

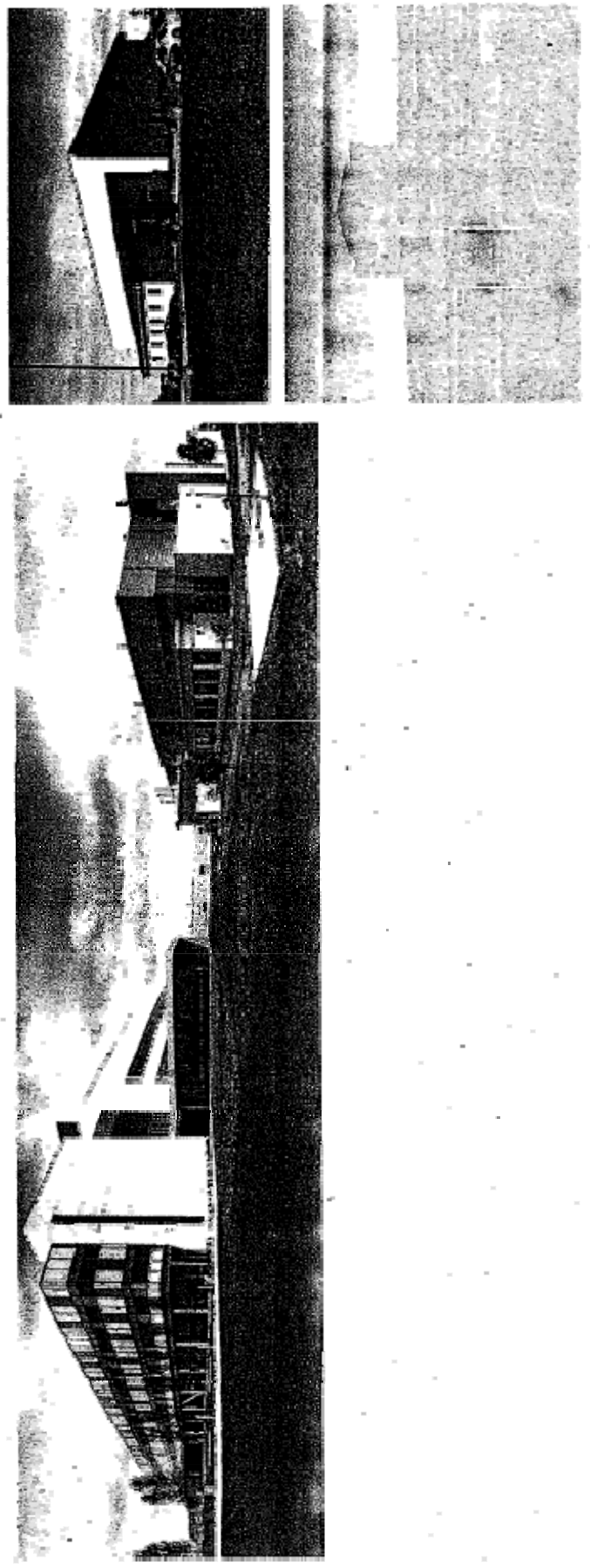
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附件 3

National Veterinary Services Laboratories

Beth Lautner, D.V.M., M.S.
Director, National Veterinary Services Laboratories
Veterinary Services, Animal and Plant Health Inspection Service
May 10, 2012





National Veterinary Services Laboratories (NVSL), APHIS
Employs ~ 280

Center for Veterinary Biologics (CVB), APHIS
Employs ~ 205

National Animal Disease Center (NADC), ARS
Employs ~ 320

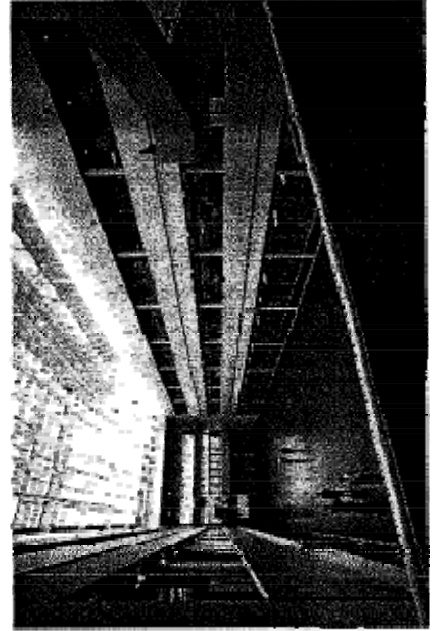
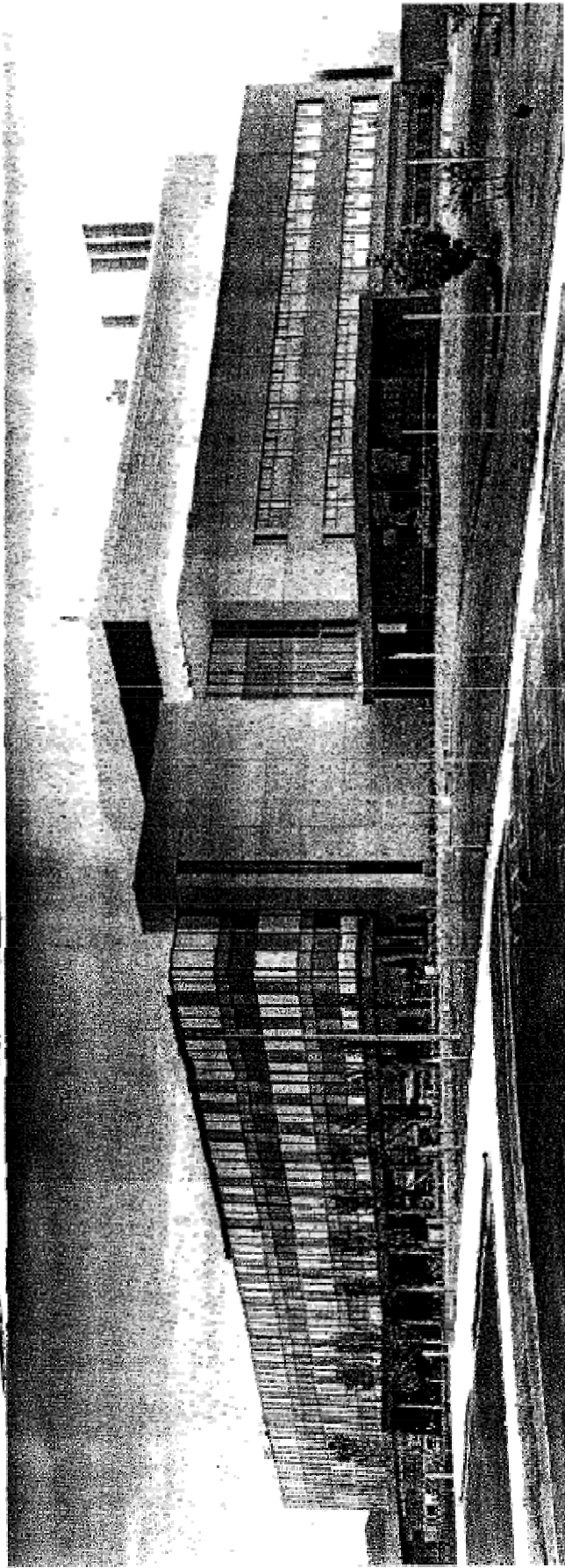
Together we meet the national needs for animal health research,
diagnosis, and product evaluation.

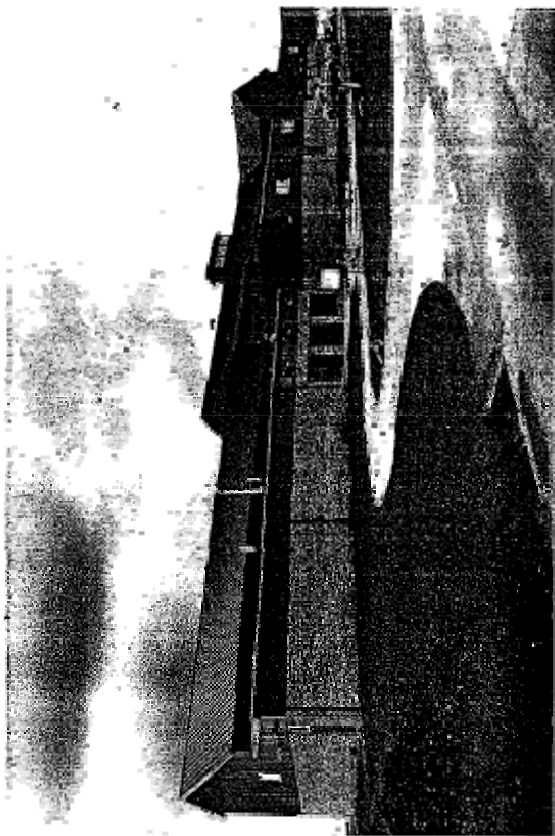




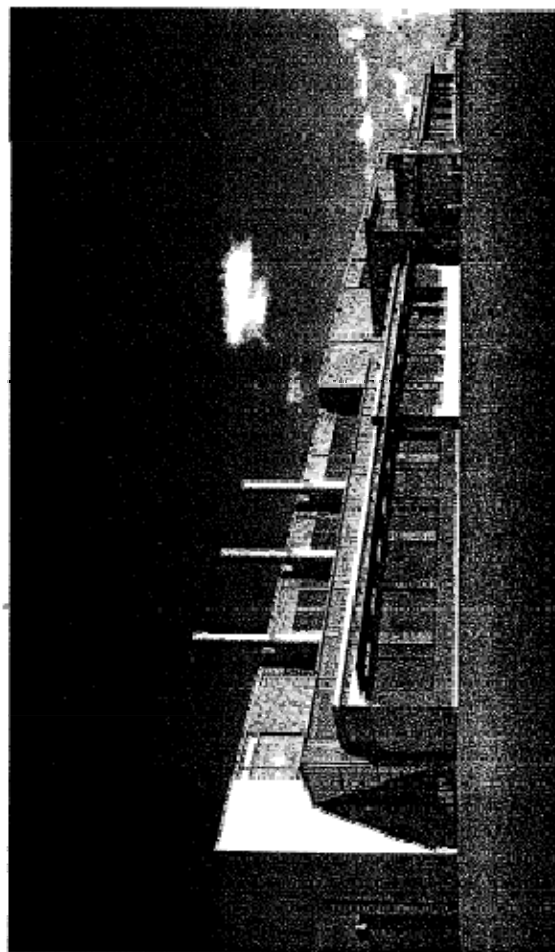
NCAH

National Centers for Animal Health





Low containment animal facility--2009



Phase 1 laboratory facility--2004



High containment animal facility--2007

NVSL Mission

- To safeguard U.S. animal health and contribute to public health by ensuring that timely and accurate laboratory support* is provided by a nationwide animal health diagnostic system.
 - Reference and confirmatory laboratory for USDA

FY11 – > 66,000 accessions and 533,000 tests approved.

* Includes both domestic and foreign animal diseases

NVSL Activities

- Conduct diagnostic testing
- Coordination of the National Animal Health Laboratory Network (NAHLN)
- Supply reference reagents to other laboratories
- Provide training in diagnostic techniques
- Conduct proficiency testing of other laboratories
- Conduct developmental projects to improve diagnostic techniques for diseases of significance
- Participate in World Organization for Animal Health (OIE) Collaborating Centre for the Diagnosis of Animal Diseases and Vaccine Evaluation in the Americas with CVB and Iowa State University
- Serve as OIE reference laboratory
 - high pathogenicity avian influenza, anthrax, pseudorabies, bluetongue, contagious equine metritis, equine encephalomyelitis, equine infectious anemia, leptospirosis, Newcastle disease, swine influenza, vesicular stomatitis and West Nile encephalitis

NVSL Structure

Laboratories

- Diagnostic Bacteriology Laboratory
- Diagnostic Virology Laboratory
- Pathobiology Laboratory
- Foreign Animal Disease Diagnostic Laboratory

Director's Office

- National Animal Health Laboratory Network
- Quality Assurance – ISO 17025 accreditation
- Calibration Laboratory

Program and Administrative Services

- NVSL program budget and user fees

Laboratory Resources Unit

- Shipping and receiving, biologics repository, warehouse, glassware, media prep

Administrative Unit

- NCAH Budget, Human Resources and Training, Procurement

Diagnostic Bacteriology Laboratory

Dr. Matthew Erdman, Director

- Serology Section
 - Dr. David Kinker
- Bacterial Identification Section
 - Vacant
- Mycobacteria & Brucella Section
 - Dr. Suelee Robbe-Austerman



Diagnostic Bacteriology Laboratory

Partial List of Testing Capability

- Brucellosis
- Bovine tuberculosis
- Johne's disease
- Anthrax
- Salmonellosis
- Leptospirosis
- Contagious equine metritis
- Piroplasmosis
- Genotyping



Diagnostic Virology Laboratory

Dr. Beverly Schmitt, Director

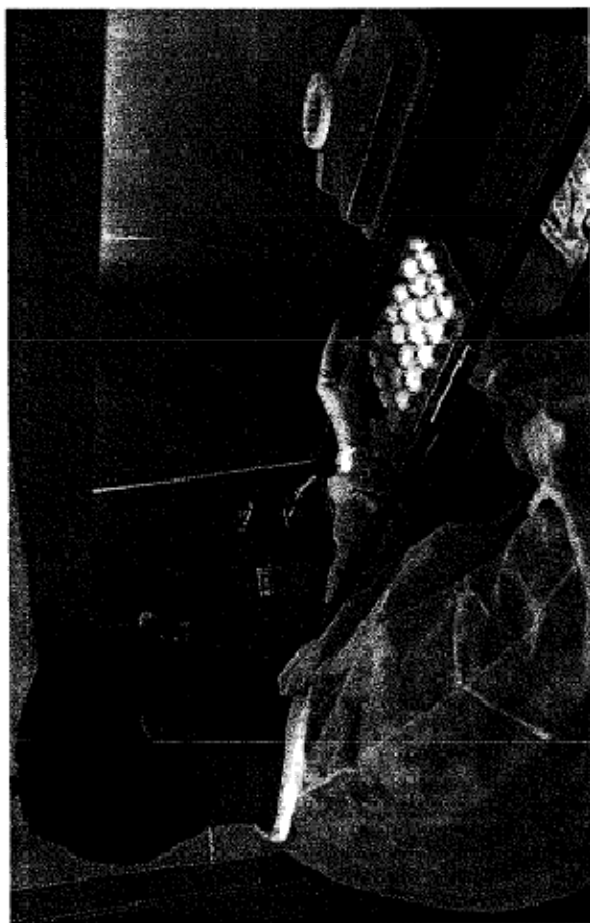
- Avian Viruses Section
 - Vacant
- Bovine, Porcine & Aquaculture Viruses Section
 - Dr. Sabrina Swenson
- Equine/Ovine Viruses Section
 - Dr. Eileen Ostlund



Diagnostic Virology Laboratory

Partial List of Testing Capability

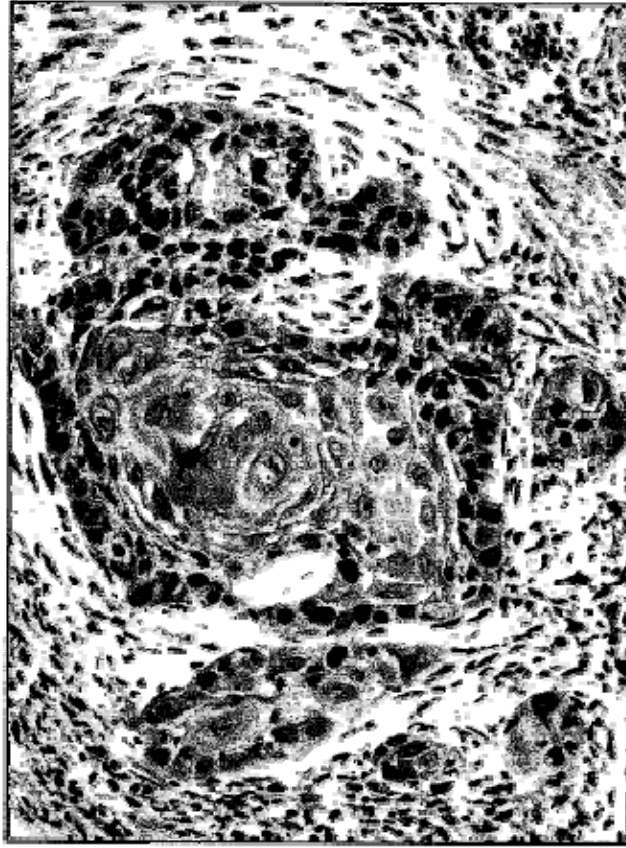
- Avian influenza
- Newcastle disease
- Pseudorabies
- Equine encephalomyelitis
(Eastern, Western,
Venezuelan)
- West Nile encephalitis
- Bluetongue
- Infectious salmon anemia
- Spring viremia of carp
- Equine infectious anemia



Pathobiology Laboratory

Dr. Arthur Davis, Director

- Pathology, Parasitology and Entomology Section
 - Dr. Mark Hall
- Chemistry and Analytical Services Section
 - Dr. Walter Hyde



Pathobiology Laboratory

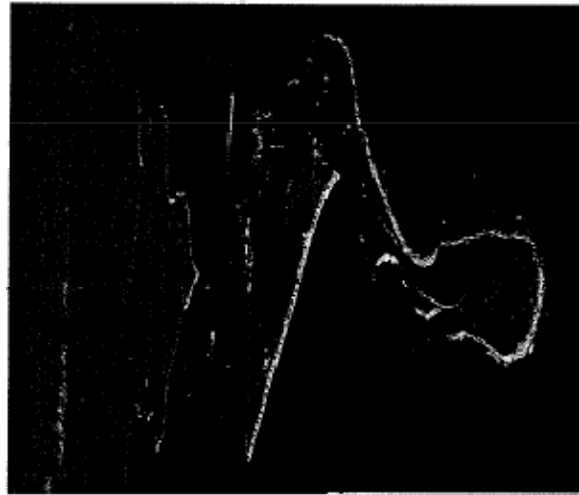
Diagnostics

- Bovine Spongiform Encephalopathy
- Scrapie
- Chronic Wasting Disease
- Bovine Tuberculosis
- Screwworm myiasis
- Tick identification



Foreign Animal Disease Diagnostic Laboratory

- Diagnostic Services Section
 - Dr. Fernando Torres-Velez
- Reagents and Vaccine Services Section
 - Dr. Wei Jia
- Proficiency and Validation Services Section
 - Dr. Mike McIntosh



Foreign Animal Disease Diagnostic Laboratory

Partial Listing of Testing Capability

- Foot and mouth disease
- Classical swine fever
- African swine fever
- Vesicular stomatitis



Quality Assurance

NVSL was the first laboratory in the world to be accredited to veterinary ISO 17025 standards by the American Association for Laboratory Accreditation (A2LA).



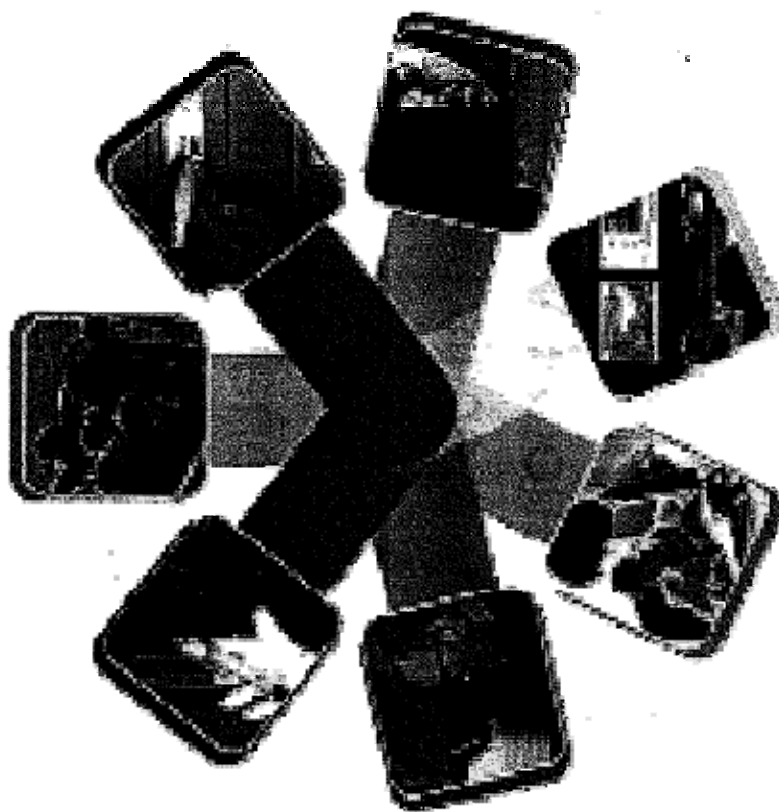
International
Organization for
Standardization

*ISO/IEC 17025 is the international
standard for testing and
calibration laboratories.*

Websites

- National Veterinary Services Laboratories
www.aphis.usda.gov/animal_health/lab_info_services
- National Animal Health Laboratory Network
www.aphis.usda.gov/animal_health/nahn

NAHLN Overview

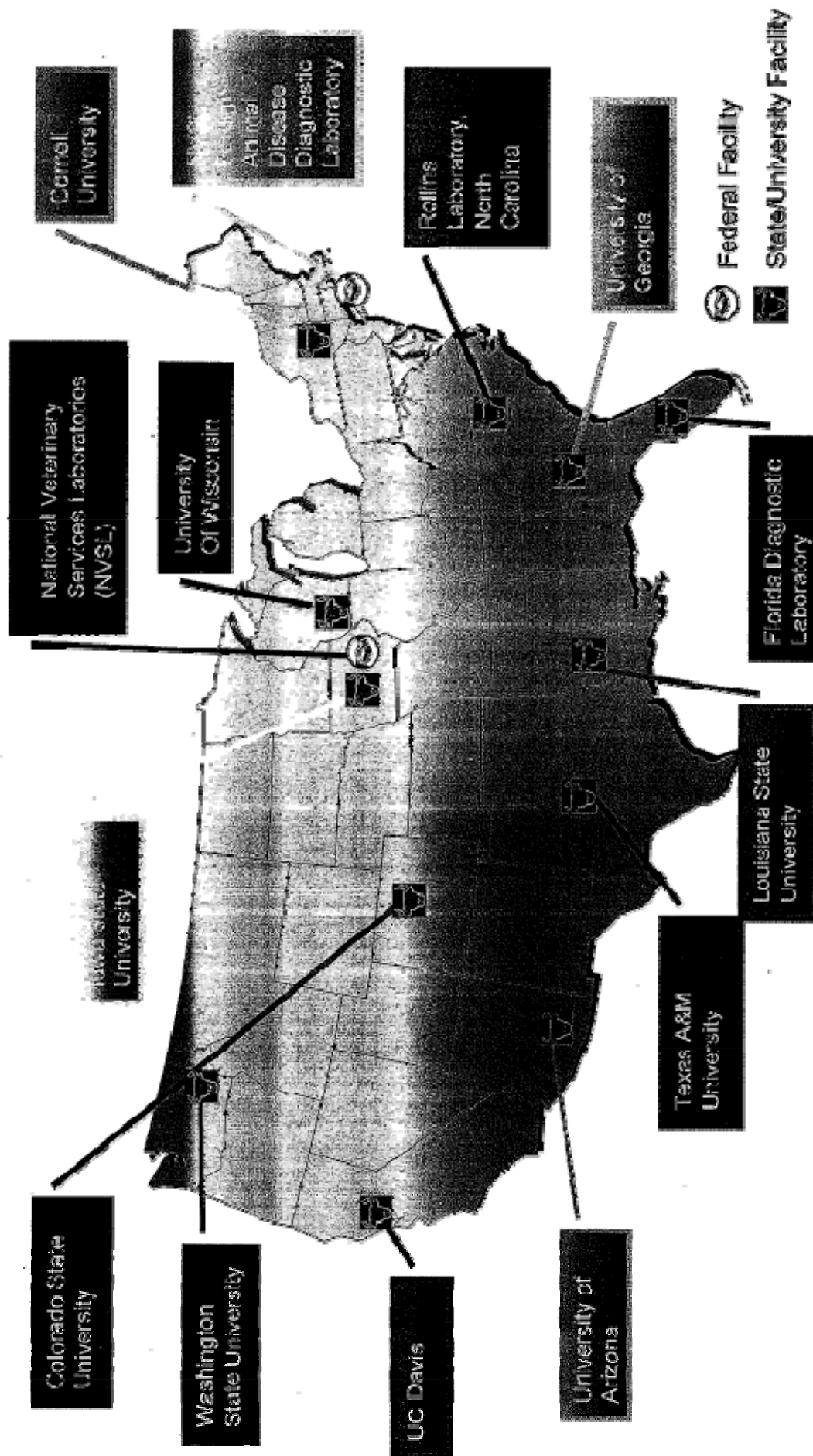


Barbara Martin, M.S.
NAHLN Coordinator
National Veterinary Services
Laboratories

National Animal Health Laboratory Network

- Formed in 2002
- Is a group of State funded veterinary diagnostic labs that deal with diseases of animals including endemic, exotic, zoonotic, and emerging diseases
- Is a **partnership** between:
 - **USDA**
 - Animal and Plant Health Inspection Service (APHIS)
 - National Institute of Food and Agriculture (NIFA)
 - **American Association of Veterinary Laboratory Diagnosticians (AAVLD)**
 - **NAHLN Laboratories**

The original 12 NAHLN laboratories



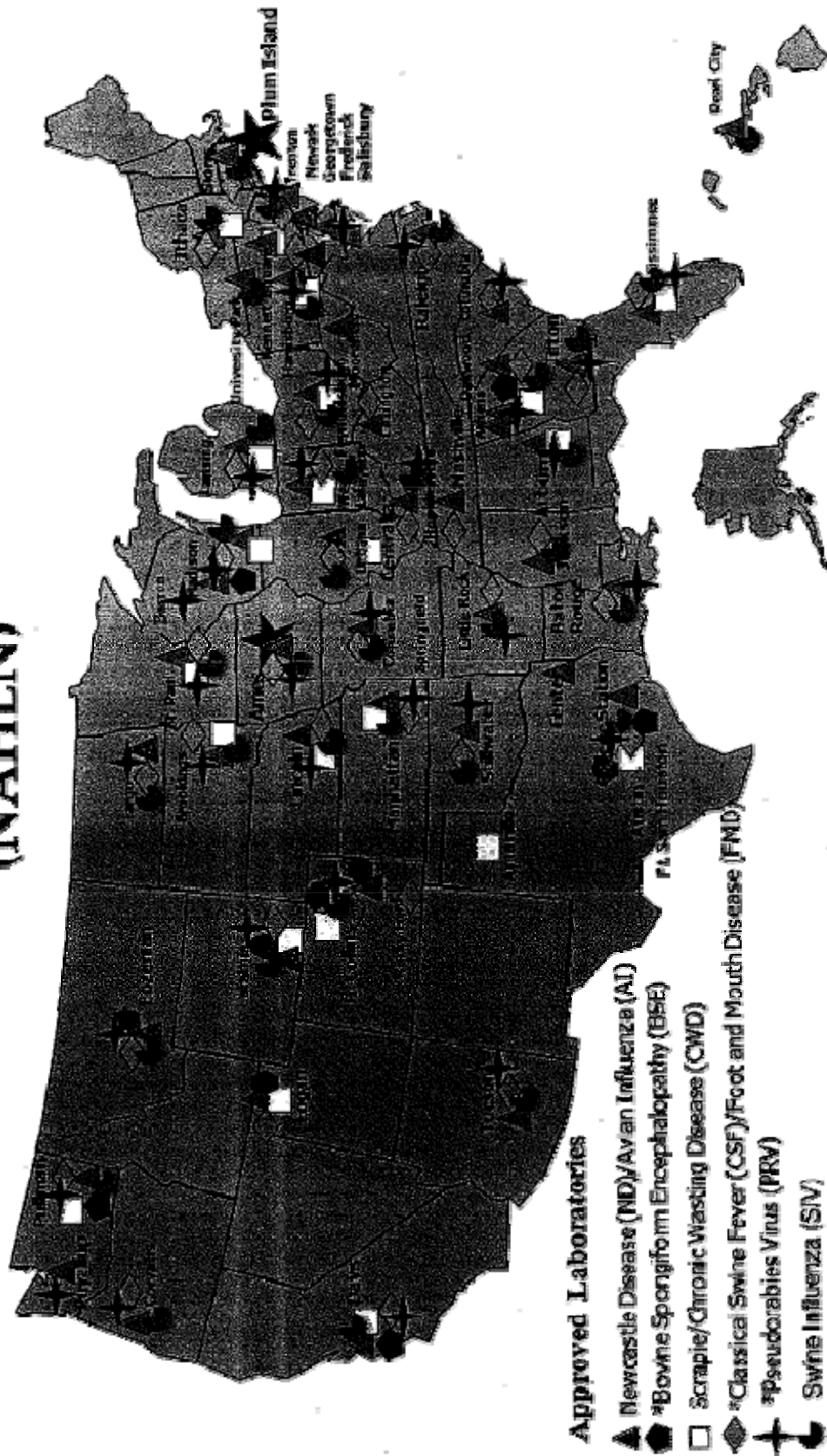
The Purposes of NAHLN

- **Early detection**
 - Targeted surveillance based on population density & risk
- **Rapid response**
 - Surge capacity to test outbreak samples
- **Appropriate recovery**
 - Large numbers of samples tested to show freedom

Founding Principles and Quality Requirements

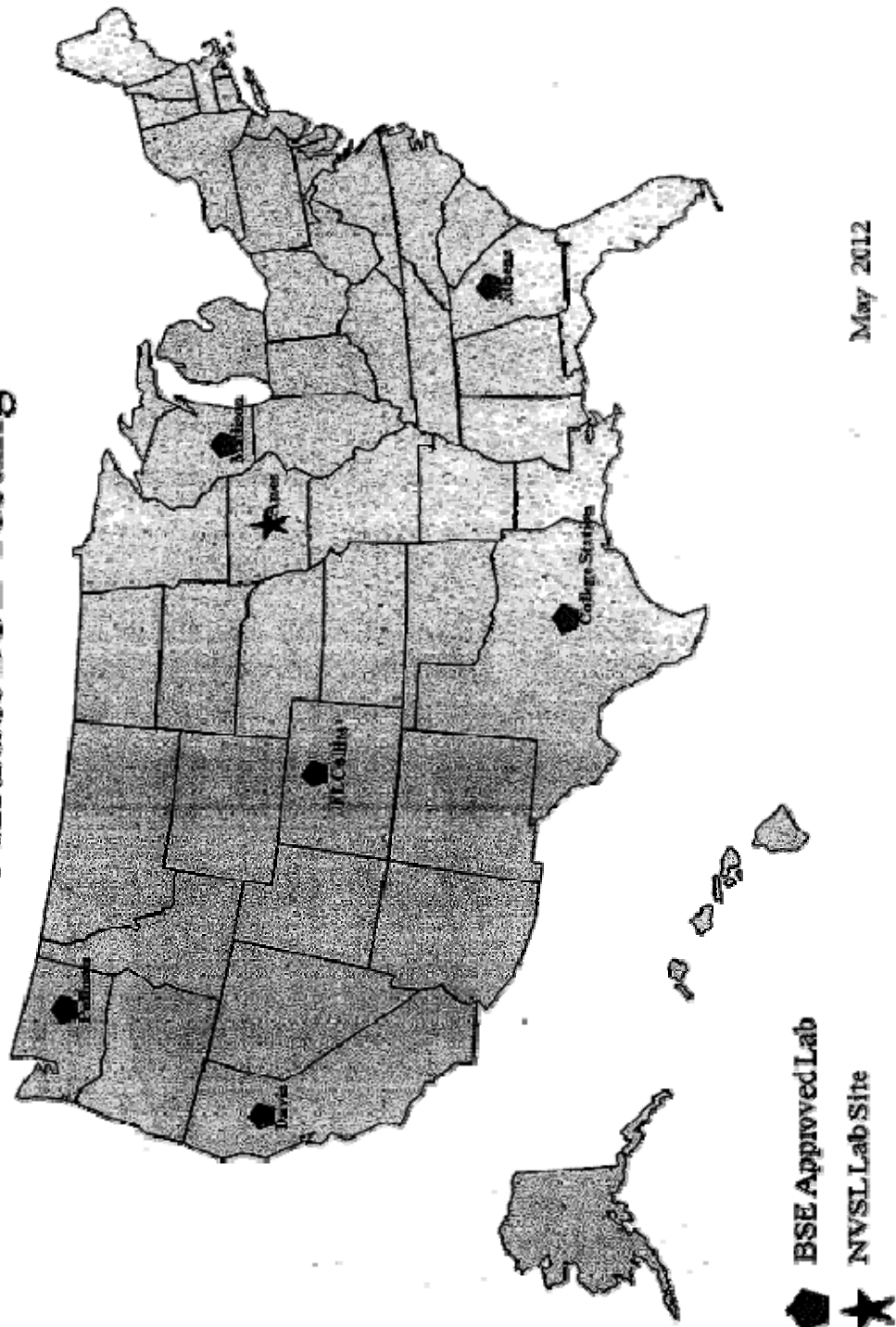
- Quality standards
- Competency of laboratory personnel
- Standardized protocols and equipment
- Adequate biosafety/biosecurity
- Secure electronic communications and reporting
- Assessment of preparedness through scenario testing

National Animal Health Laboratory Network (NAHLN)



May 2012

Laboratories Approved to Conduct BSE Testing



Applying Quality Requirements to BSE Testing

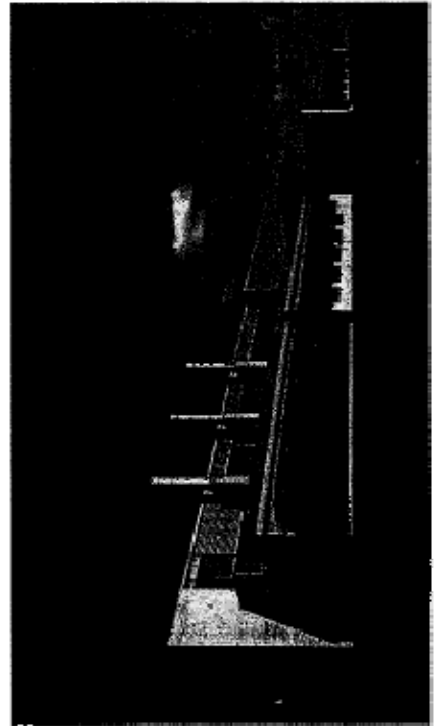
- Quality standards
 - All BSE labs are AAVLD accredited
 - 17025/OIE requirements
 - Required site visits
 - Quality Management System
- Standardized protocols and equipment
 - Licensed diagnostic kit
 - Controls and performance standards
 - Controlled copy of SOP
 - Maintenance and calibration of equipment

Applying Quality Requirements to BSE Testing

- Competency of laboratory personnel
 - NVSL – National Reference Laboratory
 - Provides training, proficiency test panels and confirmatory testing
 - Requirement - must successfully complete proficiency test process to participate in NAHLN surveillance activities
 - Annual competency assessment
- Adequate biosafety/biosecurity
- Secure electronic communications and reporting
 - Reporting requirements detailed in standard operating procedure and contract documents
 - Dedicated data system for BSE testing results from NAHLN laboratories

BSE Testing at NVSL

Bruce Thomsen, D.V.M., Ph.D., Diplomate ACVP
Supervisor, Pathology, Parasitology and Entomology Section
Pathobiology Laboratory
National Veterinary Services Laboratories



Current NVSL BSE Diagnostic Tests

- Enzyme Linked Immunosorbent Assay (ELISA) is used as screening test for surveillance on fresh brainstem (obex).
- Immunohistochemistry (IHC) and Western Blot (WB) are the approved confirmatory test methods.
 - IHC - formalin fixed tissues
 - WB - fresh tissue

First Case of BSE in the U.S.

1. December 2003

- Six and one-half (6 ½) year-old Holstein cow with history of calving paralysis in Washington
- Cow was imported from Canada in 2001
- Classical form of BSE

Second and Third Cases of BSE in the U.S.

2. 2005

- 12-year-old Brahman crossbred from Texas that died during transportation to packing plant
- H-type (high-type) BSE

3. 2006

- 10-year-old Santa Gertrudis crossbred, downer on farm, from Alabama
- H-type (high-type) BSE

All four cases were strongly positive by Bio-Rad ELISA.

Mean Optical Densities

Case 1 (WA)

Mean 1.859 N=2

Case 2 (TX)

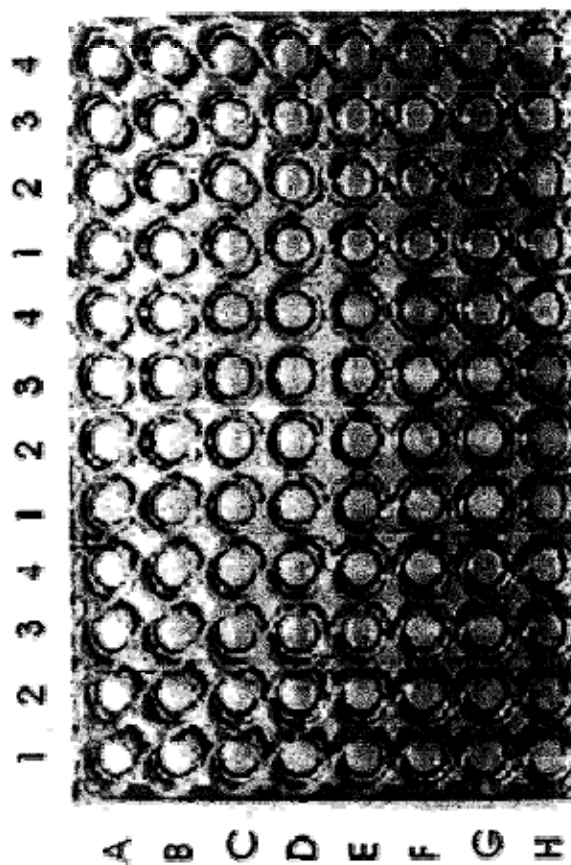
Mean 2.493 N=6

Case 3 (AL)

Mean 2.403 N=5

Case 4 (CA)

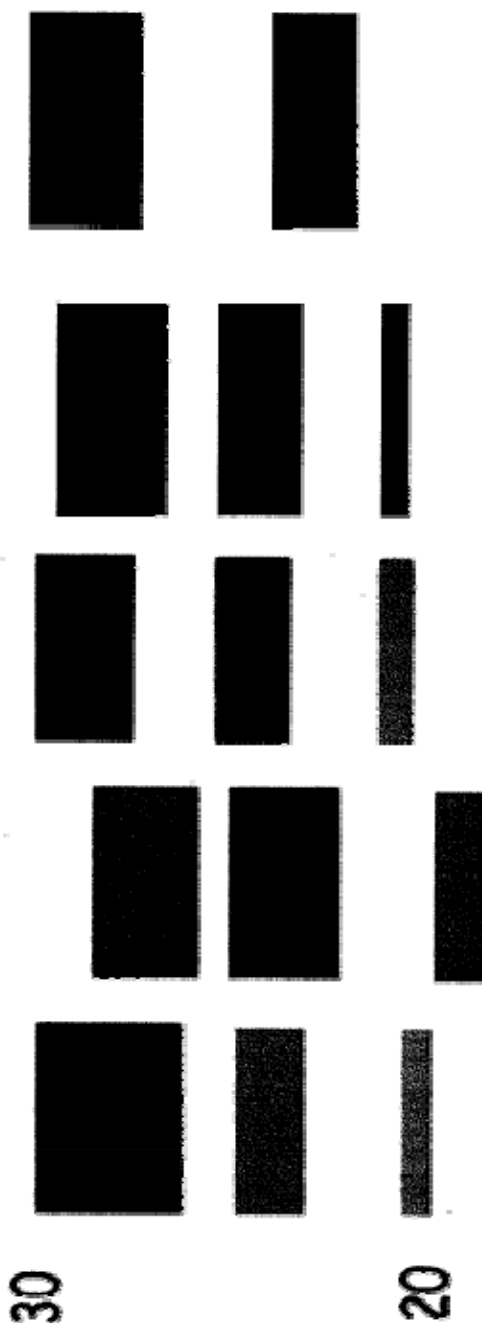
Mean 3.15 N=2



Western Blot Banding Patterns

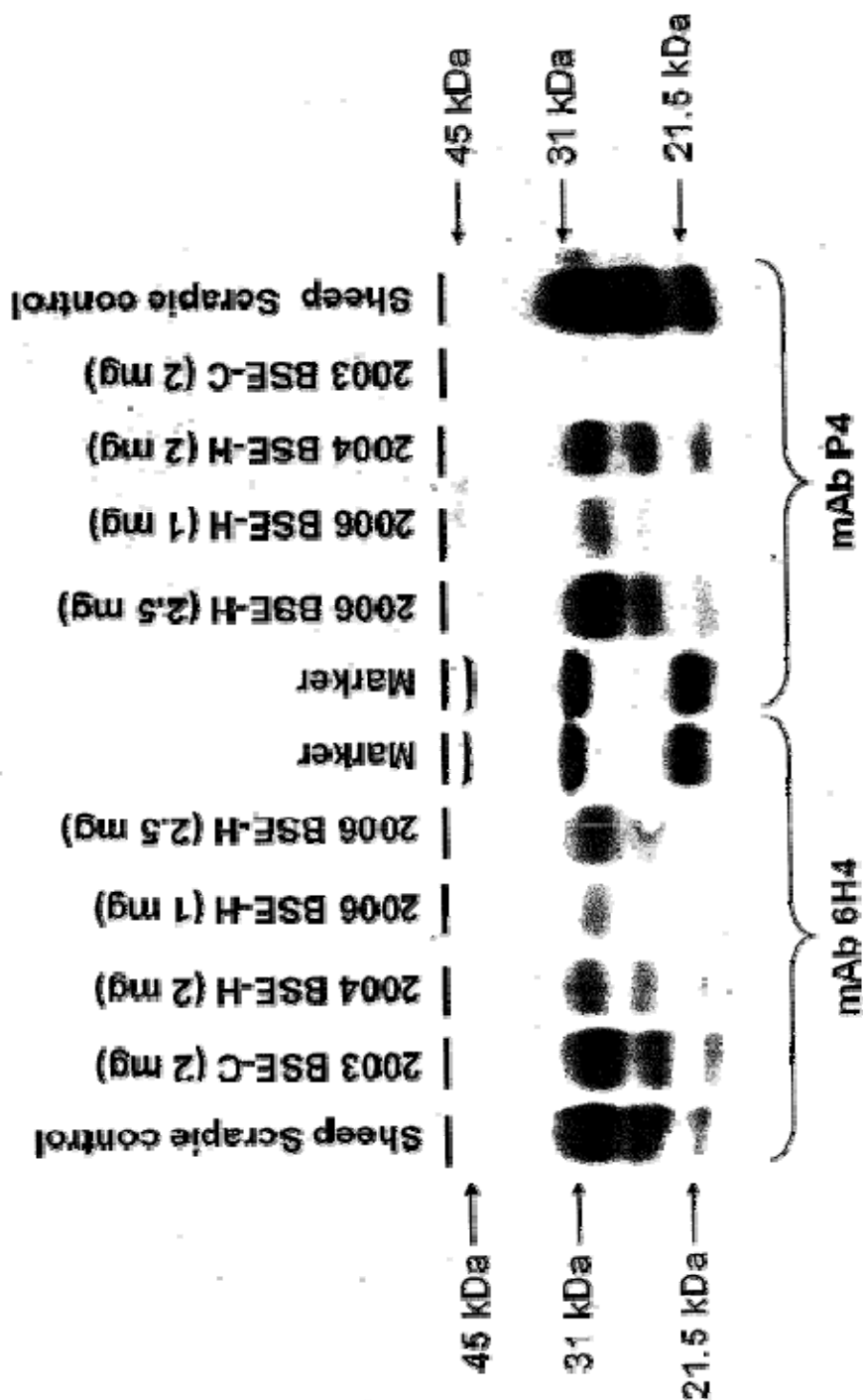
M.W. **BSE** **L type** **H type** **Scrapie** **Nor98**

Nonclassical BSE



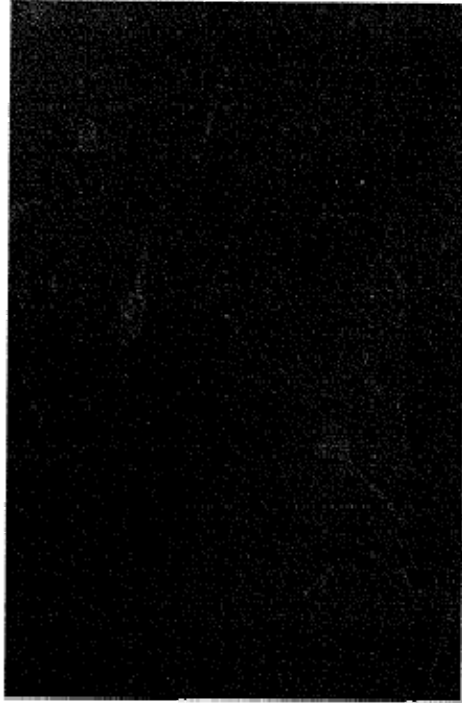
Western Blots of First Three U.S. Cases

Molecular Comparison of U.S. BSE Cases

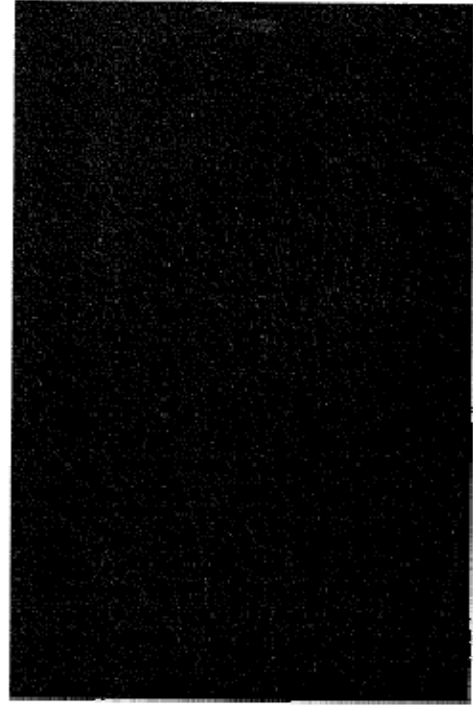


IHC Results - Case 1 vs. Cases 2 and 3

- Phenotype unusual in Cases 2 & 3
- Classical histology not seen in Cases 2 and 3
- Weaker staining by IHC in Cases 2 and 3



IHC Staining Case 1



IHC Staining Case 2



The 4th US BSE Case in Detail

Diagnostic Testing in California

- April 19: Obex sample delivered to the California Animal Health and Food Safety Laboratory (CAHFSL).
 - CAHFSL located in Davis, California.
- Later on April 19: CAHFSL reported an “inconclusive” test result to USDA.
 - Enzyme-linked immunosorbent assay (ELISA) BSE screening test used. *Bio-Rad*

Shipment of Inconclusive Sample

- April 20: CAHFSL sent sample material to the USDA National Veterinary Services Laboratories (NVSL).
 - Located in Ames, Iowa.
- April 21: Inconclusive sample material arrived at NVSL.
 - Additional items also shipped to NVSL.
 - Ear tags (collected by the renderer).
 - Sample of hide containing a brand (registered to the Tulare County dairy) – shipped later



NVSL—Ames, Iowa

Diagnostic Testing at NVSL

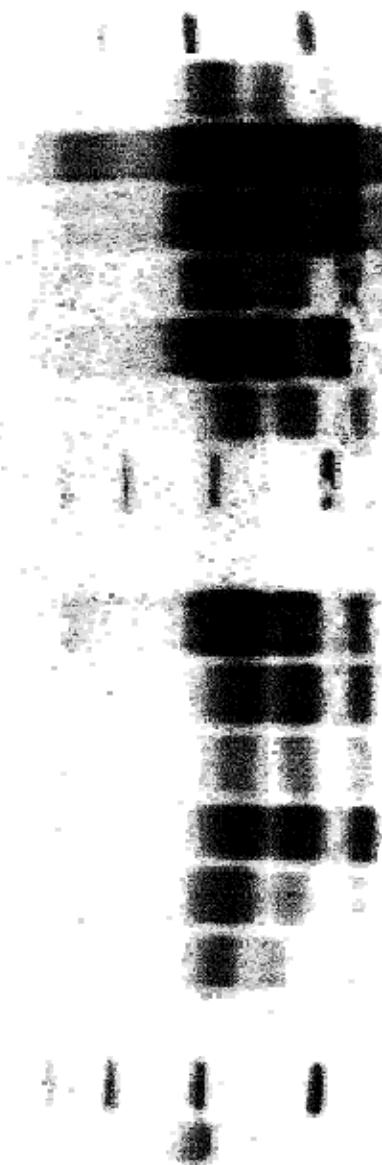
- April 21: ELISA testing for BSE was positive.
- April 23: Immunohistochemistry (IHC) and Western blot (WB) testing were positive.
 - Results indicated “L-type” (low-type) BSE, known as “atypical BSE” - not “classical BSE”.
- April 25: NVSL DNA genotyping completed matching branded hide, to tissue from ear tag, to positive obex sample.



Western Blot – 4th U.S. BSE Case

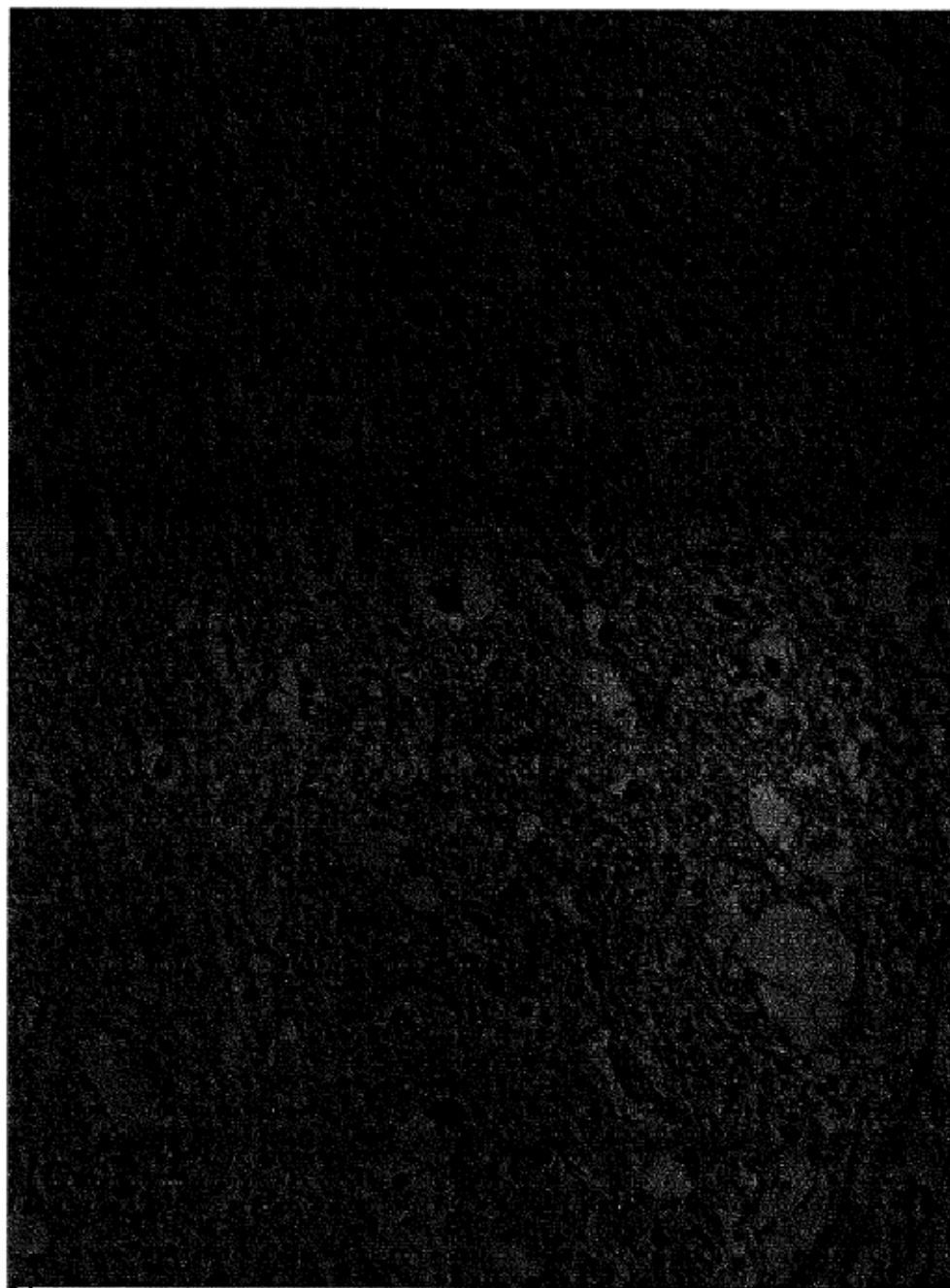
1. K
2. MM 1:10
3. neg. bows
4. high type BSE
5. classical BSE 1:20
6. sample 1:10
7. ARS 6942
8. sample 1:25
9. classical 1:20
10. neg. scrapie
11. MM 1:10
12. sample 1:50
13. high type BSE neat
14. classical BSE 1:20
15. ARS 6942 neat
16. sample neat
17. positive scrapie
18. MM 1:20

04-23-2012 12E040 with High Type and ARS 6942

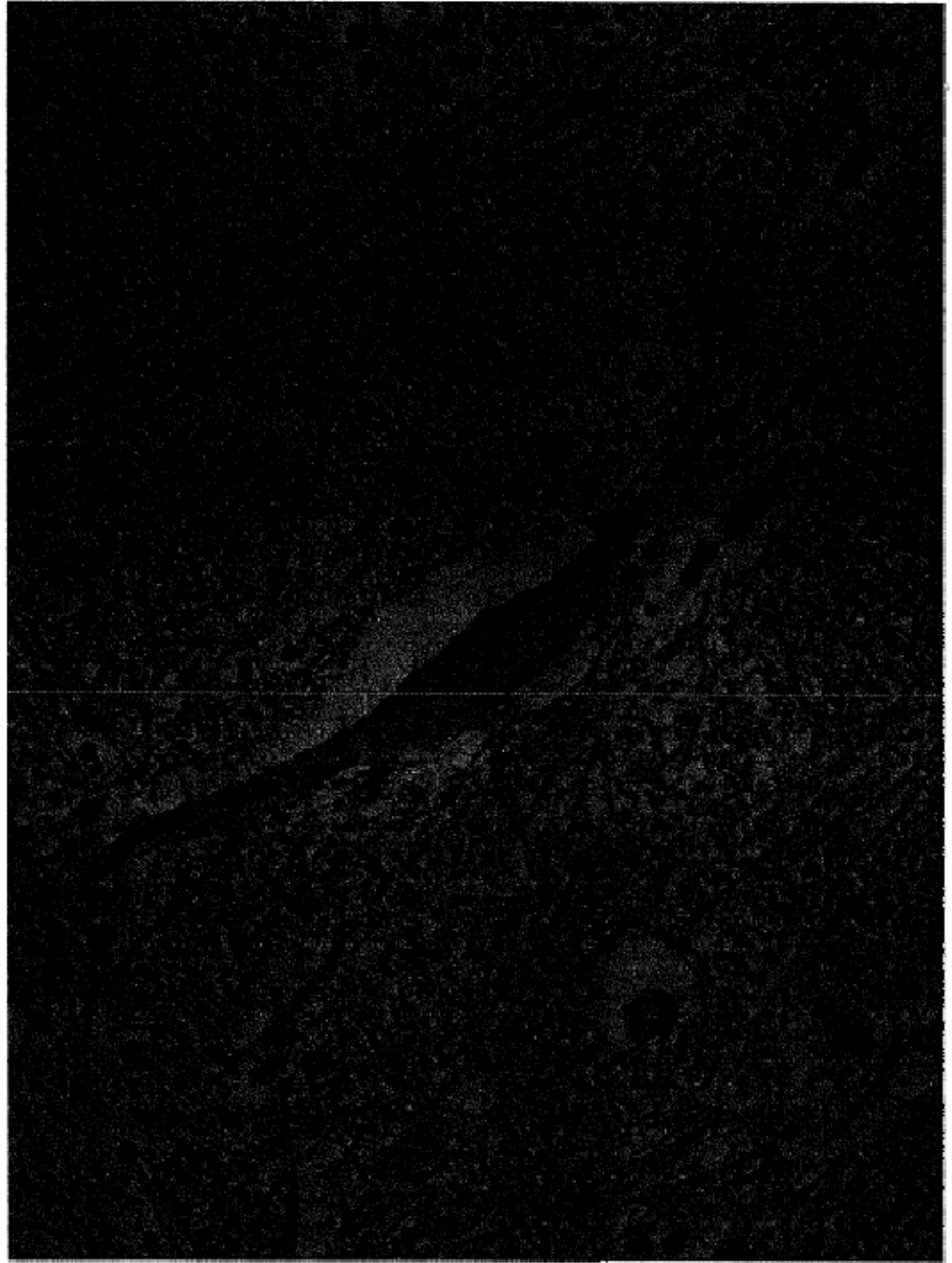


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

IHC 4th U.S. BSE Case



IHC 4th U.S. BSE Case



Progeny in Last Two Years – Testing at NVSL

- Progeny heifer
 - BSE testing was negative (ELISA)
 - DNA genotyping is pending completion

Summary

- Four Cases of BSE in US
 - First Case was Classical BSE - Imported from Canada
 - Next two cases were High-Type BSE
 - Fourth case is Low-Type BSE
- OIE BSE Labs in Canada and U.K. have received WB images and brain samples to review NVSL results.

Tissue Matching at NVSL

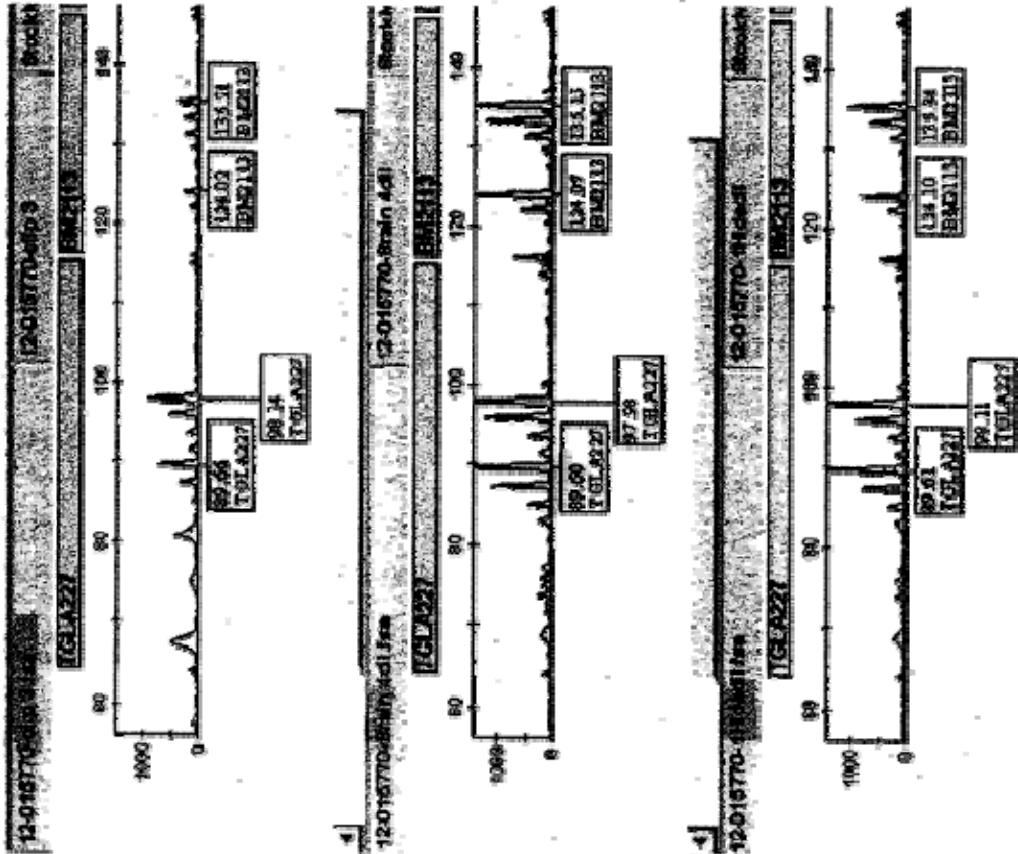
Suelee Robbe-Austerman, D.V.M., Ph.D.
Section Head, Mycobacteria Brucella
Diagnostic Bacteriology Laboratory
National Veterinary Services Laboratories



Microsatellite Genotyping (Tissue Matching)

- All newly identified cases of BSE, bovine TB, or other high consequence diseases have DNA from identification devices matched to the lesioned tissue.
- NVSL uses StockMarks™ (Life Technologies) Cattle Genotyping Kit.
 - Multiplex PCR for 11 loci that are approved by ISAG.
 - Fragment analysis on Sequencer
 - Software measures the peak location.
 - At least 7 loci must match, no mismatches
- 4th BSE case: NVSL extracted DNA from the USDA Official “Brite” ID tag, the obex tissue, and the hide.

Tissue Matching Results



	USDA Brite ID tag		Obex Tissue		Skin (H/de)	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
TGLA227	89.66	98.14	89.69	97.98	89.61	98.11
BM2113	124.04	135.21	124.07	135.15	124.10	135.30
TGLA53	163.58		163.78		163.65	
ETH10	205.18	212.94	205.25	213.04	205.20	212.93
SPS115	246.01		246.16		246.16	
TGLA126	116.01		116.05		115.97	
TGLA122	146.91	163.58	146.83	163.65	146.91	163.65
INRA23	205.07	210.71	205.27	209.82	205.20	209.83
ETH3	126.27		126.35		126.39	
ETH225	134.49	144.12	134.54	144.22	134.76	144.13
BM1824	176.32	186.80	176.38	186.93	176.37	186.83

Tissue from the Tag, Obex and Skin matched at all 11 loci, confirming they originated from the same animal.