

Chapter 4 - Reflection on Learning

The KSU Rabies Laboratory has a stellar reputation for a reason. There are many processes in place to keep from running afoul of laws and regulations. The institution is subject to many external and internal audits to make sure things are running appropriately. Even with all of this in place, there is always the question of whether more could, or should, be done.

Safety never sleeps. As more information comes to light, processes change. This can result in greater caution going forward, or action reversal if it is determined that greater action does not result in a better outcome for that stage of the process. When reviewing the process for titer monitoring in the Rabies Laboratory, it was apparent that there was very little room for error. The risk of death involved with a potential exposure was too great to allow an oversight regarding someone's protective titer, or potential lack thereof. The biggest obstacles did not involve setting up titer draws or performing the assay. The staff are highly proficient at this and the staff at Lafene Health Center are very accommodating for sample collection. Ironically, this was the main obstacle that needed to be addressed. Everyone's present familiarity with the process can lead to things not being written down for others to observe or perform. Titer monitoring itself is important, but the process to arrange blood draws, process the samples, and result them were piecemealed across multiple SOPs and were not defined explicitly. The high degree of familiarity with these processes and the individual people that conduct them could pose a problem when employees leave or change positions. This was the main reason why the SOP was drafted. Other obstacles included how to find a date for blood draws that accommodated over 25 employees, without interrupting work tasks, and how to accommodate those who miss designated draw dates. Scheduling phlebotomists to come from Lafene to the Rabies Laboratory ended up being simple to arrange and minimized the back and forth of multiple employees in the lab throughout the day. The product ordering system changed midway through the process of writing the SOP, so that was another factor to navigate. Those who miss draw days have the option of being drawn by a licensed phlebotomist within the laboratory, or they can travel to Lafene, or another healthcare provider, to collect and bring back the sample. This meant we needed a safe way for individual employees to transport blood samples to and from the Rabies Laboratory.

Rabies surveillance via diagnostic DFAT testing is performed every day, but there is always the chance that submitted samples may carry other diseases of concern. The zoonotic viruses that bats can carry tend to be severe, but non-zoonotic viruses can cause problems for other animals, which can result in economic burden if left unchecked. The only way to know is to

test. It would also be a problem if bats started carrying a disease that impacted their own ability to exist and reproduce naturally. Bats are important to the ecosystem in North America as pest control and pollinators. In the interest of public health, we must balance the knowledge of their potential disease risk with their known benefits.

Student Attainment of MPH Foundational Competencies

Most of my APE and ILE foundational competencies fall under evidence-based approaches to public health, but my courses thoroughly covered the other groupings, as well. Social and Behavioral Bases of Public Health (MPH 818) and Environmental Health (MPH 802) both covered workplace safety, which became the main topic for my project. Administration of Health Care Organizations (MPH 720) and Multidisciplinary Thought and Presentation (DMP 815) also proved helpful when writing the draft SOP as a product of my project. I had to learn how the Rabies Laboratory worked with our local campus health center and learn which tasks fell to whom. DMP 815 taught me how to write to target my audience. Once I had the required information, I was able to phrase the SOP in a way that someone with a science background, but was new to the job and the organization, could navigate the titer monitoring process.

Table 4.1 Summary of MPH Foundational Competencies

Number and Competency		Description
1	Apply epidemiological methods to the breadth of settings and situations in public health practice	Rabies-negative bats with a history of human exposure were selected for sampling. Employees that work with concentrated rabies virus and rabies-suspect necropsy cases qualified for titer monitoring.
2	Select quantitative and qualitative data collection methods appropriate for a given public health context	NGS testing was selected for bat research and the RFFIT endpoint assay was selected for rabies titer monitoring.
3	Analyze quantitative and qualitative data using biostatistics, informatics, computer-based programming and software, as appropriate	Titers were evaluated for compliance with the recommended minimum value and other significant deviations, and were recorded in the Laboratory Information Management System and Microsoft Excel.
4	Interpret results of data analysis for public health research, policy or practice	Titers below the minimum threshold resulted in employee contact from their direct supervisor and a cessation of rabies-related job duties until an effective serological response is achieved.
21	Perform effectively on interprofessional teams	I worked with multiple teams, including Molecular, NGS, Quality Control, RFFIT, and Special Projects, to find the relevant information for arranging health care, testing responsibility among sections, and how results are relayed in accordance with various regulations.

Table 4.2 MPH Foundational Competencies and Course Taught In

22 Public Health Foundational Competencies Course Mapping	MPH 701	MPH 720	MPH 754	MPH 802	MPH 818
Evidence-based Approaches to Public Health					
1. Apply epidemiological methods to the breadth of settings and situations in public health practice	x		x		
2. Select quantitative and qualitative data collection methods appropriate for a given public health context	x	x	x		
3. Analyze quantitative and qualitative data using biostatistics, informatics, computer-based programming and software, as appropriate	x	x	x		
4. Interpret results of data analysis for public health research, policy or practice	x		x		
Public Health and Health Care Systems					
5. Compare the organization, structure and function of health care, public health and regulatory systems across national and international settings		x			
6. Discuss the means by which structural bias, social inequities and racism undermine health and create challenges to achieving health equity at organizational, community and societal levels					x
Planning and Management to Promote Health					
7. Assess population needs, assets and capacities that affect communities' health		x		x	
8. Apply awareness of cultural values and practices to the design or implementation of public health policies or programs					x
9. Design a population-based policy, program, project or intervention			x		
10. Explain basic principles and tools of budget and resource management		x	x		
11. Select methods to evaluate public health programs	x	x	x		
Policy in Public Health					
12. Discuss multiple dimensions of the policy-making process, including the roles of ethics and evidence		x	x	x	
13. Propose strategies to identify stakeholders and build coalitions and partnerships for influencing public health outcomes		x		x	
14. Advocate for political, social or economic policies and programs that will improve health in diverse populations		x			x
15. Evaluate policies for their impact on public health and health equity		x		x	
Leadership					
16. Apply principles of leadership, governance and management, which include creating a vision, empowering others, fostering collaboration and guiding decision making		x			x
17. Apply negotiation and mediation skills to address organizational or community challenges		x			
Communication					
18. Select communication strategies for different audiences and sectors	DMP 815, FNDH 880 or KIN 796				
19. Communicate audience-appropriate public health content, both in writing and through oral presentation	DMP 815, FNDH 880 or KIN 796				

22 Public Health Foundational Competencies Course Mapping	MPH 701	MPH 720	MPH 754	MPH 802	MPH 818
20. Describe the importance of cultural competence in communicating public health content		x			x
Interprofessional Practice					
21. Perform effectively on interprofessional teams		x			x
Systems Thinking					
22. Apply systems thinking tools to a public health issue			x	x	

Student Attainment of MPH Emphasis Area Competencies

Competency 1: Pathogens/pathogenic mechanisms

General Virology (BIOL 730) was a great introduction to learning more about lyssaviruses and their effect on hosts. It was through this class that I learned how the virus travels through the nervous system before reaching the brain where it replicates enough to start symptoms in the host. This is also how I learned about the multiple variants and phylogroups of lyssaviruses.

Competency 2: Host response to pathogens/immunology

While multiple courses touch on host response to pathogens, Domestic Animal Immunology (DMP 850) was instrumental in giving me greater understanding about the full topic of immunology. While I had learned the basics of how rabies infects a host and replicates in BIOL 730, this class provided the background information concerning why the mechanisms are so successful in mammals, why the vaccine works, and why there is a time constraint for rabies immunoglobulin to provide protection for the infected host.

Competency 3: Environmental/ecological influences

Environmental Health (MPH 802) and One Health (DMP 710) both cover how changes in the environment can affect host and pathogen behavior. Both human-created obstacles, such as urban or agricultural development, and natural occurrences, like less snow than usual or too much rain at once, may drive hosts to seek new territory for better feeding opportunities. It could also cause them to venture closer to humans due to lack of options otherwise. This increases human-animal conflict and the risk of zoonotic disease spread. Close human proximity to wildlife/feral animals is a common precursor to spillover events and rabies exposure to both humans and domestic animals.

Competency 4: Disease surveillance

One Health (DMP 710), Introduction to Epidemiology (MPH 754), and Intermediate Epidemiology (MPH 854) each covered disease surveillance through multiple scientific studies. This allowed me to contribute ideas for the sample selection for the bat study.

Competency 5: Disease vectors

One Health (DMP 710) was great for covering disease vectors and sentinel animals in the context of public health. Most vector-borne diseases of importance to public health use parasites or insects as a vector but have separate hosts and reservoirs for the disease. Rabies is unique in having the hosts/reservoirs also serve as the vector to transmit the disease.

Table 4.3 Summary of MPH Emphasis Area Competencies

MPH Emphasis Area: Infectious Disease and Zoonoses		
Number and Competency		**Description**
1	Pathogens/pathogenic mechanisms	**Evaluate modes of disease causation of infectious agents.** Rabies travels through the nervous system of the host until it hits the brain, causing encephalitis, fever, hydrophobia, and other symptoms, until it is excreted in the saliva where it is commonly spread through bites.
2	Host response to pathogens/immunology	**Investigate the host immune response to infection.** Knowing the host response to rabies is important for understanding the need for titer monitoring among employees who work with the virus and knowing how to prevent potential infections.
3	Environmental/ecological influences	**Examine the influence of environmental and ecological forces on infectious diseases.** There are many wildlife reservoirs for rabies virus and all of them are important to the environment. Bats
4	Disease surveillance	**Analyze disease risk factors and select appropriate surveillance.** Diagnostic rabies testing is performed regularly and the information obtained from those results is utilized to observe rabies disease patterns in Kansas over time. We designed a small surveillance study to see if bat viral diseases were found in bats located near humans.
5	Disease vectors	**Investigate the role of vectors, toxic plants and other toxins in infectious diseases.** The vectors host and spread rabies virus to other potential hosts, allowing the virus to replicate anew.

References

1. Tarantola, A. *Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, Its Prevention and Its Cure.* Tropical Medicine and Infectious Disease, 2017. **2**(2): p. 5.
2. Mayor, A., *Greek Fire, Poison Arrows, and Scorpion Bombs: Unconventional Warfare in the Ancient World*. 2022: Princeton University Press.
3. McIsaac, J.H., *Hospital Preparation for Bioterror: A Medical and Biomedical Systems Approach*. 2010: Elsevier. 464.
4. U.S. Centers for Disease Control and Prevention. *Rabies*. 2025.
5. Imray, G. *Scientists in South Africa say they have identified the first known outbreak of rabies in seals*. Associated Press News. 2024. https://apnews.com/article/seals-rabies-south-africa-environment-wildlife-faafc6b19a3e211853cd50952c5c802b.
6. U.S. Centers for Disease Control and Prevention. *US Declared Canine-Rabies Free CDC Announces at Inaugural World Rabies Day Symposium*. 2007. Centers for Disease Control and Prevention.
7. Boutelle, C., et al. *Rabies surveillance in the United States during 2023.* Journal of the American Veterinary Medical Association, 2025: p. 1-8.
8. Kansas Veterinary Diagnostic Laboratory. *FAVN Test*. 2025. https://ksvdl.org/laboratories/rabies-laboratory/favn-test/.
9. Kansas Veterinary Diagnostic Laboratory. *RFFIT Tests*. 2025. https://ksvdl.org/laboratories/rabies-laboratory/rffit-test/index-1-1.html.
10. Kansas Veterinary Diagnostic Laboratory. *Diagnostic Tests*. 2025. https://ksvdl.org/laboratories/rabies-laboratory/diagnostic-test/
11. Kansas Veterinary Diagnostic Laboratory. *Laboratories*. 2025. https://ksvdl.org/laboratories/.
12. Natesan, K., et al. *Developments in Rabies Vaccines: The Path Traversed from Pasteur to the Modern Era of Immunization.* Vaccines, 2023. **11**(4): p. 756.
13. World Health Organization. *Occupational Health*. 2025. https://www.who.int/health-topics/occupational-health.
14. U.S. Centers for Disease Control and Prevention. *Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories*, in *Morbidity and Mortality Weekly Report*. 2012: www.cdc.gov.
15. Barbanel, C. *Standard Operating Procedures: Rabies Protection Program*. 2004, Boston University.
16. Heymann, D.L. *Rabies*, in *Control of Communicable Diseases Manual*, D.L. Heymann, Editor. 2022, APHA Press. p. 526-536.
17. Winkler, W.G. *Airborne Rabies Transmission in a Laboratory Worker.* JAMA: the Journal of the American Medical Association., 1973. **226**(10): p. 1219.
18. U.S. Centers for Disease Control and Prevention. *Rabies in a Laboratory Worker — New York.* MMWR. Morbidity and mortality weekly report, 1977. **26**(22): p. 183-184.
19. World Health Organization, Food and Agriculture Organization of the United Nations, World Organization for Animal Health, Global Alliance for Rabies Control. *Zero by 30: The Global Strategic Plan to end human deaths from dog-mediated rabies by 2030*. 2018.
20. Zha, R., et al. *Exploring one health-based strategies for rabies elimination: Overview and future prospects.* PLOS Neglected Tropical Diseases, 2025. **19**(6): p. e0013159.

21. Food and Agriculture Organization of the United Nations. 2013. *DEVELOPING A STEPWISE APPROACH FOR RABIES PREVENTION AND CONTROL*. Proceedings of the FAO/GARC Workshop, November 2012, Rome, Italy. 2013.

Appendix

Product 1: Draft Standard Operating Procedure

PURPOSE

A. To outline and document a standard protocol for rabies vaccination and titer monitoring for KVDL rabies employees.

DEFINITIONS

A. ACIP: The Advisory Committee on Immunization Practices
B. DFA: Direct Fluorescent Antibody
C. eQMS: Electronic Quality Management System
D. FAVN: Fluorescent Antibody Virus Neutralization
E. FDA: Food and Drug Administration
F. LIMS: Laboratory Information Management System
G. PEP: Postexposure prophylaxis
H. PO: Product Order
I. PrEP: Preexposure prophylaxis
J. RFFIT: Rapid Fluorescent Focus Inhibition Test
K. RNA: Ribonucleic acid
L. RVNA: Rabies Virus Neutralizing Antibody
M. WHO: World Health Organization

RESPONSIBILITIES

A. It is the responsibility of the Section Head and Laboratory Manager to ensure training for staff that will perform this SOP. It is the responsibility of KVDL personnel using this procedure to read, understand, receive training for and agree to follow the procedure described in this SOP.

BACKGROUND

A. Rabies is caused by a single-stranded, negative-sense RNA virus that causes encephalitis of the brain. Once symptoms appear it is typically fatal in nearly 100% of cases. Symptoms of infection in humans include anxiety, insomnia, confusion, agitation, delirium, hallucinations, hydrophobia, hypersalivation, and seizures. Dogs are the primary vector of rabies worldwide, causing over 95% of deaths due to rabies, but bats, raccoons, skunks, and foxes are the primary vectors for rabies in the U.S. with bats comprising 70% of U.S. rabies deaths. If exposure is suspected, the patient is given post-exposure prophylaxis (PEP) to prevent rabies infection.
   Rabies virus is most commonly transmitted through direct contact with infectious saliva, making bites and scratches from a rabid animal the most common routes of transmission. Other potentially infectious material include nervous tissue, cerebrospinal fluid (CSF) and lacrimal secretions. Rare routes of rabies transmission include organ transplants and airborne

transmission. Laboratory workers may have an elevated occupational risk of exposure to concentrated, aerosolized rabies virus when conducting certain lab procedures. The incubation period varies from a few weeks to months, but can last over a year in human transplant cases.

B. Rabies preexposure prophylaxis (PrEP) is recommended for those with an elevated risk of rabies exposure and is comprised of 2 doses of an FDA approved rabies vaccine given 7 days apart. The Advisory Committee on Immunization Practices (ACIP) provides a detailed list of Risk categories from 1 (highest risk) to 5 (lowest risk) describing the risk of rabies exposure and suggesting recommended rabies virus neutralization antibody (RVNA) titer checks for long-term monitoring. The ACIP and the WHO define a minimum acceptable RVNA titer for rabies as ≥ 0.5 IU/mL, but some laboratories, especially those performing work with non-rabies lyssaviruses, may require an occupationally defined higher titer as an additional protective measure.
C. PrEP is required for KVDL laboratory employees who perform any testing or research which puts them at an elevated risk for recognized and or unrecognized exposure to rabies virus (i.e. ACIP Risk Categories 1-4). In accordance with ACIP guidelines, the risk category that a person falls into determines how often their titer is tested.
    a. Risk category 1 is described as having elevated risk due to working with concentrated, live rabies virus for diagnostic testing, research, or vaccine production. Laboratory work with live virus introduces the possibility of virus aerosolizing under certain conditions with specific activities within the workspace, which maybe a unrecognized form of exposure. Those in the KVDL that perform serological testing using virus neutralization assays (e.g. RFFIT or FAVN assays), diagnostic DFA testing, or nucleic acid extraction for rabies molecular diagnostics fall into this category. Those within this risk category will get their rabies titer tested every 6 months.
    b. Risk category 2 is described as having elevated risk due to frequent contact with bats, working in environments with high-bat density, or performing animal necropsies. Performing necropsy using sharp implements on rabies-suspect animals also increases the risk of contracting a rabies infection. Those in the KVDL who perform any testing or research with bats or perform animal necropsies fall into this category. Those within this category will get their rabies titer tested every 2 years.
    c. **NOTE:** Any titer below 0.5 IU/mL requires an immediate cessation of work that puts the worker at elevated risk of rabies infection. A rabies booster will be administered by an approved health care facility, with vaccine records submitted to the employer. Work duties may not be resumed until a new titer draw is taken and a serological response of ≥ 0.5 IU/mL is confirmed.

HAZARDS AND SAFETY PRECAUTIONS

A. Refer to KSVDL-RAB-1 for specific rabies lab safety procedures. Refer to the KSU Laboratory Safety Manual, KSU Chemical Hygiene Plan, and the KSU Biohazardous/Medical Waste Management and Sharps Procedures for further instructions and for sample spills or leakage, PPE policy and hazardous waste disposal. Wear appropriate PPE, including lab coat, safety glasses, and gloves.
B. Common hazards consist of virus aerosolization (vortexing, centrifugation, opening tubes), droplet creation (mechanical pipetting, liquid culturing, jostling of assay materials, pouring

off virus), handling contaminated sharps (scalpels, broken glass, needles), and handling contaminated equipment (pipettes, pipette aids, liquid handlers, microscopes).

QUALITY CONTROL

A. Processing and data entry are double-checked for accuracy prior to or during the final review (see KSVDL-RAB-4).

PROCEDURE

A. PrEP for unvaccinated employees
   a. All employees at risk of exposure to rabies virus through their job duties must receive rabies PrEP before training in, or conducting, any tasks involving rabies virus or rabies-suspect animal carcasses or tissues.
   b. A written acknowledgement of the occupational requirement for rabies vaccination and routine titer checks may be obtained at onboarding.
   c. The employee's supervisor requests a purchase order (PO) from VDL Supply for PrEP vaccination and a blood draw after completing the vaccination series. The employee schedules the 0 day and 7 day vaccine visits at an approved health care facility. The employee also schedules a blood draw 14-28 days after the 7 day dose for the titer check and will email this schedule to their supervisor and the Medical Records Coordinator.
      1. The employee will have access to their rabies immunization records through the health care facility's online portal. The employee will download and email these vaccination records to the Medical Records Coordinator to file in the eQMS.
   d. The employee takes the PO down to the health care facility and receives the required dose(s) of vaccine and or has a blood sample collected at the outlined times based on the vaccination schedule.
   e. The employee transports their blood sample to the rabies laboratory within 2 hours of collection using a small biohazard cooler as the transport container.
   f. The employee presents their sample to the laboratory for accessioning. The processing team will accession the sample using KSU Rabies Lab account "R16050" as the clinic, Rabies Lab "R6075" as the owner, and Rabies Lab "212" as the case coordinator. The sample will be run as a RFFIT endpoint. Titer results report to the employee's Section Head, the RFFIT Technical Supervisor, and the Medical Records Coordinator. The Medical Records Coordinator informs the employee's supervisor of the results.
      1. If the employee's titer is ≥ 0.5 IU/mL, the employee may proceed with training on tasks involving rabies virus.
      2. If the employee's titer is < 0.5 IU/mL, the employee must receive a third dose and a passing titer before moving forward with training on tasks involving rabies virus. The employee's supervisor will proceed with a PO for a booster, referring to Section c above.
   g. Rabies laboratory employees must receive titer checks every 6 months to determine serological response. New employees are added to this rotation, but are not required to have a titer check if their passing titer check occurred within 3 weeks of the next 6 month rotation. For additional titer checks these will align with the schedule for the entire laboratory.

B. PEP or booster for vaccinated employees
   a. If an employee's titer falls below 0.5 IU/mL, they must cease all work with live rabies virus until their titer is ≥ 0.5 IU/mL. If an employee has a recognized exposure to rabies virus, PEP is required.
      1. A recognized exposure occurs when there is a high probability of virus contact with an open cut, wound, or mucous membranes. Each possible exposure needs to be examined on a case-by-case basis, but may include:
         i. A spill or splash of rabies virus into an open cut or mucous membranes or prolonged presence around a high concentration of aerosolized virus.
         ii. A bite or scratch from a confirmed rabid animal.
         iii. Mucous membrane or open wound contact with saliva or nervous tissue of a rabid animal. Note that contact with blood, urine, or feces alone does not constitute exposure.
         iv. Any bite or scratch from an animal, especially wildlife, in which rabies cannot be confidently ruled out. Can occur if a live bat is submitted for testing.
         v. **NOTE:** Bat bites and scratches are small and can go unnoticed. If contact or suspected contact with a bat occurs and the bat cannot be caught for testing, exposure is assumed.
      2. If a recognized exposure occurs, immediately stop work and thoroughly clean the area with soap and water for 15 minutes. If exposure occurs to the eye, proceed to the nearest eye wash station and irrigate the eyes for 15 minutes. Notify your supervisor and the laboratory director of the incident, and initiate the accident reporting procedure.
         i. Call 911 in the case of an emergency.
         ii. If non-emergency treatment is indicated, call Nurse Triage (833-756-2007) and follow their instructions. The employee completes the KSU Incident Report after treatment and presents it to their supervisor for approval.
         iii. If no treatment is required, the employee completes the KSU Incident Report and presents it to their supervisor for approval.
   b. The employee's supervisor requests a PO from VDL Supply for PEP and a blood draw at an approved health care facility. The employee schedules the vaccine(s) and a blood draw at least 14 days after the final PEP vaccine. The employee emails the schedule to their supervisor and the Medical Records Coordinator
   c. The employee takes the PO down to an approved health care facility and receives the required vaccines.
   d. The employee returns to an approved health care facility for the blood draw and transports their blood sample to the rabies laboratory within 2 hours of collection using a small biohazard cooler as the transport container.
   e. The employee presents their sample to the laboratory for accessioning. The processing team will accession the sample using KSU Rabies Lab account "R16050" as the clinic, Rabies Lab "R6075" as the owner, and Rabies Lab "212" as the case coordinator. The sample will be run as a RFFIT endpoint. Titer results report to the employee's Section Head, the RFFIT Technical Supervisor, and the Medical Records Coordinator. The Medical Records Coordinator informs the employee's supervisor of the results.
      1. If the employee's titer is ≥ 0.5 IU/mL, the employee may proceed with tasks involving rabies virus.

2. If the employee's titer is < 0.5 IU/mL, another booster may be needed. The Laboratory Director will consult a health care provider and or public health officials on the best course of action for the employee.

C. Routine titer checks of previously vaccinated employees
   a. Employees at risk of rabies infection will receive regular titer checks in accordance with the current ACIP guidelines.
      1. Risk category 1 employees will receive checks every 6 months.
      2. Risk category 2 employees will receive checks every 2 years.
      3. Risk category 3 and 4 employees will schedule their titer checks when necessary after consulting with their respective supervisors.
   b. The Medical Records Coordinator, or other designated employee, contacts the laboratory administrators at an approved health care facility at least one month prior to the scheduled titer check. They arrange a date and time for 2 or more phlebotomists to come to the rabies laboratory to draw blood samples from employees. A notice of upcoming titer draw with date and location of event is emailed to the employees at least 2 weeks prior to the scheduled date.
      1. If an approved health care facility cannot come to the rabies laboratory, the Medical Records Coordinator or other designated employee will arrange a day and time range for rabies laboratory employees to go to an approved health care facility to get their blood drawn.
      2. In this case, a volunteer from the rabies laboratory will collect the blood samples from the health care facility to return the samples to the rabies laboratory once all employees have finished getting drawn.
   c. The Medical Records Coordinator compiles a list of the employees who need their titers checked, including their birthdates, and emails this list to the point of contact at the health care facility at least a week before the scheduled blood draws.
   d. The phlebotomists draw blood from employees at the designated time and place. the employees come in to get their blood drawn.
      1. If any employee cannot attend during the this time, it is the responsibility of the employee to inform the Medical Records Coordinator and the Laboratory Director at least 2 working days prior to the set date. They will then need to arrange to get this done at the health care facility and return their sample to the laboratory for processing at an agreed upon later date. Another option is to see if any licensed phlebotomists work in the laboratory and are willing to perform the blood draw in house.
   e. The group samples are arranged in alphabetical order and accessioned before passing to the RFFIT team for processing and testing. The samples are processed under one accession number to facilitate easy tracking and reporting. The processing team will accession the sample using KSU Rabies Lab account "R16050" as the clinic, Rabies Lab "R6075" as the owner, and Rabies Lab "212" as the case coordinator. The samples will be run as RFFIT endpoints.
   f. The RFFIT team tests the samples and logs the results into the LIMS.
      1. If the RFFIT team is experiencing a staff shortage or a high load of service samples, the Special Projects team can test the samples.
   g. When results are finalized, they are emailed automatically via the Laboratory LIMS to the Laboratory Director and the Medical Records Coordinator for review.

h. The VDL Quality team then emails each employee notifying them that their titer results are finalized. This allows each employee to view their results using the eQMS.
i. Each employee's titer is input into a tracking spreadsheet by the Medical Records Coordinator for monitoring by the Laboratory Director.
j. If an employee has a titer below 0.5 IU/mL, in consultation with the Laboratory Director the Medical Records Coordinator emails the employee and cc's the rabies Laboratory Director and the employee's supervisor of the finding with notification to cease all work with rabies virus. The employee's supervisor requests a PO for a booster vaccine from VDL Supply..

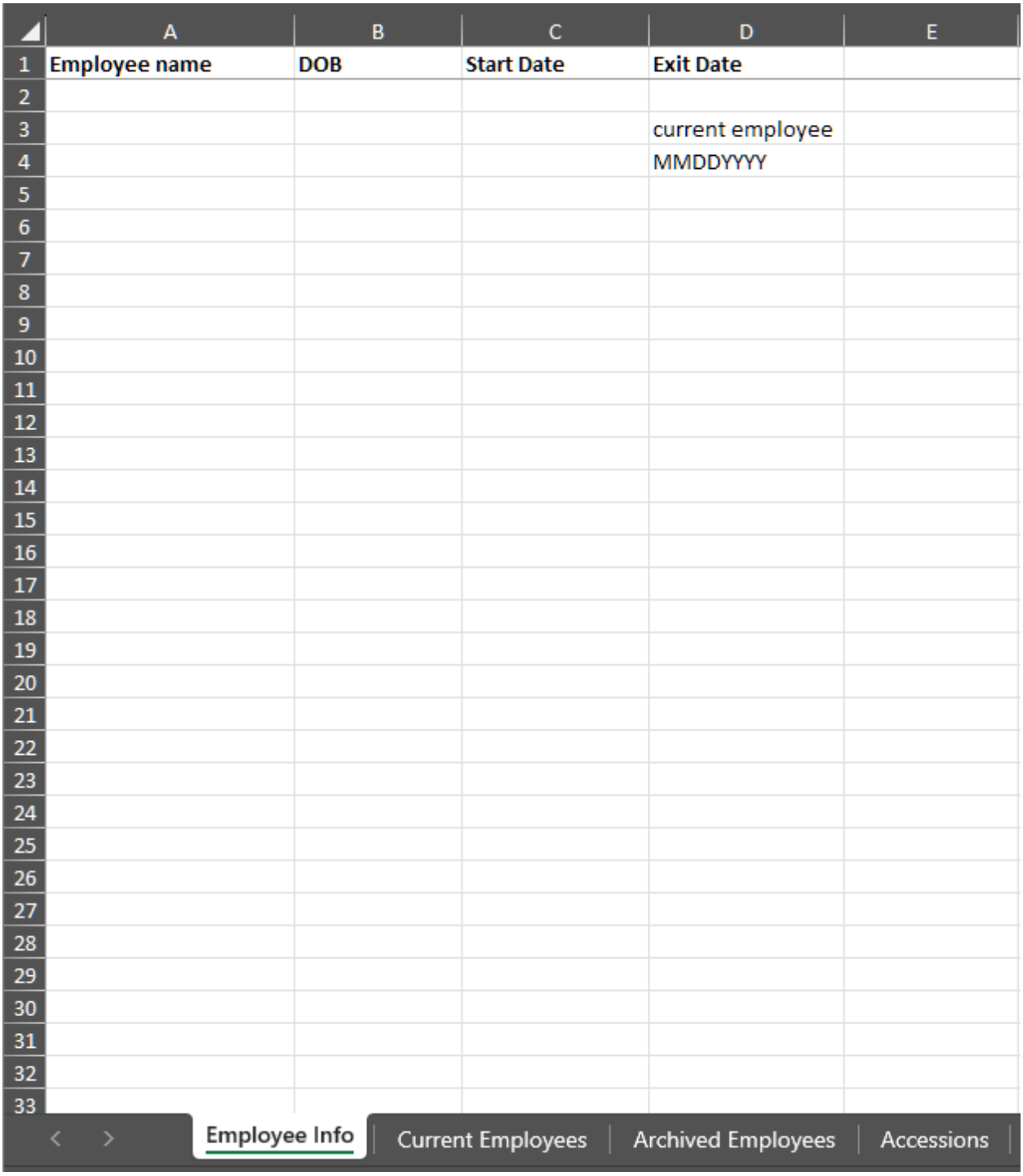
ASSOCIATED DOCUMENTS

[Due to privacy concerns, these have been redacted.]

REFERENCES

A. Centers for Disease Control and Prevention. Rabies. 2025. https://www.cdc.gov/rabies/
B. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. July 29, 1977. Vol. 26, 183-184. Rabies in a Laboratory Worker — United States, 2022.
C. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. Jan 6, 2012. Supplement/Vol. 61. Guidelines for Safe Work Practices in Human and Animal Medical diagnostic Laboratories.
D. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. May 6, 2022. Supplement/Vol. 71. Use of a Modified Preexposure Prophylaxis Vaccination Schedule to Prevent Human Rabies: Recommendations of the Advisory Committee on Immunization Practices — United States, 2022.
E. Standard Operating Procedures: Rabies Protection Program. Boston University. Mar 23, 2004. https://www.bu.edu/buohc/files/2009/07/bu_rabies_sop_3-23-04.pdf
F. Winkler WG, Fashinell TR, Leffingwell L, Howard P, Conomy JP. Airborne Rabies Transmission in a Laboratory Worker. JAMA. Dec 3, 1973; Vol. 226, No. 10:1219–1221. doi:10.1001/jama.1973.03230100043011
G. Mansfield, K. L., Andrews, N., Goharriz, H., Goddard, T., McElhinney, L. M., Brown, K. E., & Fooks, A. R. (2016). Rabies pre-exposure prophylaxis elicits long-lasting immunity in humans. Vaccine, 34(48), 5959–5967. doi.org/10.1016/j.vaccine.2016.09.058

Product 2: Titer Monitoring Spreadsheet



	A	B	C	D	E
1	**Employee name**	**DOB**	**Start Date**	**Exit Date**	
2					
3				current employee	
4				MMDDYYYY	
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					

Employee Info | Current Employees | Archived Employees | Accessions

	A	B	C	D	E	F
1	**Rabies - FT Staff**	**H1 2018** R18-XXXXXX	**H2 2018** R18-XXXXXX	**H1 2019** **R19-XXXXXX**	**H2 2019** **R19-XXXXXX**	**H1 2020** **R20-XXXXXX**
23						
24						
25						
26				0.1	15.0	
27			0.3			
28						
29						
30						
31						
32						
33	**Rabies - Students**					
34						
35				1.0		
36						
37			8.0			
38						
39						
40						
41						
42	**Molecular R&D Lab**					
43					3.0	
44						
45			1.2			
46						
47						
48						
49						
50	**Serology Lab**					
51				0.2		
52						
53						

Employee Info | Current Employees | Archived Employees | Accessions

	A	B	C	D	E
1	Former Employees	H1 2018 R18-XXXXXX	H2 2018 R18-XXXXXX	H1 2019 R19-XXXXXX	H2 2019 R19-XXXXXX
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					

Employee Info | Current Employees | Archived Employees | Accessions

	A	B	C	D	E
1	**Sample Point**	**Employee Name**	**Accession no.**	**Sample draw date**	**Sample testing date**
2	H1-2018		**R18-XXXXXX**	XX/XX/2018	XX/XX/2018
3	H2-2018		**R18-XXXXXX**	XX/XX/2018	XX/XX/2018
4	H1-2019		**R19-XXXXXX**	XX/XX/2019	XX/XX/2019
5	H2-2019		**R19-XXXXXX**	XX/XX/2019	XX/XX/2019
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					

Employee Info | Current Employees | Archived Employees | Accessions