Quality Assurance/Standards Committee

Task 1:

Develop a procedures manual

Dan McKeel has continued to work on this goal

Quality Assurance/Standards Committee

Task 2:

Identify standards for histological stains and/or immunoreactions used for Alzheimer diagnosis

Standardized Immunohistochemistry

- At present no standardization exists in IHC methodology among ADRCs
- Includes fixation, embedding materials, pretreatment protocols, reagent sources, antibody working titers, substrates used, etc.
- Hence results vary non-systematically and adversely affect comparisons among results obtained at various centers.

(From McKeel, QAS presentation, 2004)

Stains used by ADCs to identify amyloid pathology

Stain	*% of ADCs*
Silver	69
Aβ IHC	69
Thioflavin-S	34
Congo red	24
Other	7

(Data from 2003 Survey - McKeel)

Stains used by ADCs to identify tangles

Stain	*% of ADCs*
tau IHC	79
Silver	76
Thioflavin-S	34
Other	10

(Data from 2003 Survey - McKeel)

NACC desires standardization of Techniques

Questions:

1. Which stains/IHCs are most reproducible and reliable?

2. Which stains/IHCs should be recommended (or mandated) for ADC workups?

The BrainNet Europe experience

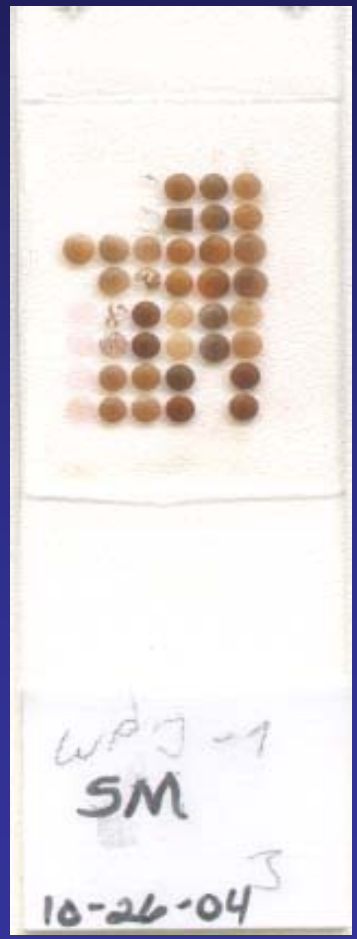
A network of 19 established brain banks across Europe, http://www.brainnet-europe.org/

2004-2005: Microarrays were prepared in one center and distributed for local staining. Results ranged from excellent to poor. This was attributed to local optimization of stains for local tissue procedures (“It works on my tissue”)

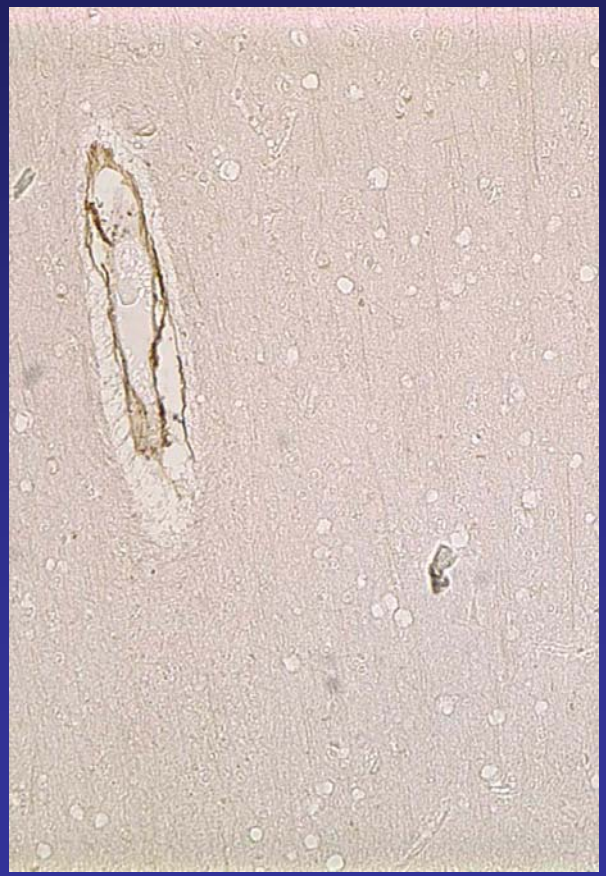
The BrainNet Europe experience

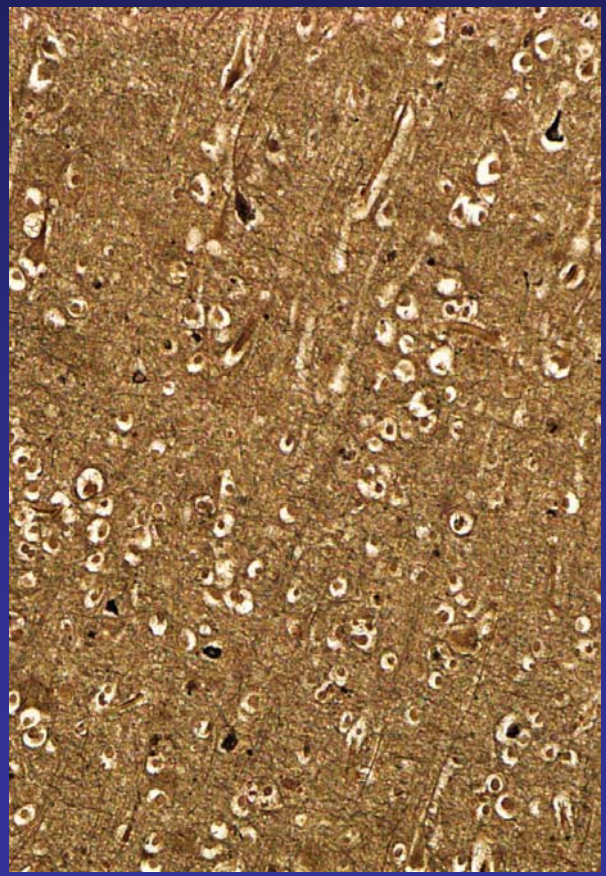
2000-2003: Microarrays were prepared using tissues from all centers to compare staining on tissues with different preparative techniques

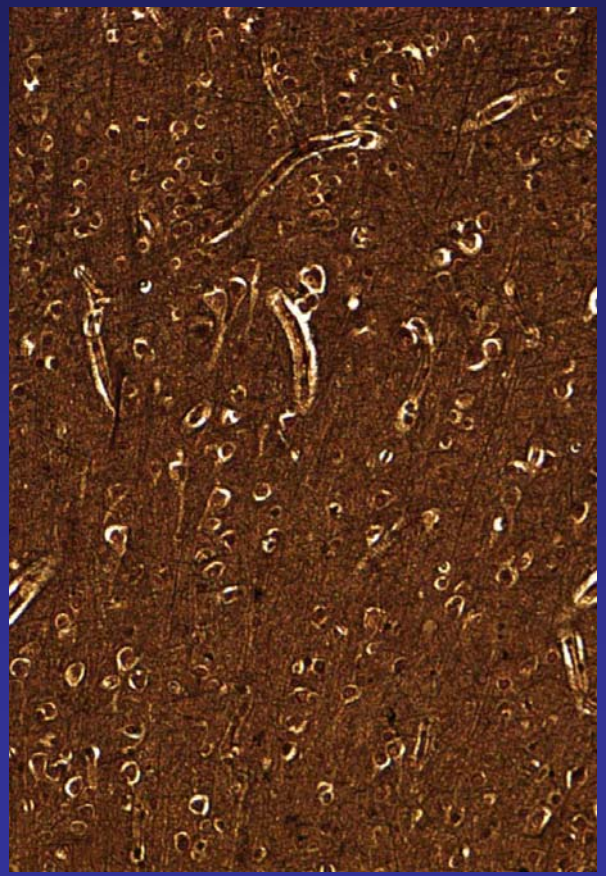
A small number of these microarrays were made available to our US ADC NP Steering Committee



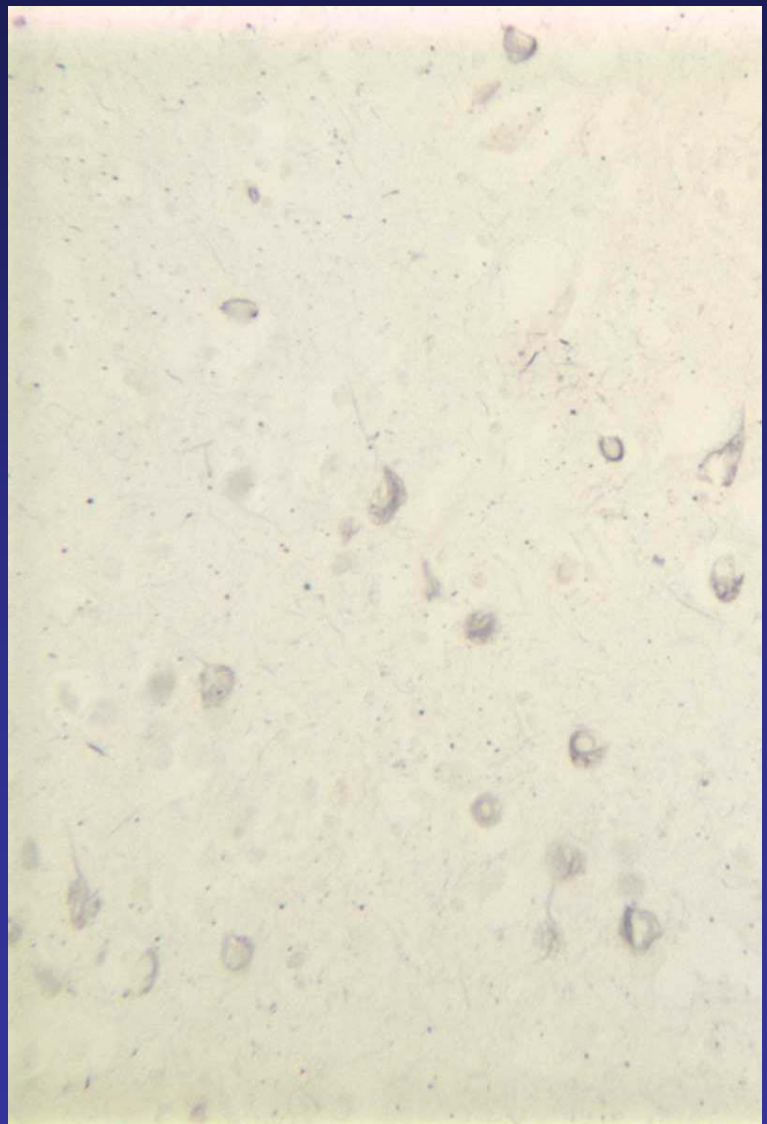
Stain variations: Bielschowsky

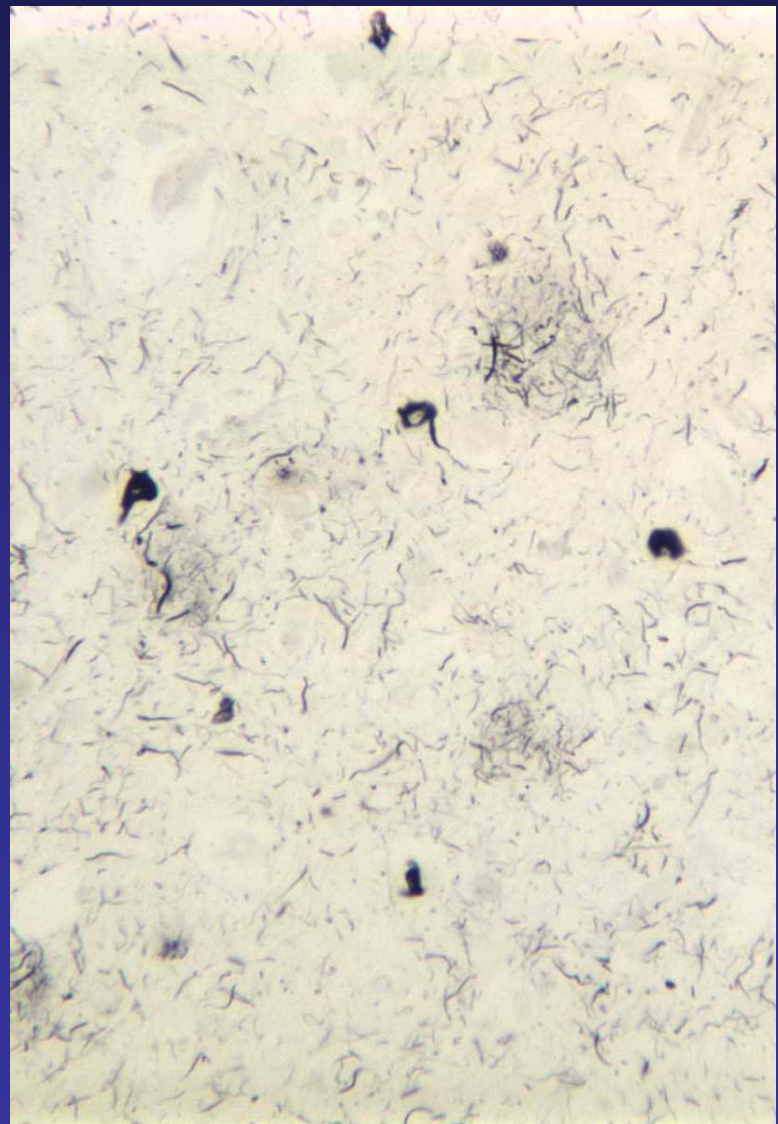




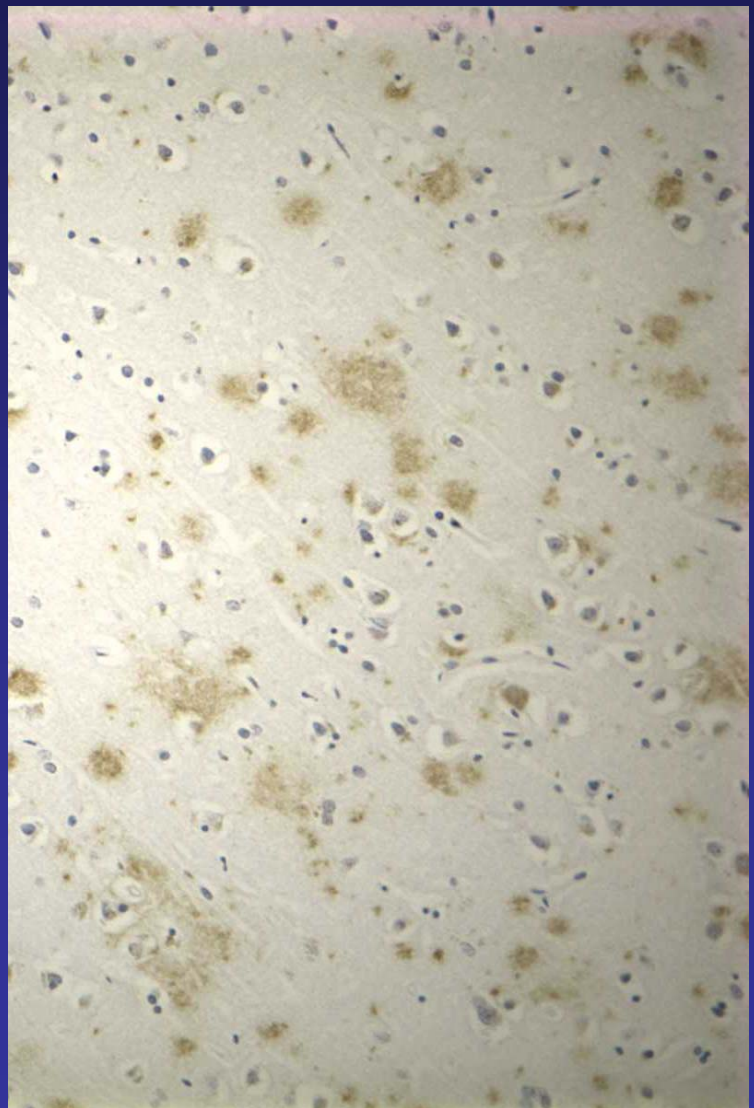


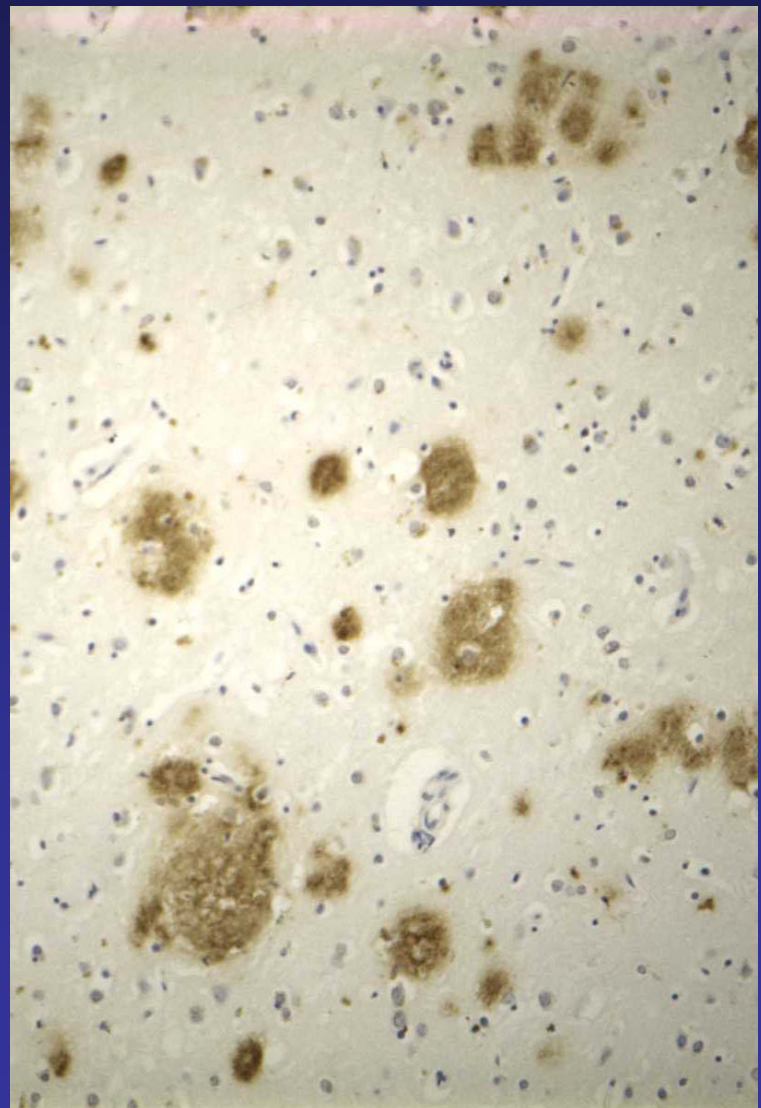
Stain variations: Gallyas



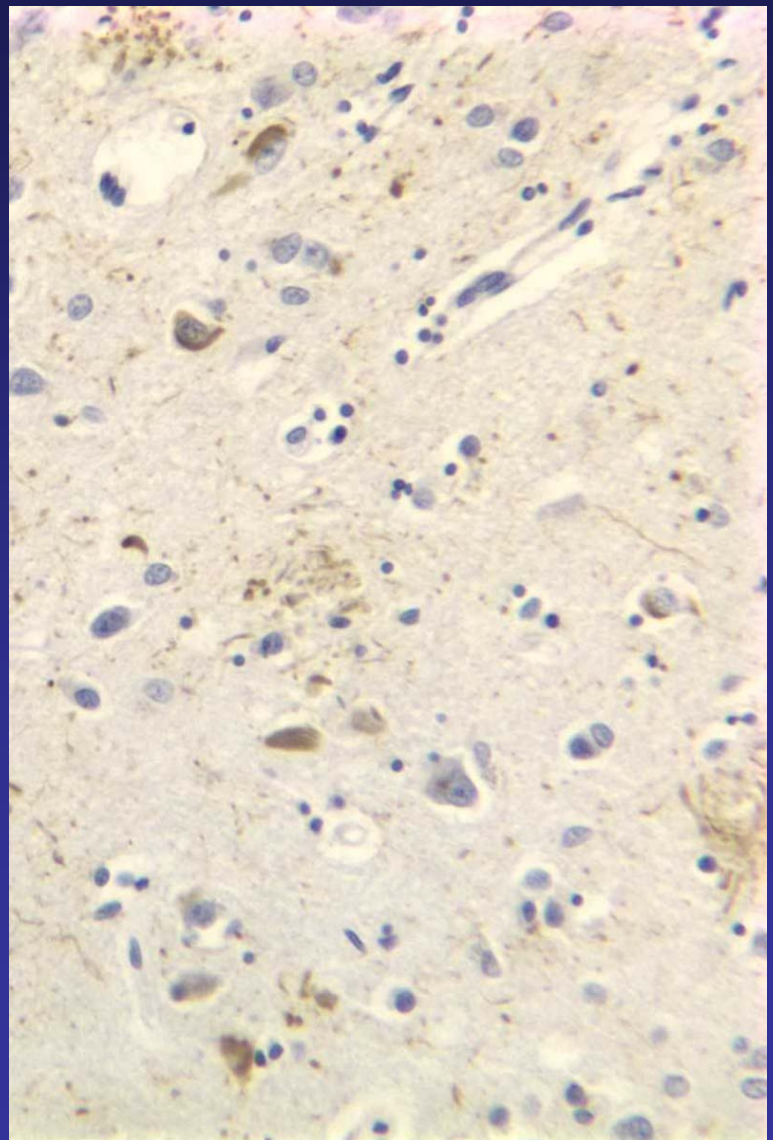


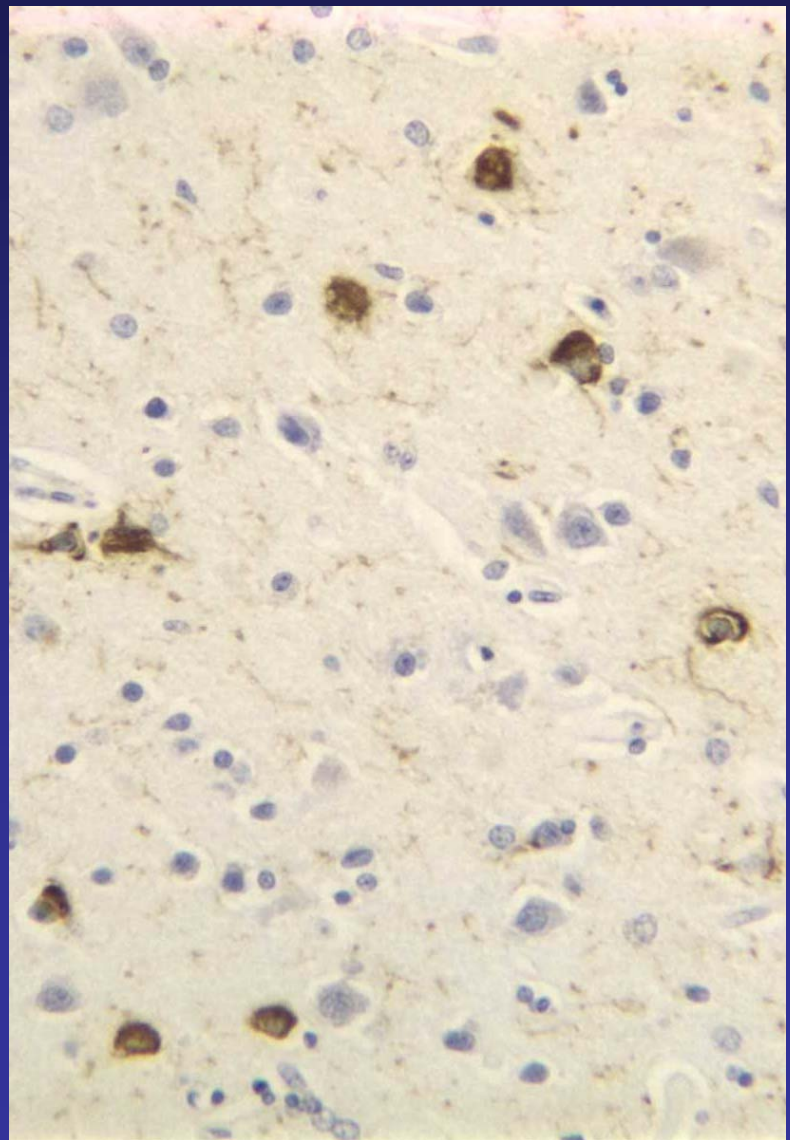
Stain variations: Aβ (4G8)





Stain variations: *tau* (AT8)





Number of microarray cells judged to be of poor stain quality

Stain/IHC	*# of poor quality*	
Bielschowsky	14	(38%)
Gallyas	6	(16%)
Aβ (4G8)	4	(11%)
Tau (AT8)	1	(3%)

Discrepancies in interpretations

Counts of plaques or tangles subjectively graded as:

- no lesions
- sparse lesions
- moderate lesions
- frequent lesions

Major discrepancies were defined as those with a difference of 2 grades or more:

- None v moderate
- Sparse v frequent
- None v frequent

Intra-institutional comparisons

	Cells with major discrepancies
Institution A:	
Plaques (Bielschowsky v AT8)	5 (14%)
Tangles (Bielschowsky v AT8 v Gallyas)	5 (14%)
Institution C:	
Plaques (Bielschowsky v tau2)	4 (11%)
Tangles (Bielschowsky v tau2 v Gallyas)	6 (16%)

Intra-institutional comparisons

15 cells discrepant at EITHER institution

BUT only 2 cells discrepant at BOTH institutions

Patterns noted:
Tangles: *tau* IHC consistently showed more tangles than Gallyas. Bielschowsky agreed with Gallyas at Institution A and agreed with *tau* at Institution C

Plaques: No consistent pattern at either Institution

Inter-institutional comparisons

	Cells with major discrepancies
Bielschowsky (A v C)	
Either plaques or tangles	13 (35%)
Plaques alone	11 (30%)
Tangles alone	4 (11%)

Inter-institutional comparisons

	Cells with major discrepancies
Gallyas (A v C)	
Tangles	1/37 (3%)
Amyloid IHC (A v B v C)	
Plaques	1/37 (3%)

Inter-institutional comparisons

	Cells with major discrepancies
Tau IHC	
Tangles (A v C)	0
Plaques (A v C)	3 (8%)
Threads (A v C)	2 (6%)
Plaques/tangles/threads or tau load (A v B v C)	6 (16%)

Hypothesis generated

1. Tissue preparation techniques are at least as important as stain techniques

2. The Bielschowsky technique is much more susceptible to case-to-case variation in quality than are the other techniques

3. Gallyas is a more reliable technique for tangles

4. Amyloid IHC gives very reproducible results between institutions

5. Tau IHC is reasonably consistent between institutions for neuritic plaques, tangles, and threads, but consistently labels more neurons than does Gallyas

Issues for discussion

- Should we evaluate and try to standardize tissue preparation techniques?
- Should we assess inter-institutional reproducibility of diagnoses, perhaps with a microarray-type study?
- Should we assess inter- and intra-institutional variability of staining, perhaps with the same microarrays?
- If, perchance, we come to the conclusion that IHC should replace silver stains, will this impact on established CERAD and Braak criteria that are, after all, based on silver stains?

Quality Assurance/Standards Committee

Bill Ellis (U C Davis)
E. Tessa Hedley-White (Mass. General)
Bob Mrak (U Arkansas Med Sci)
Charles White (U Texas Southwestern)
Tom Wisniewski (NYU)