RABIES: A GLOBAL THREAT

“Taking a Lead on Education and Scientific Initiatives”

Mylissia R. Smith
Masters of Public Health Defense
Emphasis: Infectious Disease and Zoonoses
April 17th 2014

“For centuries man has felt terror after bites by rabid dogs”.

Dr. George M. Baer (1/12/36 - 6/2/09)
RABIES: A GLOBAL THREAT

✓ INTRODUCTION
  - HISTORY
  - VIRUS & PATHOGENESIS
  - DIAGNOSIS
  - VACCINE & PROPHYLAXIS
  - HUMAN CASE
  - GLOBAL BURDEN

✓ FIELD EXPERIENCE

✓ CAPSTONE EXPERIENCE

✓ RECOMMENDATIONS

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History of Rabies

- Ancient Literature: Traced back more than 4000 years…
  - **2300 BC**: Eshnunna – 1st Documentation. If a dog is mad, authorities notify owner, dog not kept in, dog bites man, results in man’s death then owner pays 2/3 a mine of silver. (Baer, 2007)
  - **500 BC**: 1st Documentation Case of Canine Rabies by Greek Philosopher Democritus. (A Short History of Rabies, 2013)
  - **800-700 BC**: “The Iliad” written by Homer (A Short History of Rabies, 2013)
  - **400 BC**: Natural History of Animals, Author Aristotle (Hernandez, 2009)
History of Rabies

- Ancient Literature: Traced back more than 4000 years…
  - **001-100 AD**: Rabies Spread Across Roman Empire
  - **1271**: 1st Outbreak Recorded – 30 human deaths, Germany from wolves.
  - **1400s**: Spreads Across Spain
  - **1700s**: Spreads Across Europe During 18th Century
  - **1703**: 1st Noted in Americas by a Priest in Mexico
  - **1734-5**: Canine Rabies Appears in England
  - **1800**: European Villagers Dying from Contact with Mad Wolves, Foxes and Canines.
  - **1835**: Appears in Chile and Kills Many
  - **1953 AD**: 1st Case Bat Rabies USA by CDC

*Reference: A History of Rabies, 2013*
The word rabies stems from the Latin word rabere which is defined as rave or rage. It is believed that the word rabere is rooted to the Sanskrit word rabhas which is defined as doing violence ("A short history of rabies", "2013").

Myths: Cause, Transmission and ‘Cures’ - play instrumental role in society.

Myths Include:

- Physically sucking wounds to remove poisons
- Placing the rabid animal hair over the human wound = a cure
- Infected children consuming raw heart & liver of infected K-9 = cure
- Placing the heart or tongue of infected animal in shoe would prevent rabies
- St. Hubert’s Key
- Witches cause rabies by spells and evil spirits
- Consumption of maggots from rabid animal = a remedy
Rabies is an acute (neurotropic virus)
Zoonotic – primarily spread through an infected bite
Routes of transmission: infected bite, aerosol transmission, mucosal contact (mouth, nose, eyes), licking of broken skin, transplantations of organs and corneas, penetration of skin from scratch with bleeding
Non-bite transmission = rare in humans, but do and can occur
Excluding transplantations – human to human cases never documented
Best described as enveloped, rod or bullet shape
Rhabdoviridae family → Lyssavirus genus
Greek language: rhabdos = ‘rod’, lyssa = ‘rage’
Two Forms:
◦ Paralytic (“Dumb”)
◦ Hyperactivity: Aggressive / Furious

Negatively Stained Rabies (Rhabdovirus) through electron microscope (CDC, 2014)
Virus Introduction & Pathogenesis

- Size: 180 x 75 nanometers
- Encodes 5 proteins
  - (N) – Nucleoprotein
    - Encapsidates (encloses) RNA
  - (P) – Phosphoprotein
    - Aid in replication process
  - (L) – Polymerase
    - Aid in replication process
  - (G) – Glycoprotein
    - On surface, spiked, attaches to host membranes
  - (M) – Matrix Protein
    - Key in assembly and egress
    - Important role in giving the ‘bullet’ shape

The order and the size of the proteins is imperative as this makes up the virus. (Centers for Disease Control and Prevention, 2014).

Life cycle of rabies infection = 3 phases: 1) Attachment of virion to susceptible membrane, 2) Replication of virion, most difficult, and 3) final replication and budding. (Wunner, 2007)
Recall: Highly neurotropic
→ Virus enters subject – replicates at or near entry site
↓ Enters peripheral nerves
↓ Travels to CNS (replication continues)
↓ Travel out to organs, including salivary glands
   Salivary glands = primary exit portal to be passed on to hosts

Clinical Symptoms:

* Rabies = 99.9% Mortality Rate (GARC, 2014)
- Headache, fever, sore throat
- Increased sensitivity to noise and light
- Hallucinations, ↑libido, nightmares
- Aerophobia, hydrophobia, depression
- Encephalitis
Diagnosis

DFA – Direct Fluorescent Antibody (Gold Standard)

- Post-Mortem
- Brain Stem – Cerebellum (Must Test Negative), Hippocampus (CDC, 2014)
- Utilizes Antibodies to Attach to the Rabies Virus (Antigen)
- Brain Tissue Required as Virus Resides in Nervous Tissue (Not Blood as Other Viruses)

* Mammals: Excluding Euthanasia = 10 day Quarantine

Anti-mortem Diagnosis - Human: Detect Antigen, Nucleic Acids or Virus Isolation

- Saliva
- Corneal Impressions
- Eye Wash Fluid
- CSF
- Tears
- Nuchal Biopsies

Figure 1.7: DFA test viewed through a fluorescent microscope that is positive for rabies, (Centers for Disease Control and Prevention, 2014).
## Vaccine, Prophylaxis and Immunoglobulin

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Nature of Risk</th>
<th>Typical Population</th>
<th>Pre-exposure Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>Virus present continuously, often in high concentrations. Specific exposures likely to go unrecognized. Bite, nonbite, or aerosol exposure.</td>
<td>Rabies research laboratory workers; rabies biologics production workers.</td>
<td>Primary course. Serologic testing every 6 months; booster vaccination if antibody titer is below acceptable level.</td>
</tr>
<tr>
<td>Frequent</td>
<td>Exposure usually episodic, with source recognized, but exposure also might be unrecognized. Bite, nonbite, or aerosol exposure.</td>
<td>Rabies diagnostic lab workers, spelunkers, veterinarians and staff, and animal-control and wildlife workers in rabies-enzootic areas. All persons who frequently handle bats.</td>
<td>Primary course. Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level.</td>
</tr>
<tr>
<td>Infrequent</td>
<td>Exposure nearly always episodic with source recognized. Bite or nonbite exposure.</td>
<td>Veterinarians and terrestrial animal-control workers in areas where rabies is uncommon to rare. Veterinary students. Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care including biologics is limited.</td>
<td>Primary course. No serologic testing or booster vaccination.</td>
</tr>
<tr>
<td>Rare (population at large)</td>
<td>Exposure always episodic with source recognized. Bite or nonbite exposure.</td>
<td>U.S. population at large, including persons in rabies-epizootic areas.</td>
<td>No vaccination necessary.</td>
</tr>
</tbody>
</table>

**Pre-Exposure Prophylaxis**
**Vaccine, Prophylaxis and Immunoglobulin**

<table>
<thead>
<tr>
<th>Type of vaccination</th>
<th>Route</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Intramuscular</td>
<td>Human diploid cell vaccine (HDCV) or purified chick embryo cell vaccine (PCECV); 1.0 mL (deltoid area), one each on days 0, *3, 7, and 14</td>
</tr>
<tr>
<td>Booster†</td>
<td>Intramuscular</td>
<td>HDCV or PCECV; 1.0 mL (deltoid area), day 0 and 3</td>
</tr>
</tbody>
</table>

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“...rabies has one of the highest case-fatality ratios of any infectious disease”

(Compendium of Animal Rabies Prevention and Control, 2011)
Private First Class Kevin Shumaker: 24 years, Bite to R hand from feral/community K-9 while deployed in Afghanistan in January 2011. August 14th = 1st Clinical Symptoms.
Human Rabies Case - Timeline

Global Burden of Rabies

- Rabies is found on 6 of the 7 world continents (OIE, 2014)
- Canines = main global source (GARC, 2013)
- PRP Study Reflects ~61,000 Human Deaths Annually (WHO, 2012)
- 95% Africa / Asia - - India = Highest Reported Incidences (GARC, 2013)
- 60% → Children 15 yrs. and younger (GARC, 2013)
- 84% Deaths Occur is Rural Areas (Poorest & Most Remote) (GARC, 2013)
- 3.3 Million People Live with Rabies Risk Daily (GARC, 2014)

Globally, dogs are the main source of rabies as over 95% of human deaths result from infected dogs. (Dr. Deborah Briggs, 2013)
Global Burden of Rabies

Distribution of risk levels for humans contacting rabies, worldwide, 2011

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2012. All rights reserved

Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD) World Health Organization
Due to the result of growing populations of humans and canines around the world, the Economic cost of rabies along with the burden of human deaths will also continue to grow. (WHO, 2012)
## Rabies Free / Controlled Regions

### Countries and Political Units Currently Rabies Free/Controlled  (CDC, 2013)

<table>
<thead>
<tr>
<th>Region</th>
<th>Countries/Localities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Africa</strong></td>
<td>Cape Verde, Mauritius, Reunion, Sao Tome, and Principe, Seychelles</td>
</tr>
<tr>
<td><strong>Americas</strong></td>
<td>North: Bermuda, Saint Pierre and Miquelon&lt;br&gt;Caribbean: Antigua and Barbuda, Aruba&lt;br&gt;The Bahamas, Barbados, Cayman Islands, Dominica, Guadeloupe, Jamaica, Martinique, Montserrat, Netherlands, Antilles, Saint Kitts (Saint Christopher) and Nevis, Saint Lucia, Saint Martin, Saint Vincent and Grenadines, Turks and Caicos, Virgin Islands (UK and US)</td>
</tr>
<tr>
<td><strong>Asia and the Middle East</strong></td>
<td>Hong Kong, Japan, Kuwait, Malaysia (Sabah), Qatar, Singapore, United Arab Emirates</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td>Albania, Austria, Belgium, Corsica, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Gibraltar, Hungary, Iceland, Ireland, Isle of Man, Liechtenstein, Luxembourg, Monaco, Netherlands, Norway (except Svalbard), Portugal, Slovakia, Slovenia, Spain (except Ceuta and Melilla), Sweden, Switzerland, United Kingdom</td>
</tr>
<tr>
<td><strong>Oceania</strong></td>
<td>Australia², Cook Islands, Fiji, French Polynesia, Guam Hawaii, Kiribati, Micronesia, New Caledonia, New Zealand, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, Vanuatu</td>
</tr>
</tbody>
</table>

1. Bat lyssaviruses have been reported throughout Europe, including areas that are reportedly free of rabies in other wild mammals.
2. Most of Pacific Oceania is reportedly “rabies-free”, with the exception of Australia, where lyssaviruses in bats have been reported, as well as fatal human rabies cases.
Rabies Free / Controlled Regions

In order for a country to be identified as rabies free, or rabies controlled, per the OIE the following provisions must be achieved:

✓ The disease is notifiable (reported to appropriate authorities).
✓ Effective disease surveillance has been implemented and is in operation.
✓ Regulatory measures for control and prevention have been implemented. This is to also include effective importation procedures.
✓ Two years free from having an indigenous case of rabies in animals and man. It is noteworthy to mention that isolating Australian or European Bat Lyssavirus would not affect this status.

No imported case of carnivores outside of the quarantine station has been confirmed with rabies for the previous six months.
The OIE specifically outlines the following recommendations in order to ship dogs and cats into rabies-free regions from countries that are considered infected with rabies:

- Veterinary authorities must provide an international veterinary certificate that confirms the animal has not shown clinical signs of rabies within the past 48 hours of shipment. The animals must be permanently identified (such as tattoo or microchip) and that identifier must be listed on said certificate.

- The animal must be vaccinated for rabies, and received not less than six months or more than one year prior to shipment. The initial rabies vaccine should have been administered while the animal was at least 3 months old in age. The vaccine is to be from an inactivated virus vaccine or from a recombinant vaccine that expresses the rabies virus glycoprotein.

- The animal must be subjected to an antibody test not less than three months or more than twenty-four months prior to shipment. The antibody test must reflect that the animal is carrying an antibody response of at least 0.5 IU/mL, which meets WHO recommendations. Animals that have not been vaccinated following the above noted criteria, the importing country can require the animal be placed in quarantine at a quarantine location within that countries territory for six months.

Recommendation & Provisions: Strictly Enforced. Failure to adhere to these guidelines can, and has, resulted in euthanasia of the animal.
Field - Experience

Global Alliance For Rabies Control

- Non-Government Entity (NGO)
- Mission: One day eliminating human deaths from rabies and to relieve the burden of rabies in animals, especially dogs. (Stukey, 2012)
- World Rabies Day Campaign

As we are aware that rabies is 100% preventable in humans, GARCs vision is “a world free of human rabies” (GARC, 2014)
Field - Experience

Zach Jones Memorial Fund

→ Non-Profit 501c3 established 2006
→ Established by Larry & Connie Jones of Humble Tx.
→ Promote Rabies Education
→ Zachary ‘Zach’ Ross Jones
→ Raise Fund for Rabies Awareness, Early Detection and Scholarships to Graduating Seniors at Atascocita High School
Grant Proposal:

**Project Intent:** Secure 50% of Funds to Promote Rabies Awareness - Relationship between Bats and Rabies (their role in transmitting Rabies)

**Target Audience:** Adolescents

**Objective:** Educate: What is Rabies, How to Prevent It, and What To Do In the Event of and Exposure / Possible Exposure.

**Goal:** 1 Million Adolescents

**Design:** PSA & Short Video (English and Spanish)

Proposal Developed and Submitted through GARC

Immediately Accepted by ZJMF....
# Field - Experience

Zach ‘Z’ Jones US Rabies Education Proposal

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>19”X25” Panel Size Poster/4 Color Process Inks/80# Gloss Coated Text Paper</td>
<td>3,711.04</td>
</tr>
<tr>
<td>Envelopes for shipping</td>
<td>2,237.44</td>
</tr>
<tr>
<td>Non-profit Postage</td>
<td>743.31</td>
</tr>
<tr>
<td>Including DVD /Computer Compatible Video in Packet for Mailing</td>
<td>4,600</td>
</tr>
<tr>
<td>Copying of DVD/Computer Compatible Video (Includes protective jacket)</td>
<td>20,700</td>
</tr>
<tr>
<td>Electronic Survey (GARC will handle logistics &amp; statistics)</td>
<td>400</td>
</tr>
<tr>
<td>Poster Design (Handled by GARC &amp; Independent Graphic Design Artist)</td>
<td>700</td>
</tr>
</tbody>
</table>

Cost per packet that is mailed out comes to 1.06

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Program design, coordination and implementation</td>
<td>12,000</td>
</tr>
<tr>
<td>Administration 3%</td>
<td>1,353</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$46,445</strong></td>
</tr>
</tbody>
</table>

ZJMF immediately accepted proposal and submitted check for $18,000.
Field - Experience

PSA was not to allow for interpretations and questioning as topic was critical!
Direct and factual with limited space.

Small working group: GARC and CDC, Included experts, translator, graphic designer and scientists.
Field - Experience

- PSA “Bats Can Have Rabies’ Finalized: Immediate Global Distribution
  GARC Websites, List-serves and Social Media Accounts
- USA Outreach Campaign to SPHV
  No Shipping or Printing $
  Spanish and English (11”x17” and 18”x24”)
  Developed Request Form and Handled Distribution Logistics
  PSA Distributed: Maine, Florida, North Carolina, Kentucky, Minnesota and Arizona

Video Segment of the ZJMF Grant Immediately Ensued

“Zach Jones is defined not by the way he died, but by the way he lived” (zachjonesmemorial.org)
Field - Experience

Bats and Rabies Video
Plan of Action: Mirror PSA → What is Rabies, How to Prevent It, and What To Do In the Event of and Exposure or Possible Exposure PLUS Educate on the Importance of Bats in our Ecosystem.

Step 1: Solicit and Identify Production Team
Step 2: Outline Project Goals and Secure Quote - $
Step 3: Finalize Contract w/ Production Team – Knowlera Media, LLC
Step 4: Arrange for 50% of Invoice
Step 5: Consultation w/ Knowlera: Project Intent, Objective, Goal & Audience
  ▶ Adolescence
  ▶ Short Video (~ 12 minutes)
  ▶ Bats and Rabies
  ▶ Outline Discussion Topics
Field - Experience

Bats and Rabies Video

Step 6: Topics were broke out to video segments and script writing began
Step 7: Narrators and expert Identified
Step 8: Identify and Secure Photos, B-Roll Footage, etc…
Step 9: Identify Film Location (Bat Conservation International, Austin Texas)
Step 10: Travel Dates, Plans and Time Line Outlined

Outlined Video Segments:

- Segment 1: Introduction to Rabies
- Segment 2: About Rabies (general scientific information)
- Segment 3: Rabies and Bats
- Segment 4: Preventing Exposures
- Segment 5: Exposures
- Segment 6: How Bats Benefit The Ecosystem
- Segment 7: Closing

Many hours and edits later…. 2 films completed – 7:22 minutes (for private distribution) and 2:00 minutes (for internet distribution).

Full Video: https://www.youtube.com/watch?v=Zjg_FHqlFnU
Mass Vaccine Distribution Initiative

**Project Intent:** Mass donation: animal rabies vaccine. Distributed through GARC across the USA.
- Promote vaccine incentives & increase involvement of the WRD.

**Target Groups:** Companion Animals for Adoption, Feral Felines and Low-Income Families.

**Objective:** 250 Doses of Animal Rabies Vaccines Per Organization
Field - Experience

Pfizer Animal Health (Zoetis)

Step 1: Solicit Proposal to Distributor – Pfizer Animal Health
Step 2: Build Team within PA to handle Internal Logistics
Step 3: Develop Application, Upload to GARC Websites, Social Media and Listserves
Step 4: Market Application
Step 5: Review Applicants and Build Data Base of Accepted Applicants
Step 6: Submit Database to Pfizer Animal Health for Packaging and Distribution

Outcomes:
Distributed Vaccine to 32 US States
Vaccinated 250,000 + animals (Companion, Feral Felines and Limited Food Animal and Equines).
Increased Awareness on the WRD Campaign & Physical Involvement.
Virus Testing Methodologies

Testing Crucial for Public Health Management:
- Testing Animals for Travel
- Establishing Herd Immunity
- Determine Vaccine Efficacy

Testing Crucial for Potential Human Rabies Subject

Testing Crucial for Maintaining ‘Adequate Protection’
- Rabies Laboratory Staff
- Veterinarians and Staff
- Animal Control Officers
- Immunocompromised Subject

WHO Recommendation: 0.5 IU/mL Immune Response for Adequate Protection

Public health concern for countries considered infected with rabies & rabies-free regions.
**Virus Testing Methodologies**

Testing Methodologies – Measuring Immune Response:

- **Antigen-Binding Assay**

  - Rabies virus glycoprotein + Anti-rabies virus glycoprotein → Primary complex
  - Enzyme-labeled antoglobulin or protein A → Secondary complex
  - Detection system (i.e., substrate) (Moore, 2007)

- **Virus-Neutralizing Assay**

  - Live rabies virus + Rabies virus neutralizing antibody → Neutralization
  - Rabies virus infection of cells → FITC anti-RNP → RNP replication in cell → Fluorescent detection of viral RNP (Moore, 2007)

- ELISAs and IFAs
**Virus Testing Methodologies**

**Virus-Neutralizing Assay vs Antigen-Binding Assay**

Similarities – YES: Both test are measuring an immune response

**Differences – YES!**
- Virus Neutralization → Measures Neutralizing Antibody (Is Virus Escaping Neutralization…)
- Antigen-Binding → Measures Antigen Binding Antibody (detected, quantified, & characterized by their ability to bind to various rabies virus antigens (Moore, 2013).

**Important Note:** results will not be consistent between subjects for reasons of Ig subclasses, various affinities, neutralizing abilities and unique polyclonal responses (Moore, 2013).

As these two testing methodologies differ in their identifications and measurements; results should not be equally comparable to one another (Moore, 2013).

» **Recall:** WHO Recommendation: 0.5 IU/mL ⇔ RFFIT and MNT, 1978

Assigning the same cut-off level for both test methods will never result in agreement for all individuals. The relationship of RFFIT and ELISA results over time points post vaccination, … demonstrates further that one cut-off is not appropriate (Susan Moore, 2013).
Investigating the Performance of a Rabies ELISA Test

Zoetis - SERELISA® Rabies Ab Mono Indirect ELISA

**Long-term Objectives:** 1) Investigate performance to determine adequate immune response levels to assess potential differing cut-off values in human samples. 2) Establish Standardization Curves for different reference sera.

Step 1: Consult: KSU Director
Step 2: Consult: Zoetis Director
Step 3a: USDA Permit (#VB-139848)
Step 3b: KSU IRB (#7012)
Step 4: Project Protocol
Step 5: Receive the Kits…

* WHO Recommendation = 0.5 IU/mL
<table>
<thead>
<tr>
<th>Reference Sera</th>
<th>IU/mL</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>OIE</td>
<td>6.7</td>
<td>Batch 3</td>
</tr>
<tr>
<td>WHO-1</td>
<td>59.0</td>
<td>Lot R3 US Standard</td>
</tr>
<tr>
<td>WHO-2</td>
<td>30.0</td>
<td>RAI</td>
</tr>
<tr>
<td>KSU</td>
<td>59.0</td>
<td>TRP-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>OIE</th>
<th>WHO-1</th>
<th>WHO-2</th>
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</thead>
<tbody>
<tr>
<td>IU/mL</td>
<td>6.7</td>
<td>59.0</td>
<td>30.0</td>
<td>59.0 → 17.0</td>
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<tr>
<td>Dilution 1</td>
<td>6.7</td>
<td>5.9</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>2.23</td>
<td>1.97</td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>Dilution 3</td>
<td>0.67</td>
<td>0.59</td>
<td>0.6</td>
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<tr>
<td>Dilution 4</td>
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<td>0.39</td>
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<tr>
<td>Dilution 5</td>
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<tr>
<td>Dilution 6</td>
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<tr>
<td>Dilution 7</td>
<td>0.0223</td>
<td>0.0197</td>
<td>0.02</td>
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</table>
### PLATE 10 (1st Half – Set Up)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>N 1:10</td>
<td>N 1:10</td>
<td>WHO1 1:300,000</td>
<td>WHO1 1:300,000</td>
<td>KSU Ref #1 1:100</td>
<td>KSU Ref #1 1:100</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>P 1:10</td>
<td>P 1:10</td>
<td>OIE 1:100</td>
<td>OIE 1:300</td>
<td>KSU Ref #2 1:100</td>
<td>KSU Ref #2 1:100</td>
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<td><strong>C</strong></td>
<td>WHO1 1:1,000</td>
<td>WHO1 1:1,000</td>
<td>OIE 1:1,000</td>
<td>OIE 1:1,500</td>
<td>KSU Ref #3 1:100</td>
<td>KSU Ref #3 1:100</td>
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<td><strong>D</strong></td>
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<td>WHO1 1:3,000</td>
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<td>OIE 1:10,000</td>
<td>KSU Ref #4 1:100</td>
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<tr>
<td><strong>E</strong></td>
<td>WHO1 1:10,000</td>
<td>WHO1 1:10,000</td>
<td>OIE 1:30,000</td>
<td>WHO2 1:500</td>
<td>KSU Ref #1 1:200</td>
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<tr>
<td><strong>F</strong></td>
<td>WHO1 1:15,000</td>
<td>WHO1 1:15,000</td>
<td>WHO2 1:1,500</td>
<td>WHO2 1:5,000</td>
<td>KSU Ref #2 1:200</td>
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<tr>
<td><strong>G</strong></td>
<td>WHO1 1:30,000</td>
<td>WHO1 1:30,000</td>
<td>WHO2 1:7,500</td>
<td>WHO2 1:15,000</td>
<td>KSU Ref #3 1:200</td>
<td>KSU Ref #3 1:200</td>
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<td><strong>H</strong></td>
<td>WHO1 1:100,000</td>
<td>WHO1 1:100,000</td>
<td>WHO2 1:50,000</td>
<td>WHO2 1:150,000</td>
<td>KSU Ref #4 1:200</td>
<td>KSU Ref #4 1:200</td>
</tr>
</tbody>
</table>

### Expected Results

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td>0.0197 IU/ml</td>
<td>0.0197 IU/ml</td>
<td>0.15 IU/ml</td>
<td>0.15 IU/ml</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td>6.7 IU/ml</td>
<td>2.23 IU/ml</td>
<td>2.8 IU/ml</td>
<td>2.8 IU/ml</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>5.9 IU/ml</td>
<td>5.9 IU/ml</td>
<td>0.67 IU/ml</td>
<td>0.447 IU/ml</td>
<td>0.5 IU/ml</td>
<td>0.5 IU/ml</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>1.97 IU/ml</td>
<td>1.97 IU/ml</td>
<td>0.223 IU/ml</td>
<td>0.067 IU/ml</td>
<td>0.1 IU/ml</td>
<td>0.1 IU/ml</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>0.59 IU/ml</td>
<td>0.59 IU/ml</td>
<td>0.0223 IU/ml</td>
<td>6 IU/ml</td>
<td>7.5 IU/ml</td>
<td>7.5 IU/ml</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>0.39 IU/ml</td>
<td>0.39 IU/ml</td>
<td>0.2 IU/ml</td>
<td>0.6 IU/ml</td>
<td>1.4 IU/ml</td>
<td>1.4 IU/ml</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>0.197 IU/ml</td>
<td>0.197 IU/ml</td>
<td>0.4 IU/ml</td>
<td>0.2 IU/ml</td>
<td>0.21 IU/ml</td>
<td>0.25 IU/ml</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.059 IU/ml</td>
<td>0.059 IU/ml</td>
<td>0.06 IU/ml</td>
<td>0.02 IU/ml</td>
<td>0.05 IU/ml</td>
<td>0.05 IU/ml</td>
</tr>
</tbody>
</table>
Kit Performance: Indirect ELISA Performed According to Kit Insert

Final Step: Automated plate reader: Optical Density (OD) measured bichromatically (450 and 630 nm). Data submitted to Zoetis for internal review and interpretation.

Outcomes:
1st Run: WHO-1 and WHO-2 serial dilutions too similar in results; no other detailed points were noted. → Plate scheme updated serial dilutions for Plate 1, Plate 2 remain as is.

Plate 1 Scheme →

(Expected Results)
Outcomes, 1st RUN: Good, consistent & clean - excess of variation detected between few of the serial dilutions than projected. Resulted in one reference sera validation.
Values = 86.2, 97.2, 94.8, 83.8, 91.7 and 88.3 for Plate 1 and 92.6, 94.3, 87.9, 81.1, 85.1 and -19.5 for Plate 2.

⇒ Re-run Plate 1 / Plate 2: Remove some reference dilutions & cut WHO-1 and WHO-2 dilutions in half

Outcomes, 2nd RUN: Results reflected WHO-1 and WHO-2 = improved results. Values = 93.2, 94.6, 93.1, 86.9, and 93.5 for Plate 1 and 96.9, 94.8, 89.0, 84.4, 90.9 and -13.6 for Plate 2. (Concern did not weigh heavily on the -13.6…).
Second validation obtained.

⇒ Slightly alter next run. Discussed: plates in parallel with multiple technicians, additional plate washings, and running duplicate reference sera in triplicate.
→ Identified washing steps: manually vs machine.
  ** Run duplicate sera in triplicate and use automated washer for wash steps.
**Capstone**

**Plate Scheme: ‘3rd’ Run:**

<table>
<thead>
<tr>
<th></th>
<th>Manual Wash</th>
<th>Automated Wash</th>
<th>Manual Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WHO1 1:150,000</td>
<td>WHO1 1:500</td>
<td>WHO1 1:150,000</td>
</tr>
<tr>
<td>B</td>
<td>WHO2 1:250</td>
<td>WHO2 1:500</td>
<td>WHO2 1:250</td>
</tr>
<tr>
<td>C</td>
<td>WHO1 1:750</td>
<td>WHO1 1:500</td>
<td>WHO1 1:750</td>
</tr>
<tr>
<td>D</td>
<td>WHO2 1:2,500</td>
<td>WHO2 1:750</td>
<td>WHO2 1:2,500</td>
</tr>
<tr>
<td>E</td>
<td>WHO1 1:500</td>
<td>WHO1 1:250</td>
<td>WHO1 1:500</td>
</tr>
<tr>
<td>F</td>
<td>WHO2 1:750</td>
<td>WHO2 1:250</td>
<td>WHO2 1:750</td>
</tr>
<tr>
<td>G</td>
<td>WHO2 1:150,000</td>
<td>WHO1 1:150,000</td>
<td>WHO2 1:150,000</td>
</tr>
<tr>
<td>H</td>
<td>WHO1 1:50,000</td>
<td>WHO2 1:75,000</td>
<td>WHO2 1:250</td>
</tr>
</tbody>
</table>

**Outcome - Pending:** Analysis & interpretation by Zoetis still pending. As I continue to move forward in this project with Zoetis and KSU, we are all optimistic final goals & objectives will result in success.

* WHO Recommendation
  = 0.5 IU/mL
Recommendations

“As human and canine population continue to grow, the cost and economic burden will also continue to grow” (WHO, 2012)

► Additional efforts for vaccine, ORV & contraceptive programs for free roaming K-9s
► Ensuring prompt wound management & proper PEP and RIG
► Further use of effective blue prints & international advocacy
► Implementing national programs w/ effective surveillance & control efforts
► Minimize human exposures to infected animals

In Reference to Research

► Additional science based studies for development of recommendation
► Well designed studies to further pursue:
  - Potential virus shedding in milk
  - PEP for animals,
  - Viral shedding in livestock
  - Ecology of rabies in wildlife.

Take – Home Messages…

I want you to leave here today with two things in reference to rabies – if you leave here today with nothing else, please take home these messages ...

1) Rabies is 100% preventable in humans by proper administration of prophylaxis!

2) In any event you receive a bite / scratch, immediately wash the wound for several minutes w/ soap and water (decreases the viral load!). Or, if you come in contact (or are in the presence of) a bat please report it!

PS: Always be a responsible pet owner and VACCINATE!
I have the utmost admiration for all individuals mentioned in this acknowledgement. They have made impressions upon me that I hope to instill in others. Although, thank you simply doesn’t reflect my appreciation – I say thank you, to each and every one of you. Mylissia R. Smith ~
QUESTIONS....?
“NEVER DOUBT THAT A SMALL GROUP OF COMMITTED PEOPLE CAN CHANGE THE WORLD.

INDEED, IT IS THE ONLY THING THAT EVER HAS.”

-DR. MARGARET MEAD

Dr. Margaret Mead: American Cultural Anthropologist
(December 16, 1901 - November 15, 1978)
References


References


References


Google Images. (2014). *Cerebellum*. Retrieved from https://www.google.com/search?q=images+of+cerebellum&tbm=isch&tbo=u&source=univ&sa=X&ei=lcVKU6a7MamG8QHg7IHADg&sqi=2&ved=0CCgQsAQ&biw=1366&bih=611#facrc=_&imgdii=ozORlmp3zn5npM%3A%3B0FQH8UZ9UInAUM%3BzoRlmp3zn5npM%3A&imgrc=0zoRlmp3zn5npM%253A%3BKegywezp_O4KL%253Bhttp%253A%252F%252Fs-n-s.org%252Fwp-content%252Fuploads%252F2011%252F05%252Fcerebellum.bmp1.gif%3Bhttp%253A%252F%252Fs-n-s.org%252Fforum-2%252Fart-2%252F2cerebellum-by-ann-falk%252F%3B960%3B720.


MPH – Spring 2014
Mylissia R. Smith