

ADRC Standards Subcommittee

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Stds Subcommittee Members

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- **Ron Hamilton** (U. Pittsburgh)
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- **Jean-Paul Vonsattel** (Columbia U.)
- **Chuck White** (U. Texas Southwest-Dallas)

Why Are We Needed?

- Achieve more standardized diagnostic & brain banking practices across centers
- NACC Methods Survey- establish common goals across centers for histologic staining, immunocytochemistry methods, brain and bodily fluids collection, storage, distribution
- Publish a “how to” manual (expansion of and patterned after CERAD path manual)

Neuropathology Data Update Manual

Version 2.00, October 2002

NUMERICAL INDEX OF VARIABLES
Neuropathology Data Update Manual, version 2.00
(In order by variable number)

Variable Number	Variable Name	For Details See Page	Variable Number	Variable Name	For Details See Page
0	ADCID	page 16	12h	NPAVAS	page 28
1	PTID	page 17	12i	NPARTER	page 29
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2b	NPFORMDY	page 17	12k	NPOANG	page 30
2c	NPFORMYR	page 18	12L	NPVOTH	page 30
3	NPID	page 18	13	NPLEWY	page 31
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Methods Survey Results - 1.

1a. # NP-related data elements maintained in each Center's Database.

# Variables Stored	Count (N=28)	Percent (%)
1-25	5	18
26-50	2	7
51-75	4	14
76-100	5	18
> 100	12	43

Frequency Missing = 1

Methods Survey Results - 2.

1d. Categories of maintained NP Data (N=29).

Type of Data	Yes	Percent (%)
AD pathological diagnosis	27	93
Senile plaques / NFTs	25	86
PD pathological diagnosis	27	93
Lewy bodies	26	90
Infarcts	26	90
Other diagnoses	26	90
Immunohistochemistry	17	59
Genetics	16	55

Methods Survey Results - 3.

3a. Do you regularly obtain circumstance of death information?

CIRDTH	Count (N=29)	Percent (%)
Yes	12	41
No	7	24
For some, not all, subjects	10	35

4. Average time from death to preservation of brain tissue (i.e Post-Mortem Interval).

POSTMORT	Count (N=29)	Percent (%)
0 - 4 hrs	3	10
5 - 8 hrs	10	35
9 -12 hrs	16	55

SPECIMEN AND TISSUE PREPARATION

5. Frequency of Centers (N=29) regularly collecting specimens from autopsy.

Specimen	Fixed N (freq)	Paraffin N (freq)	Frozen N (freq)
Hippocampus	25 (0.86)	29 (1.00)	27 (0.93)
Entorhinal cortex	24 (0.83)	28 (0.97)	25 (0.86)
Amygdala	24 (0.83)	24 (0.83)	23 (0.79)
Frontal cortex	25 (0.86)	29 (1.00)	28 (0.97)
Temporal cortex	25 (0.86)	29 (1.00)	26 (0.90)
Parietal cortex	25 (0.86)	29 (1.00)	25 (0.86)
Occipital cortex	23 (0.79)	26 (0.90)	26 (0.90)
Thalamus	23 (0.79)	26 (0.90)	22 (0.76)
Hypothalamus	20 (0.69)	19 (0.66)	18 (0.62)
Basal ganglia	25 (0.86)	28 (0.97)	26 (0.90)
Nucleus basalis	23 (0.79)	26 (0.90)	21 (0.72)
Olfactory nerve/bulb	17 (0.59)	11 (0.38)	14 (0.48)
Cerebellum	23 (0.79)	28 (0.97)	27 (0.93)
Substantia nigra	24 (0.83)	29 (1.00)	23 (0.79)
Midbrain (not sub. nigra)	23 (0.79)	24 (0.83)	16 (0.57)
Pons	26 (0.90)	28 (0.97)	19 (0.66)
Medulla	25 (0.86)	26 (0.90)	19 (0.66)
Pineal gland	10 (0.35)	3 (0.10)	6 (0.21)
Pituitary gland	12 (0.41)	8 (0.28)	4 (0.14)
Dorsal root ganglia	3 (0.10)	2 (0.07)	3 (0.10)
Spinal cord	10 (0.35)	9 (0.32)	9 (0.32)
Peripheral nervous tissue	2 (0.07)	2 (0.07)	1 (0.03)
Visceral organs	4 (0.14)	4 (0.14)	2 (0.07)
Skeletal muscle	5 (0.17)	3 (0.10)	4 (0.14)
Cerebrospinal fluid (post-mortem)	1 (0.03)	0 (0.00)	19 (0.66)

Methods Survey Results - 5.

SPECIMEN AND TISSUE PREPARATION (continued)

6a. Fixative most often used for paraffin-embedded (PE) tissue.

Type of Fixative	Count (N=29)	Percent (%)
Alcohol	2	7
10% buffered formaldehyde	20	69
4% paraformaldehyde/buffer	9	31
Other	3	10

6b. Average time tissue in fixative prior to PE.

Time	Count (N=29)	Percent (%)
1 day	1	3
2 days	3	10
3-7 days	3	10
8-14 days	15	52
> 14 days	7	24

7a. Is tissue stored in cryoprotective solution?

CRYOPROT	Count (N=29)	Percent (%)
Yes	10	34
No	19	66

7b. Tissue used for initially preserving tissue prior to cryoprotection.

Type of Fixative	Count (N=10)	Percent (%)
Alcohol	0	0
10% buffered formaldehyde	0	0
4% paraformaldehyde/buffer	9	90
Other	1	10

7d. Cryoprotective solution most commonly used.

Cryoprotective Solution	Frequency
16% sucrose	1
2% DMSO/10% glycer for 2 days; 2%/20% for 2 days;	1
20% glycerol, 2% DMSO in 0.1 M PBS	1
20% glycerol, DMSO	1
30% sucrose in PBS	1
40% Sucrose	1
Isopentane-liquid Nitrogen	1
PBS-0.3 M Sucrose with Thiomerosal	1
PLP	1
Sucrose/ethylene glycol in phosphate buffer	1

Methods Survey Results - 7.

9a. Commonly used method for dissecting tissue.

Method	Count (N=29)	Percent (%)
Hemibrain frozen en bloc	1	3
Slabbed brain sections frozen	19	66
Dissected small samples frozen	15	52
Other	2	7

Other method	Frequency

Liquid nitrogen or fixatives	1
PROTOCOL, SOME SLICES, SOME DISSECTED	1

9b. Commonly used method for freezing tissue.

Method	Count (N=28)	Percent (%)
Dry ice (chilled aluminum plates)	7	25
Flash frozen (liquid nitrogen)	9	32
Ultra low freezer ($\leq -70^{\circ}$)	14	50
Other	4	14

Frequency Missing = 1

Methods Survey Results - 8.

Other method	Frequency
AL PLATES AT -80 IN ULTRA LOW FREEZER	1
Dry ice only	1
Dry ice slabs (no aluminum plates)	1
Dry ice, no plate	1
Liquid Nitrogen Vapor	1
Over nitrogen vapor	1
Preparing to use liquid Nitrogen	1

9c. Commonly used method for storing frozen tissue.

Method	Count (N=29)	Percent (%)
Regular Freezer	0	0
Ultra-low Freezer	29	100
Liquid Nitrogen Tank	1	3
Other	0	0

Methods Survey Results - 9.

10. Biological Specimens Banked.

Specimen Type	Count (N=27)	Percent (%)
DNA	17	63
Serum	13	48
Buffy coat lymphocytes	8	30
Lymphoblastoid ICL	2	7
Plasma	9	33
Antemortem CSF	5	19
Other	1	4

Frequency Missing=2

Methods Survey Results - 10.

HISTOPATHOLOGY

11a. Stains used for identifying beta-amyloid pathology.

Stain	Count (N=29)	Percent (%)
Silver Stain *	20	69
Thioflavin-S	10	34
Congo Red	7	24
A β immunostain **	20	69
Other ***	2	7

* Specific types of silver stain include Bielschowsky, Bodian, Campbell-Switzer, Gallyas, Hirano, and Microwave King.

** Specific types of A β antibodies include Athena 10D5, Signet 6E10, Chemicon AB5074P, DAKO monoclonal mouse, Elan 4G8, GFAP, Tau (AT8), Novocastra beta-amyloid

*** Other stains/antibodies include APP, Reusch, Tau

Methods Survey Results - 11.

12. Stains used for identifying neurofibrillary tangles.

Stain	Count (N=29)	Percent (%)
Silver Stain *	22	76
Thioflavin-S	10	34
Tau immunostain **	23	79
Other ***	3	10

* Specific types of silver stain include Bielschowsky, Bodian, Gallyas, Hirano, and Microwave King.

** Specific types of tau antibodies include Tau-1, Tau-2, PHF-1, PHF-6, Sigma P6402, Sigma monoclonal T5530, recombinant Tau, Dako Tau A0024, and AT8.

*** Other stains/antibodies include Ubiquitin, TG-3, and MC1.

Methods Survey Results - 12.

HISTOPATHOLOGY

13. Stains used for identifying any other neuronal/glial pathology.

Stain	Count (N=29)	Percent (%)
α -crystallin	5	17
α -synuclein *	26	90
Neurofilament **	11	38
Neuroserpin	0	0
Ubiquitin ***	23	79
Other	3	10

* Specific types of α -synuclein antibodies include Chemicon AB5038, Zymed LB509, Chemicon affinity purified polyclonal formic acid, SYN202, SYN203, Novocastra NCL-ASYN, Novocastra clone KMS1, Rabbit anti-synuclein, Rectin, and Signet 4D6.

** Specific types of neurofilament antibodies include Dako 2F11, SMI34, Zymed 13-1300, anti-NT, and SNA31.

*** Specific ubiquitin antibodies include Chemicon MAB, Dako Z0458, Dako rabbit polyclonal, East Acres polyclonal, and Novocastra clone.

Methods Survey Results - 13.

14. Is PRP immunostain used for identifying prion pathology

	Count (N=29)	Percent (%)
Yes *	6	21
No	23	79

* Specific PRP antibodies include 3F4, Chemicon MAB 1562, Chemicon PRP27-30 N-terminus.

Goal #1 - Methods Manual

- Extend and provide continuity with the 1988 and 1994 “Guide to the CERAD Protocol for the Neuropathological Assessment of Alzheimer’s Disease”
- Add sections on tissue banking, staining methods and newer disease classifications
- Develop in close partnership with histology technicians to promote career development

CERAD GUIDE
to the
Neuropathological
Assessment of
Alzheimer's Disease
and
Other Dementias

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Revised, 4/94

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Consortium **to**
Establish a
Registry for
Alzheimer's
Disease

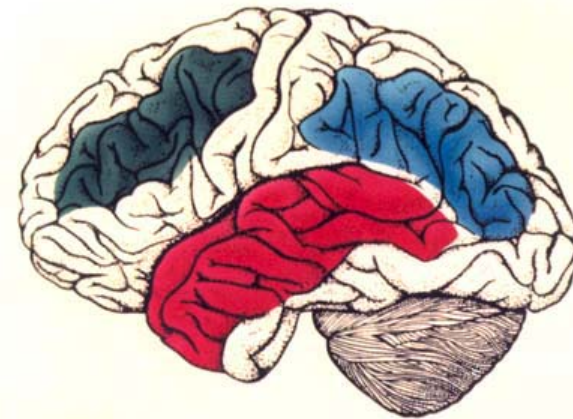
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New Standards Manual

- Stain protocols (ex: Gallyas silver)
- Antibody methods
 - alpha-synuclein
 - PHFtau
 - a-beta



- Superior and Middle Temporal Gyri
- Middle Frontal Gyrus
- Inferior Parietal Lobule

Figure 1. Three recommended neocortical regions for sectioning are depicted.

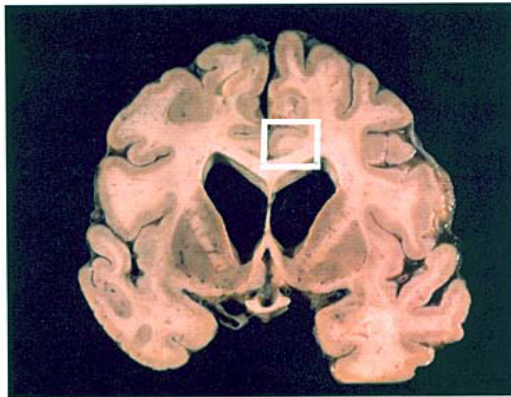


Figure 2. A section of anterior cingulate cortex is also recommended because of the frequency with which cortical Lewy bodies are detected in this region.

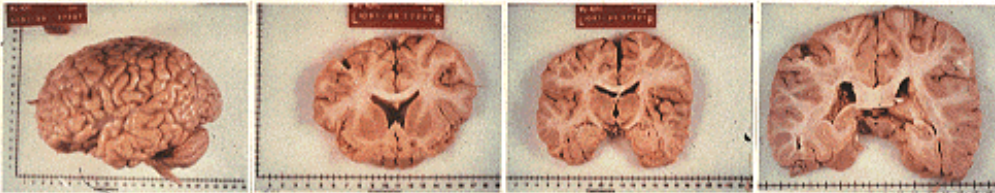
More Content Ideas ...

- Inclusions illustrated
- New disease classifications with key literature citations
- Brain and Tissue banking protocols (cryo protection)
- Laser capture tissue prep

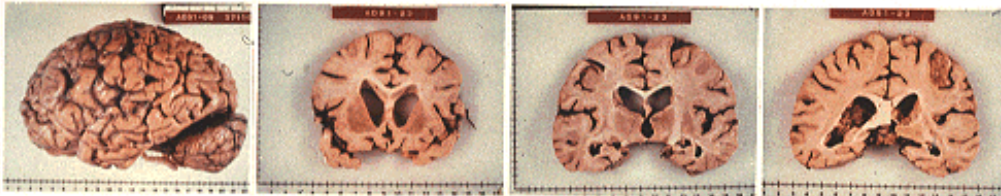
Gross 5b: Anatomy Visual Standards

BRAIN ATROPHY VISUAL STANDARDS

GRADE = 1 (NONE, NL FOR AGE)



GRADE = 2 (MODERATE)



GRADE = 3 (SEVERE)



frontal horns

body/temporal horns

trigone

AD100-54 Grade 2



McKeel DW, Gado M. A visual standards based system for scoring Alzheimer and aging-related human brain atrophy at autopsy (abstr. P34-11). Brain Pathol 1994;4:544

Brain Size and Dementia

Table 1. Ventricular Score Summary (FH+TH+BO+TRI) versus Brain Wt, Braak NFP Stage, and Expiration CDR

CDR_E group	Correlative measures of Brain Size and AD Severity		
	Fresh Brain Wt (g)	Braak NFP Stage	VENTR_score ¹
0 [n=4]	1204	2.4	4.0
0.5 [n=8]	1252	3.1	5.2
1 [n=10]	1262	2.8	5.6
2 [n=11]	1196	5.0	5.9
3 [n=84]	1074	5.0	7.6

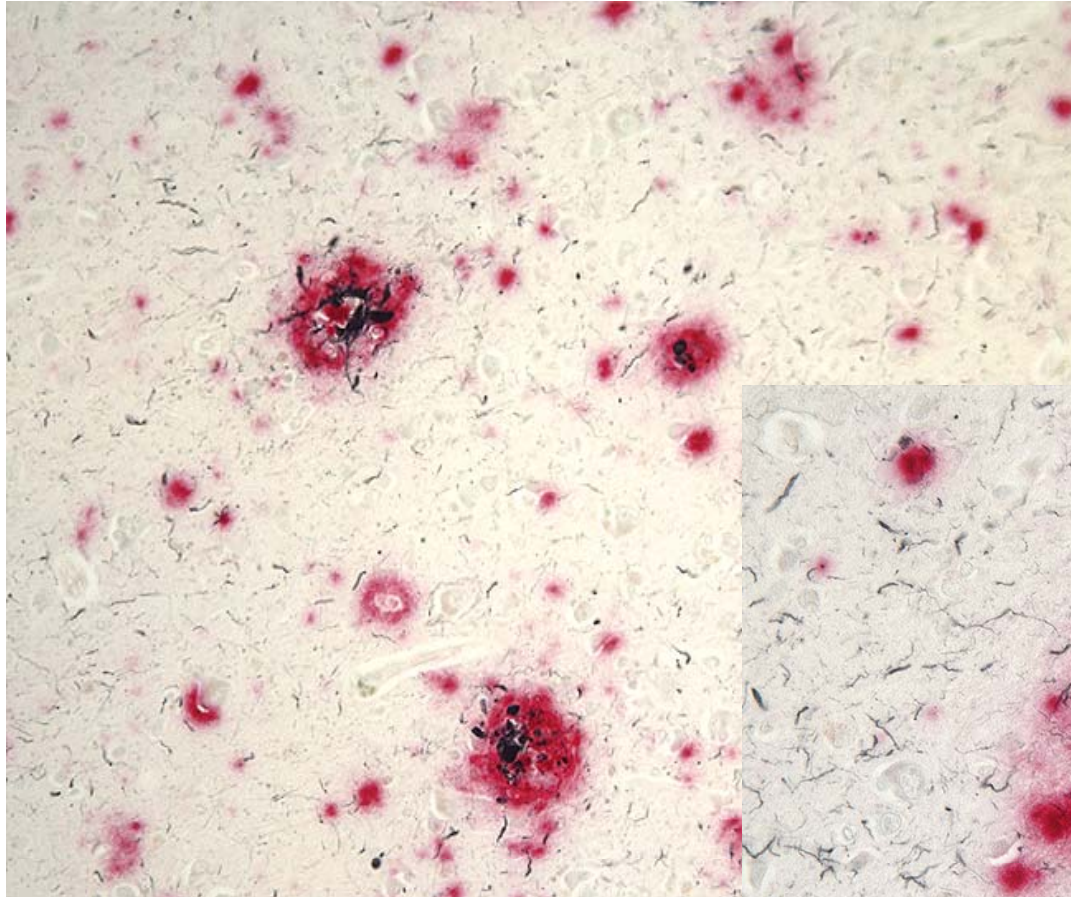
¹ Ventricular Score Summary (McKeel-Gado FH+TH+BO+TRI) [range 0-12]

CASE REPORT

PRECLINICAL ALZHEIMER'S

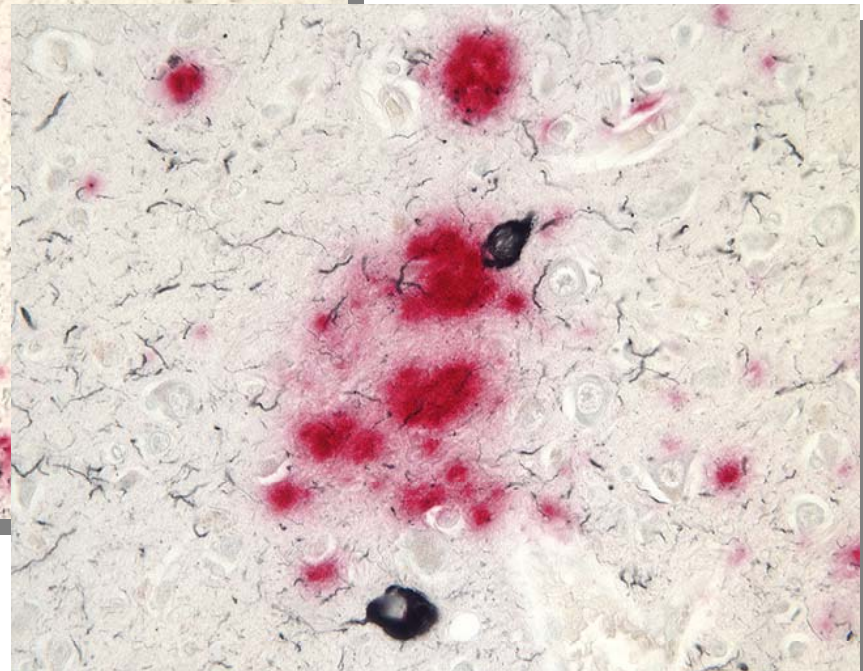
- **Washington U ADRC clinical “feedback” conference**
- **Subject: 93M - CDR 0
nondemented at expiration**
- **apoE 34 18 clinical visits**

10D5 A β red + PHF1 Tau black



**Tangles plus
Neuropil
Threads**

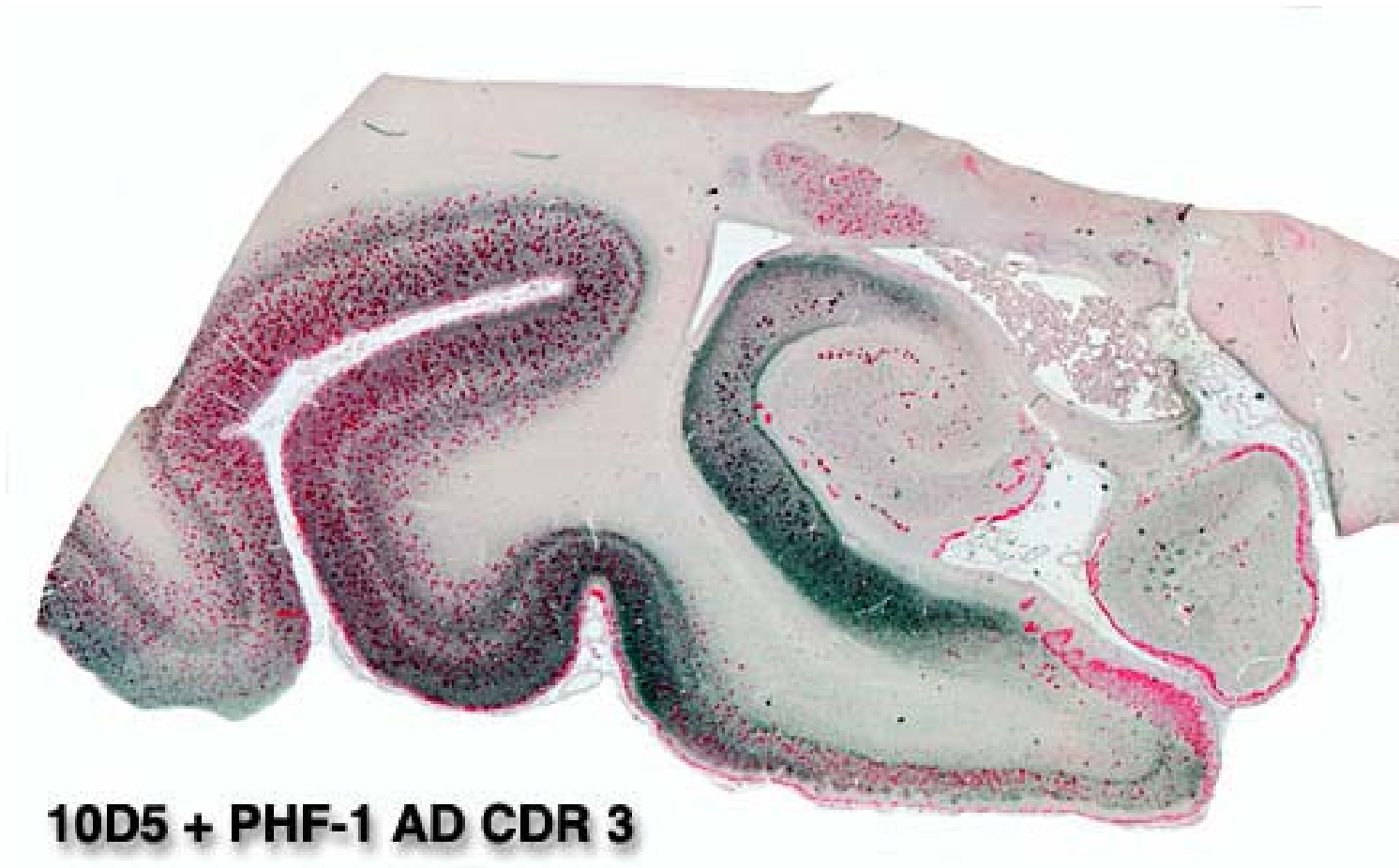
Neuritic plaques



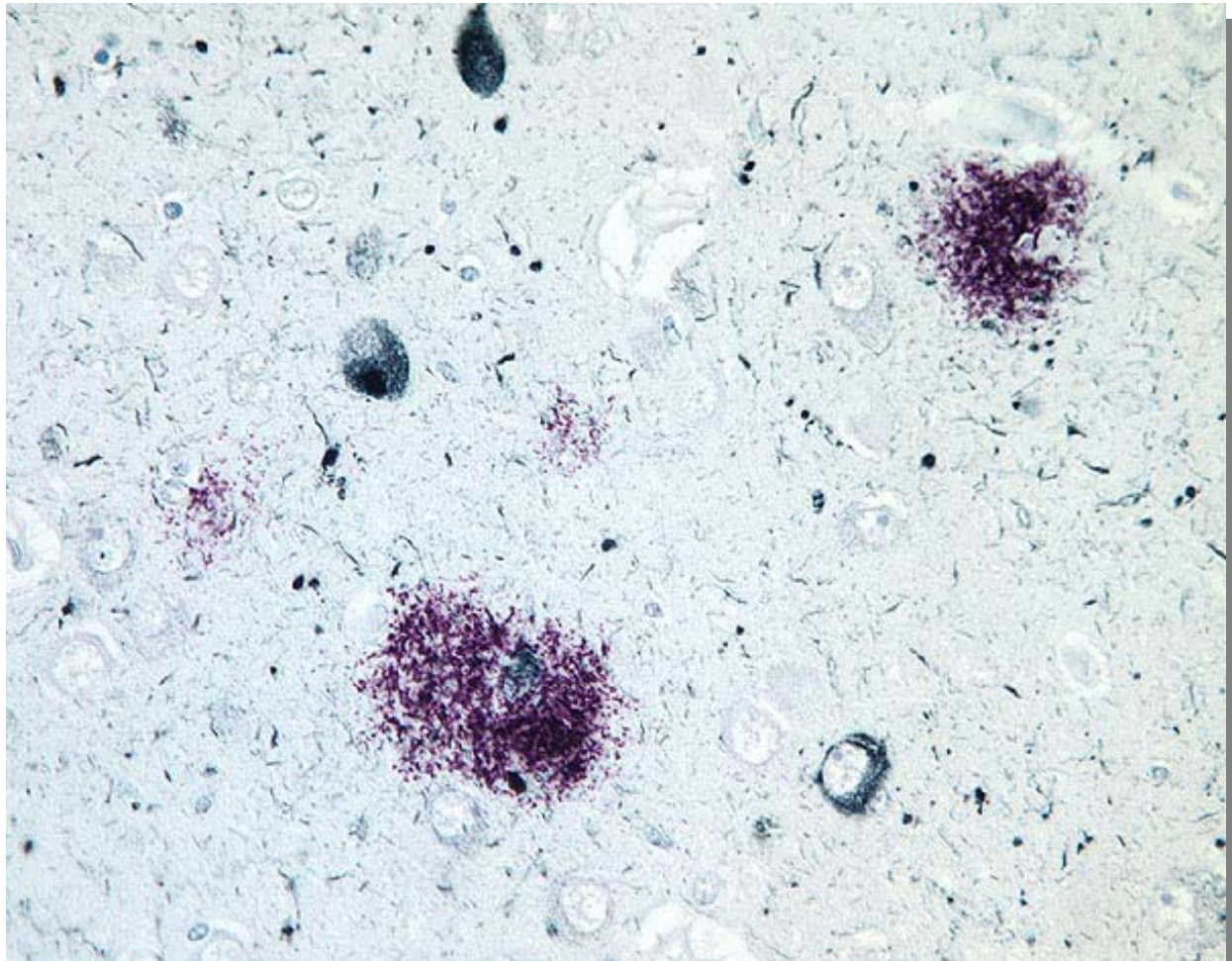
Hippocampus: 10D5 (**red**) A β *SP*
and PHF-1 tau (Black) *Tangles*



CDR 3 AD Hipp: 10D5 A β + PHF1



AD100-66: 10D5 A β + PHF-1 Tau+ Grains, Tangles & Plaque Neurites



WUSM ADRC Standard Blocks

1. Frontal cortex
2. STG + MTG
3. Inf. Parietal ctx
4. Primary visual
5. Hippocampus/ERC
ten levels
6. Striatum
7. Mamillary bodies
8. Thalamus
9. Nigra, rostral
10. Nigra, caudal
11. Pons, 3 levels
12. Medulla, 2 levels
13. Spinal Cord
14. Cerebellum + Dent. N.
15. Cerebellar vermis
16. Hypothalamus



17. Nucleus basalis
18. Orbitofrontal ctx
19. Ant. Cingulate
20. Inf. Temporal ctx
21. Primary motor ctx
22. Primary sensory ctx
23. Amygdala
24. Olfact. Tract & Bulb
& ant. olf. nucleus
25. Optic chiasm & nerve
26. WM, deep frontal
27. WM, mid portion
28. WM, occipital
29. Caudate, putamen &
globus pallidus
30. Posterior cingulate
- 31ff - Pathologic lesions

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