



**Presentation 11b – Anil Prasad**



## GULF WAR VA BIOREPOSITORY TRUST

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Southern Arizona VA Health Care System (SAVAHCS)

Co-Director, Molecular Diagnostic & Research Laboratory, SAVAHCS

## GULF WAR VA BIOREPOSITORY TRUST

- MAVERIC Core Lab (Blood processing), Boston, MA
- MAVERIC Informatics, Boston, MA
- Southern Arizona Core Tissue Laboratory (SACTL), Tucson, AZ
- ALS Tissue Registry
- Gulf War Researchers
- Gulf War Veterans



## Southern Arizona Core Tissue Laboratory (SACTL), Tucson, AZ

### Southern Arizona Veterans Affairs Health Care System (SAVAHCS):

- 1928; 300 Bed Tertiary Hospital
- 150,000 Veterans
- 1,400 Health Care Professionals
- 7,900 Inpatients
- > 410,802 Outpatients



- Affiliated principally University of Arizona Colleges of Medicine, Pharmacy and Nursing
- Affiliated with the Arizona Cancer Care Center
- Comprehensive Health Care Service to 8 Counties

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SACTL Start Date: May 8, 2006

### Project Phases:

- ❖ Planning: Facility preparation
- ❖ Personnel: Hiring
- ❖ Equipment: Evaluating quotes and specifications
- ❖ IT: Evaluating quotes and specifications
- ❖ Purchasing and procurement
- ❖ Installation and set up
- ❖ Equipment testing phase
- ❖ Procedures: SOPs
- ❖ IRB

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**SACTL Team**

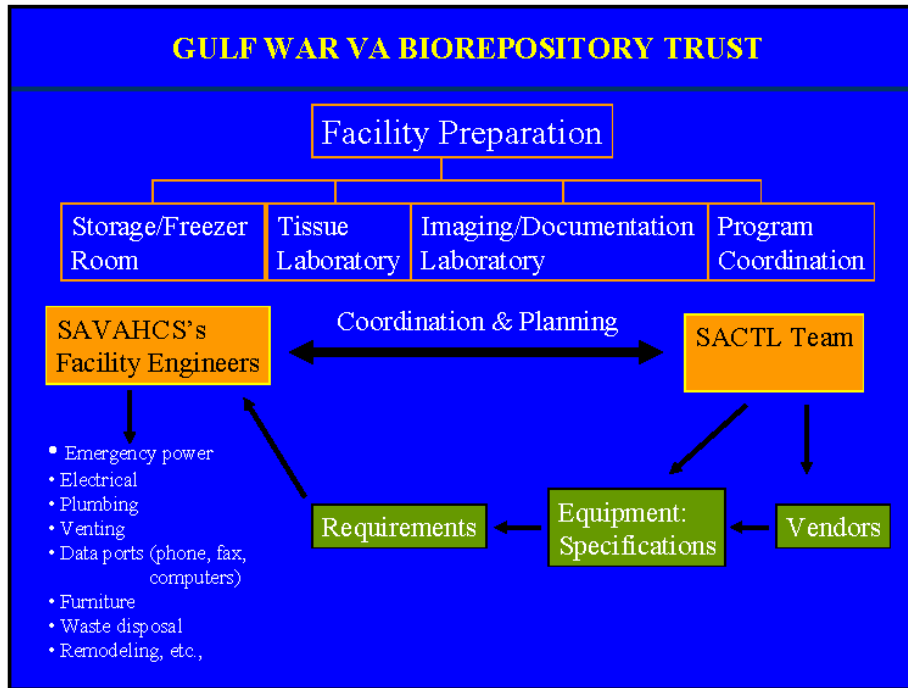


Donna Wolk, PhD                      Michael Kelly, MS                      Monika Schmelz, PhD  
Ewa Sikorksi, BS                      Anil Prasad, MD

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Facility preparation and equipment is defined by workflow:

- Receiving Tissue
- Data Entry
- Gross examination
- Sample=Aliquot preparation/Characterization:
  - snap-freezing
  - formalin fixation
  - DNA/RNA
- Aliquot Parameters Data Entry
- Storage
- Distribution



### GULF WAR VA BIOREPOSITORY TRUST

3 x 28 ft<sup>3</sup> freezers



Following NBN Blueprint Good Practice Guidelines:

- ❖ 2 Freezers used for storage
- ❖ 1 Freezer kept empty as back up

**Current Storage capacity:**

- 1 Freezer: 600 boxes
- ❖ 1 Brain yields on the average (covering the Brodman areas) ~ 150 brain tissue samples
- ❖ Spinal cord: total 6 samples
  - two of each: - cervical
  - thoracic
  - lumbar
- ❖ 8 boxes filled by one brain with spinal cord
- ❖ 75 brains including spinal cords per freezer
- ❖ 2 Freezers capacity: 150 brains incl. spinal cords

**Total storage capacity:**  
 23,400 samples/150 brains

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### SACTL: Equipment Purchases:

- Thermo Electron Gross Station
- Macropath Digital Imaging System
- IT Equipment (Servers, thin clients, computers etc.)
- Cryostat
- Microtomes, Waterbath and other Accessories
- Freezers x 4 (-80°C)
- Refridgerator (4C)
- Cryovessels
- Dry Ice Storage and Liquid Nitrogen Tanks
- Wireless Alarm System
- Embedding Station
- Archival Paraffin Block Storage
- Digital Camera
- Staining equipment and reagents
- Drying oven

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**SACTL: Freezer Storage**



All samples are standardized at the point of collection

Barcodes

- human error is estimated at 1 per 300 keystrokes
- no decoding of someone's handwriting
- speeds up processing
- decreases QC issues

All -80°C freezers are on


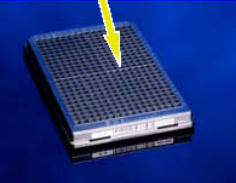
- liquid nitrogen backups
- generator emergency power
- freezer phone alarm system


All samples are

- electronically tracked by freezer, shelf rack, box and position location
- only Biorepository authorized users have access to physical inventory
- allows for fewer exceptions to the rule
- increases efficiency in utilizing freezer space
- consumption history tracking

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Dry DNA Storage



**Benefits of Whatman FTA paper**

- Isolate/preserve DNA for years (16 so far) at room temperature
- Conventional shipping methods, no biohazard

**GenVault Innovation**


- Pre-punching into plate
- GenCode sample tagging
- DNA recovery method



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**Digital Imaging**


Automated Scanner: [Ariol SL 50, Applied Imaging Corp.](#)  
 50 slide capacity



Additional:

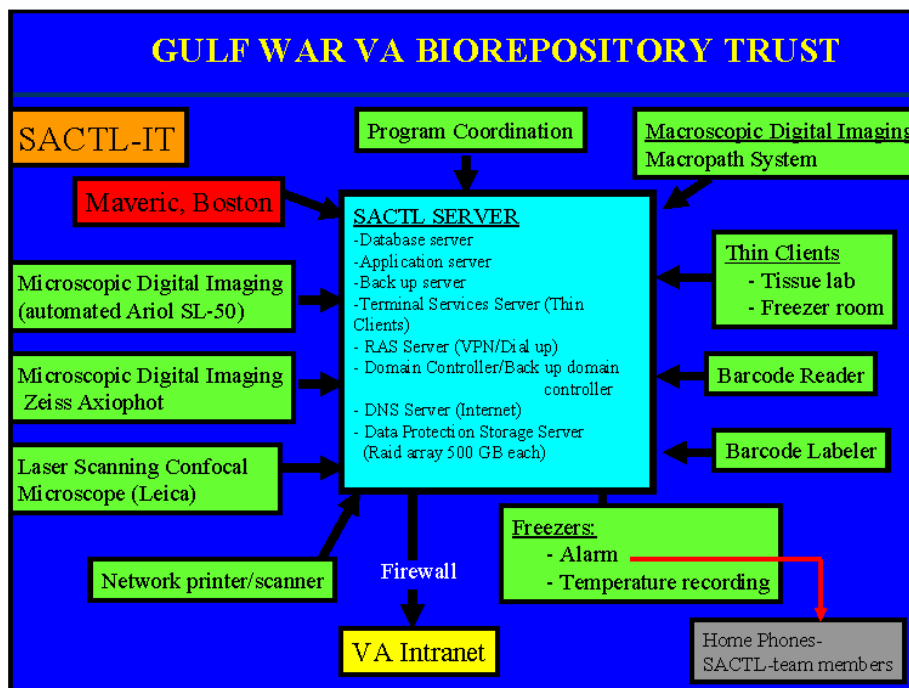
- Dual headed Zeiss Axiophot with digital camera
- Dual headed Nikon microscope

Digitalized H & E Images



**Automated Digital Image Analysis for IHC Stained Tissue:**

- Customization of the Scoring Criteria to fit Individual Protocols
- Morphometric Analysis
- Karyometry
- Tissue Microarray
- High Throughput

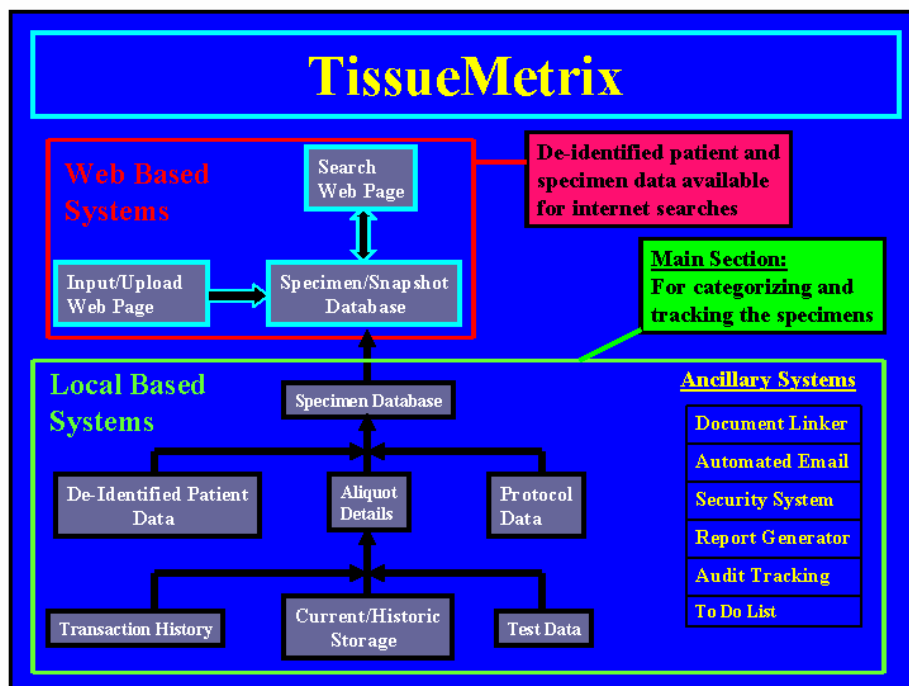


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**SACTL Database**      **TissueMetrix**

**Biospecimen Management Software**

- Configurable tissue banks and storage devices
- Efficient sample registration system
- Ability to restrict users' functional privileges
- Ability to restrict users' access to specific data
- Configurable interface with user-defined data fields
- Digital signatures for data authenticity verification
- Active contention control for data editing

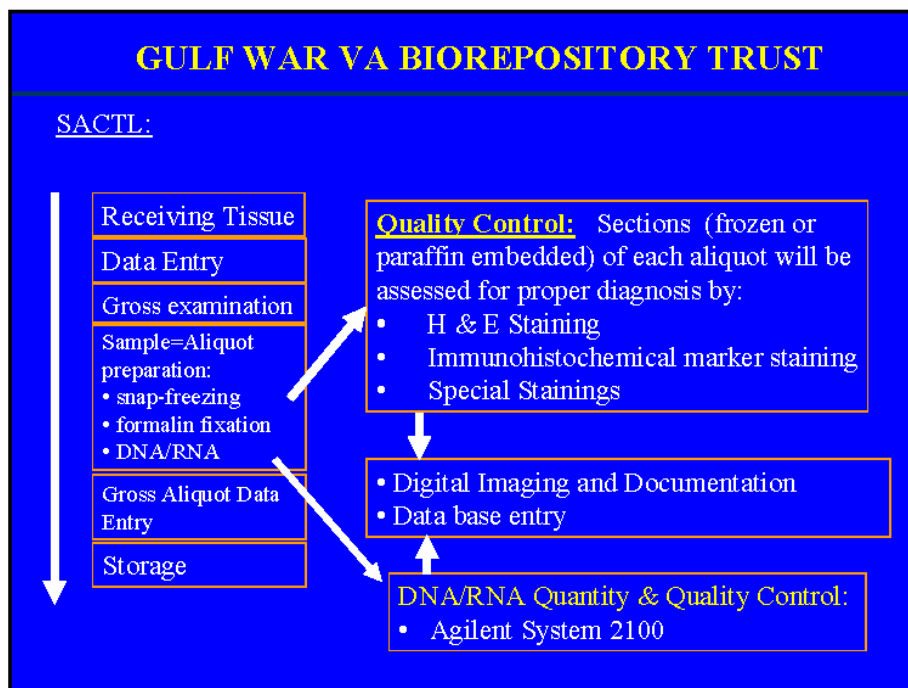




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**SACTL Database**

Microsoft Windows graphical user interface



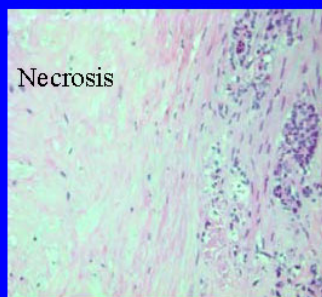
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### Key issues for banked samples

- **samples received from outside sources** may not be of the type or disease state advertised, or may be of poor quality, i.e. necrotic
- **certain cell types within a sample**, such as epithelial lining or an inflammatory component, may need to be **isolated specifically**, rather than the whole tissue being digested in an expression assay
- **non-neoplastic tissue adjacent to tumor** must be regarded carefully if intended as a "normal" control
- **pigments, inflammatory infiltrates, preservation conditions**, or other features may affect the intended performance of the tissue in an expression assay.

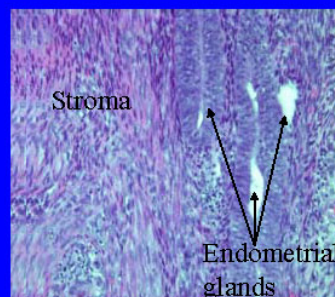
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### "Garbage in- Garbage out"



**Metastatic adenocarcinoma in the liver**

NOT IDEAL FOR RESEARCH  
ESPECIALLY FOR RNA  
EXTRACTION



Target of interest is endometrial glands. Specific isolation of the desired cell type may be needed, for example through laser-capture microdissection, if a digestive assay is planned (H&E, 400x)

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### Role of Pathology:

- **Characterization of tissues for research** - review of H&E-stained slide corresponding to tissue specimen (frozen or fixed); this serves a quality assurance purpose
- **Interpretation of studies** - assist researchers by interpreting studies (such as immunohistochemistry) performed on banked specimens
- **Educational role** – serve an educational role for investigators using banked tissues, especially with regard to pathology principles and appropriate use of tissues
- **Resource allocation** – make resource allocation decisions for precious human tissue samples

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### Immunohistochemistry Core



Automated Nemesis 7200 IHC stainer

- Open system
- Customized & standardized protocols
- Reagents



- Single stainings
- Double stainings
- Triple stainings
- Quadruple stainings

- Biomarker correlations
- Multiple marker analysis in one tissue section

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**Immunohistochemistry Core:**

**Marker in question**  
**CD 45**  
p63

Overview

**$\alpha$ -Methylacyl-Coenzyme A  
Racemase**

Quadruple IHC

### VABT Requirements for Tissue Bank Submission

- VA collection sites should follow established internal guidelines and Procedures for the collection of specimens and for the handling and Disposal of specimens.
- Supplies and Equipment:
  - Submission Forms
  - Fedex Packaging
  - Dry ice for frozen specimens
  - Protective mailing containers
  - Resealable bags
  - Biohazard stickers
- On site freezing i.e. embedding tissue in OCT, snap-freezing block in isopentane
- Placing block in polypropylene plastic container and shipping in a rigid container, a styrofoam cooler within a cardboard box
- Cooler to be filled with 2-5 lbs of dry ice prior to shipping
- Including details of Surg.Path. report, consent forms, etc.

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### Procedures & protocols for VABT Brain Bank:

Clinical and pathologic findings determine the categorization of the brain

Half of the brain is fixed in 10% formalin for diagnosis, the other half processed to be snap-frozen for research

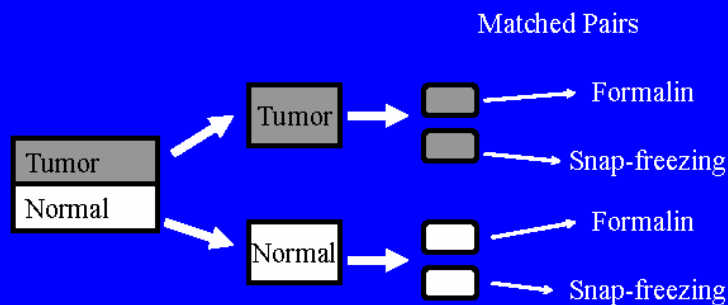
- A) No abnormality identified on external examination:
- even days of reception of the specimen: left half brain
  - odd days of reception of the specimen: right half brain
- B) If a lesion or abnormality is found on gross examination of the external surface:
- the half brain with abnormality is fixed for diagnosis
  - the other half is for research (snap-frozen)

To minimize the effects of autolysis, the half brain selected for research is processed first.  
The weight of the half brain prepared for research is recorded

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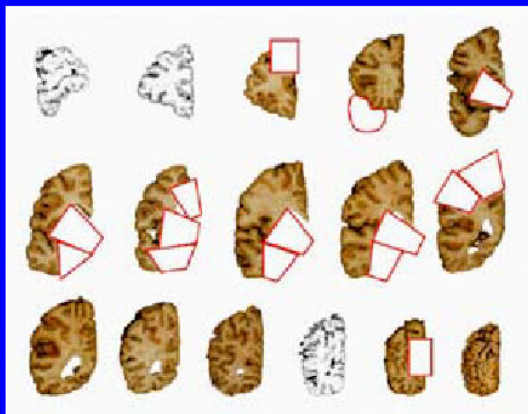
### SACTL: tissue procurement

Frozen & Fixed Mirrored-Image Samples



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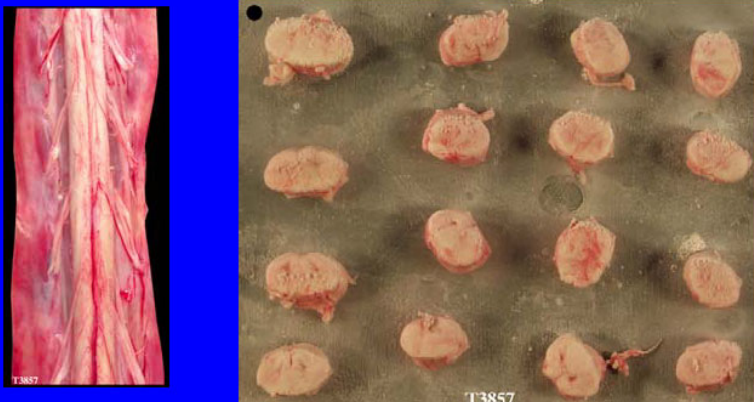
SACTL: Gross Digital Imaging with the Macropath System



Visual report linked to each sample

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ALS Protocol: Spinal Cord gross examination and cutting

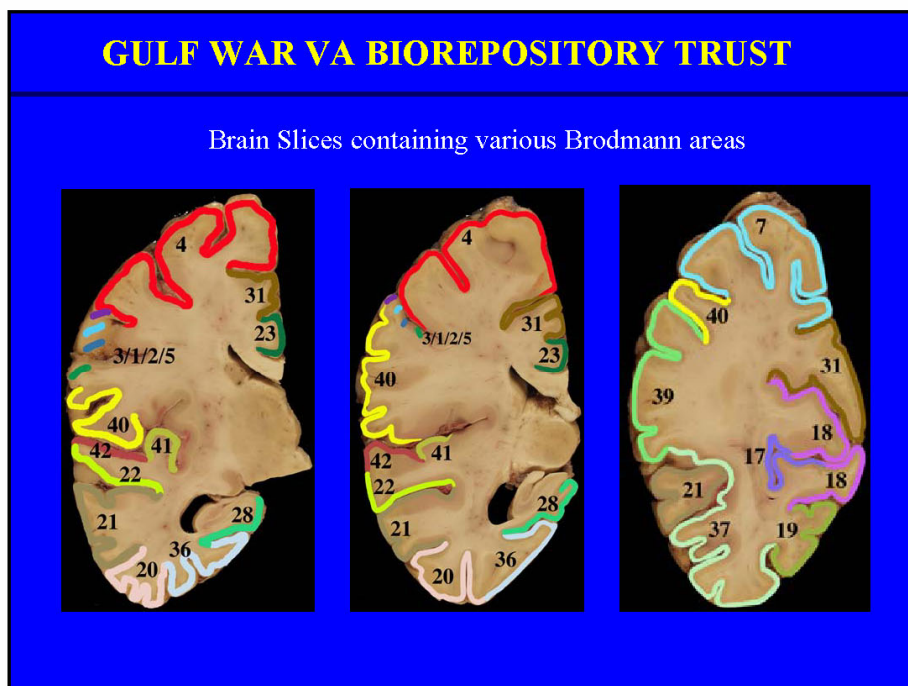
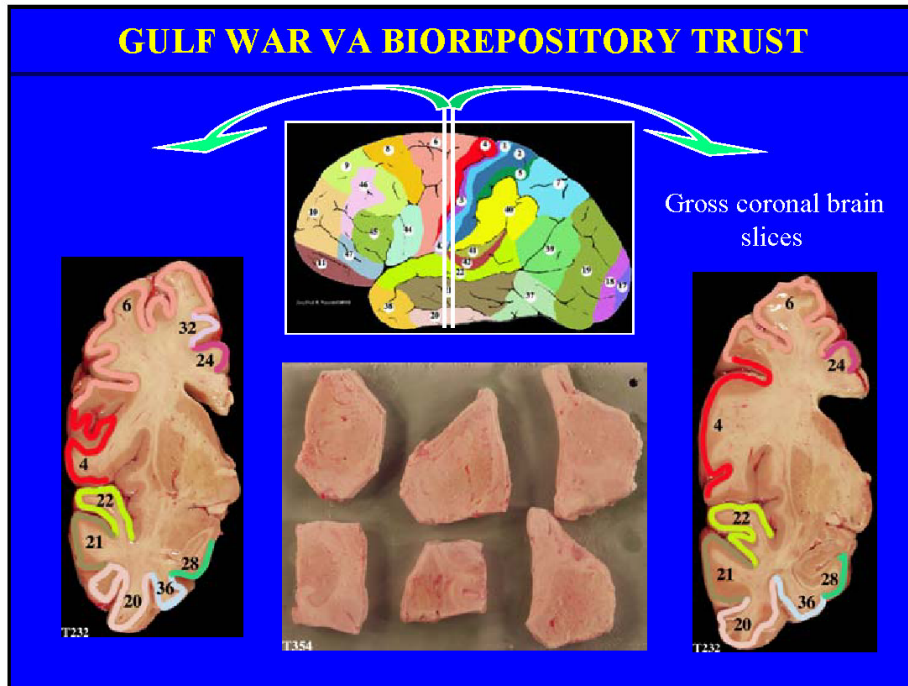


Alternate segments : Frozen -> research / Fixation -> diagnosis



<b>GULF WAR VA BIOREPOSITORY TRUST</b>		
<b>Brodman areas and structures</b>	<b>Standardized Brain Blocks (SBB)</b>	
<b>Structure</b>	<b>Brodman areas</b>	<b>Sample ID</b>
Superior frontal cortex	BA 8, 9	1.0
Posterior frontal cortex	BA 4	2.0
Parietal cortex	BA 1, 3, 5, 40, 7	3.0
Calcarine cortex	BA 17, 18 & 31	4.0
Hippocampal formation with lateral geniculate body and tail of CN		5.0
Caudate, putamen, and accumbens (CAP)		6.0
Globus pallidus and putamen with claustrum		7.0
Amygdala		8.0
Thalamus (level of anterior nucleus)-dementia)		9A

<b>GULF WAR VA BIOREPOSITORY TRUST</b>		
<b>Brodman areas and structures</b>	<b>Standardized Brain Blocks (SBB)</b>	
<b>Structure</b>	<b>Brodman areas</b>	<b>Sample ID</b>
Thalamus (level of centrum medianum-striatum degeneration-)		9B
Midbrain with red nucleus		10.1
Midbrain with decussation of superior cerebellar peduncle		10.2
Midbrain (if the level obtained is undetermined)		10.0
Upper pons (level of locus coeruleus)		11.0
Lower pons (at inferior border of V cranial nerve)		12.0
Medulla oblongata		13.0
Temporal pole, and prepole	BA 38, 20, 21, 22	15.0
Cingulate gyrus with cingulum	BA 24, 32, 6	17.0



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**SACTL: Standard Brain Block # 4 including the Brodmann's areas it contains**

Parieto occipital sulcus

Cuneus

Calcarine sulcus

Precuneus (homotypical cortex)

Calcarine (heterotypical cortex)

Brodmann's areas

○ Transition between homotypical and heterotypical cortices

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**SACTL: Standard Brain Block # 5 including the Brodmann's areas it contains**

Tail of caudate nucleus

Collateral sulcus

Occipito-temporal gyrus

Hippocampus

Lateral geniculate body

Entorhinal cortex

Parahippocampal gyrus

Brodmann's areas

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**SACTL: Quality Control**

Review of clinical data

- SOPs for case review
- Standardization and coding
- Correlation of clinical data with histopathology

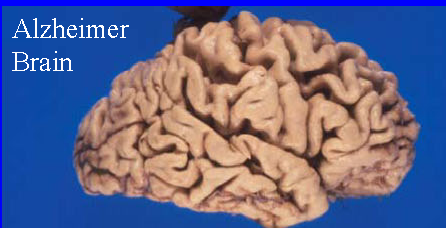
Quality control of preserved tissue samples

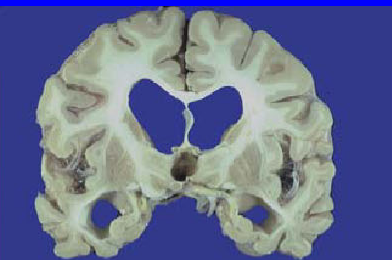
- Confirmation of histopathology
- Confirmation of DNA/RNA quality
- Confirmation of sample usability

## GULF WAR VA BIOREPOSITORY TRUST

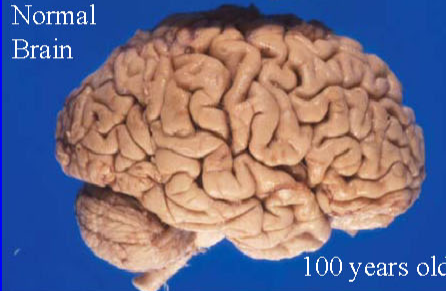
**Microscopic Examination: Alzheimer's (AD)**


Alzheimer  
Brain





Normal  
Brain



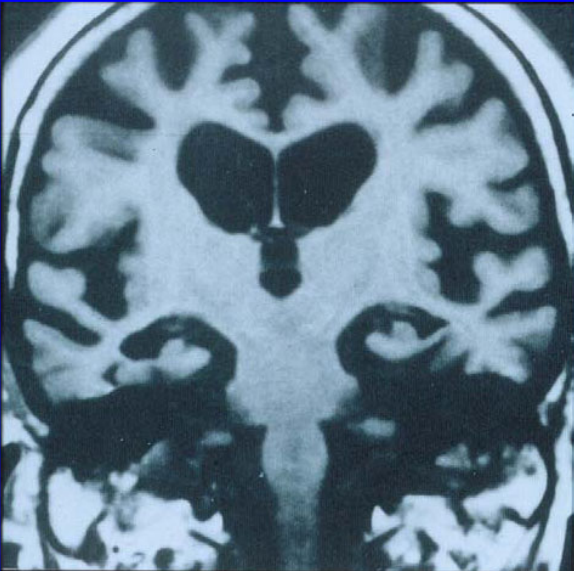


100 years old

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Neuroimaging

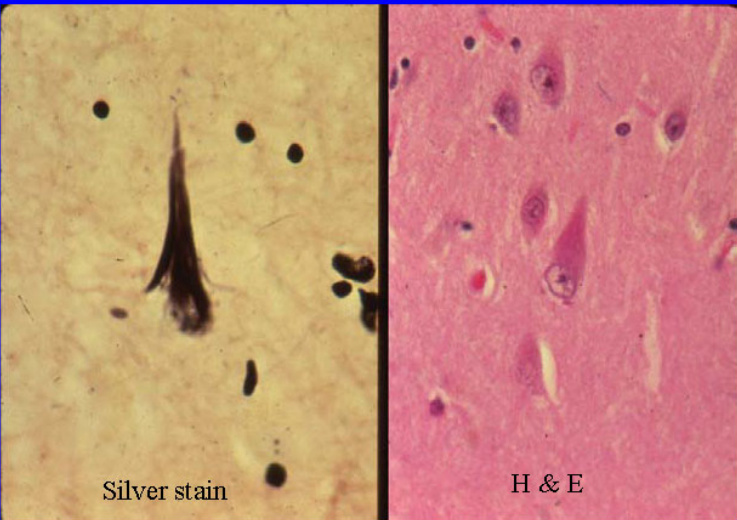
Brain Atrophy



This is a coronal MRI scan of the brain. The image shows a significant reduction in the volume of brain tissue, particularly in the hippocampal region, which is characteristic of brain atrophy. The ventricles appear enlarged, and the overall brain structure is less dense than normal.

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Microscopic Examination: AD



Silver stain

H & E

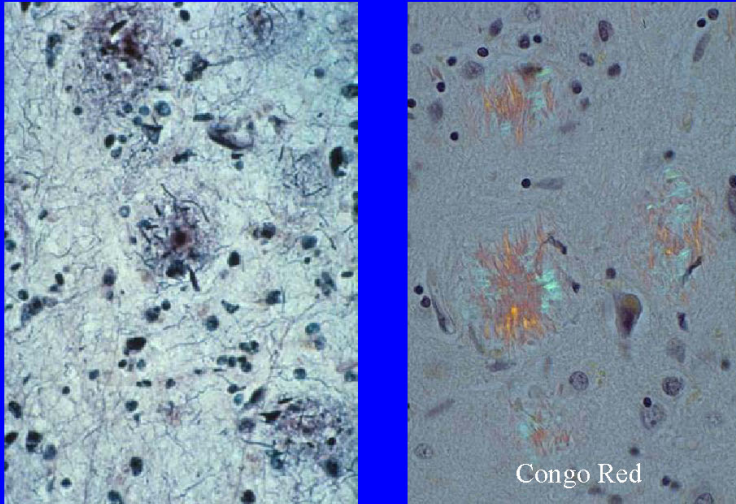
This block contains two side-by-side microscopic images of brain tissue. The left image is a silver stain, showing dark, elongated, and somewhat pointed structures, likely representing amyloid plaques. The right image is an H&E stain, showing pink and purple cellular structures, likely representing neurons and glial cells.



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**Microscopic Examination: AD**

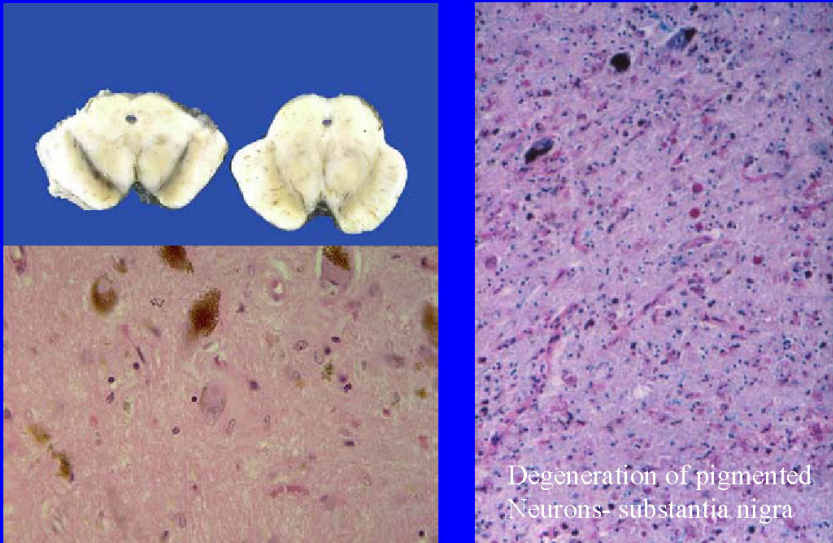
Neuritic plaques



Congo Red

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**Microscopic Examination: Parkinson's Disease**



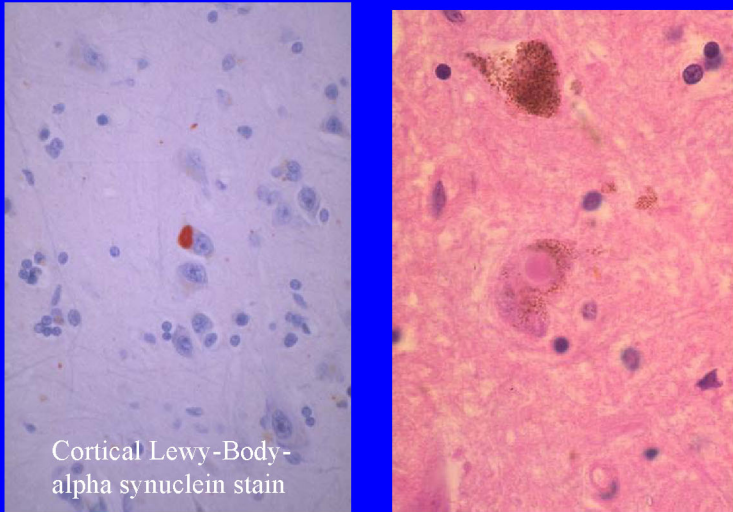
Degeneration of pigmented  
Neurons- substantia nigra



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Microscopic Examination: Parkinson's Disease

Lewy-Bodies

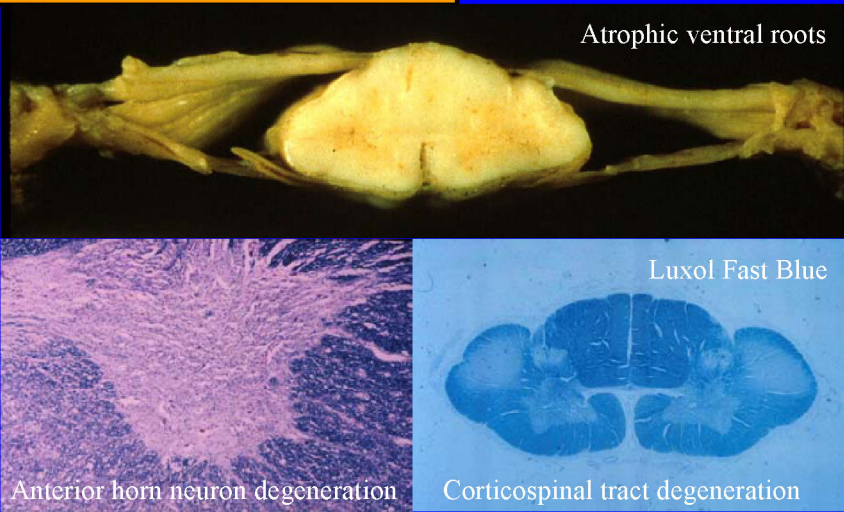


Cortical Lewy-Body-alpha synuclein stain

Lewy-Bodies

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Gross & Microscopic Examination: ALS



Atrophic ventral roots

Luxol Fast Blue

Anterior horn neuron degeneration

Corticospinal tract degeneration



## Molecular Core Services

- Nucleic Acid Extraction
- Real Time PCR
- Genetic Testing and Mutation Testing (SNP)
- Fluorescent Microscopy with Digital Imaging
- Fluorescent In-Situ Hybridization (FISH)
- GLP Testing/Consulting/Method Development and Verification
- Biorepository



## Molecular Lab






## Existing Infrastructure: Automated Nucleic Acid Extraction



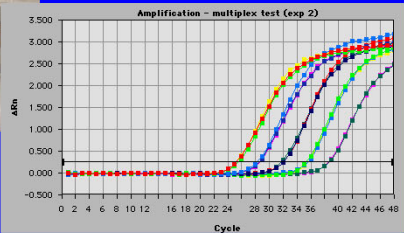
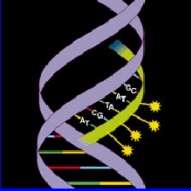

## The MagNA Pure LC instrument and Total Nucleic Acid Isolation Kit

- designed for the preparation of highly purified total nucleic acid
- variety of sample materials (serum, plasma, unseparated whole blood, and buffy coats).
- purified total nucleic acid can be used in a variety of molecular methods:
  - blockcycler PCRs
  - restriction digests
  - single nucleotide polymorphism detection (SNP)
  - real-time PCRs
  - Southern blots
  - gene expression assays




## Real-time PCR

### ABI Sequence Detection System



## GULF WAR VA BIOREPOSITORY TRUST

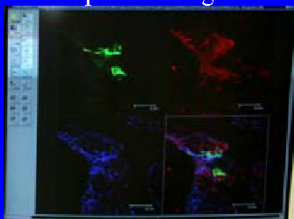
**Confocal Microscopy:**



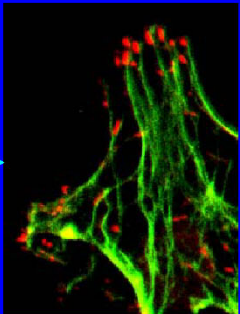
Leica TCS SP-DM IRBE


- Krypton Laser (568 nm)
- Argon Laser (488/514 nm)
- Helium/Neon Laser (633 nm)
- LCS TCS SP/NT Software


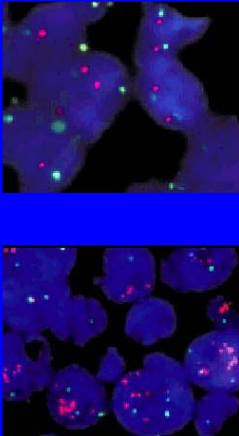
**Triple stainings**




**Focal Contacts:**  
Dual staining  
**Vinculin**  
**Actin**  
(Phalloidin-FITC)





 **Core: Microscopy & DNA Probes for Cancer (FISH)**

Her-2-Neu positive 

**GULF WAR VA BIOREPOSITORY TRUST**  
Tissue Array Analysis  
300 Samples  
IHC: Biomarkers (Ki67, Tau protein, GFAP)

 VS 



**Standard IHC**  
900 Assays

**Tissue array**  
900 Assays

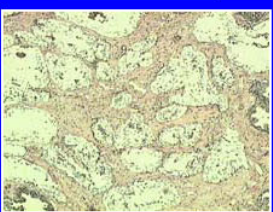
1. IHC slides = 900	1. IHC slides = 3
2. Assay time = 360 hrs (45runs x 8hr)	2. Assay time = 4 hrs (1run x 4hr)
3. Assay cost = \$22,500 (900 x \$25)	3. Assay cost = \$75 (3 x \$25)
4. Assay storage = 33.264 cu mm	4. Assay storage = 56.25 cu mm



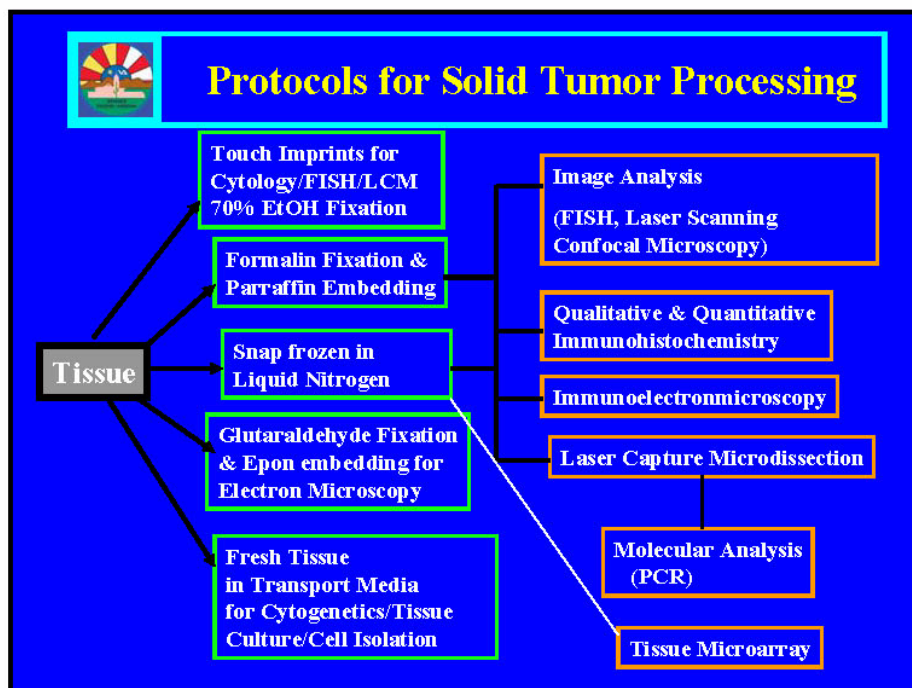
## Arcturus Laser Capture Microdissection System



Before LCM




After LCM





## SUMMARY



### AIM OF GULF WAR VABT

- **Collect**
- **Process**
- **Store**
- **Distribute**

In a standardized fashion along with patient related data including all lab results.



## VABT Role:

- Provision of high quality histology services to Gulf War researchers
- Establish database with sufficient clinical information on patient samples such that basic research findings can be correlated with clinical data without any compromise of patient confidentiality
- Provide expert pathology consultation



**Thank You**