Appendix 1 (as supplied by the authors): Supporting concepts and data

I. Basic principles of evidence-based diagnosis

The central principle of evidence-based diagnosis is the progressive revision of diagnostic opinion (defined as the probability of presence or absence of a particular condition or class of conditions), using at each step an estimate of initial diagnostic probability, results of an investigation, and an estimate of the investigation's discriminatory power to calculate a post-investigation probability. Although most clinical or pathological observations and investigations can be interpreted within this framework, it is particularly well suited to laboratory tests.

Pre-test diagnostic probabilities may be based on expert opinion or, more objectively, on an estimate of the target condition's prevalence in the clinical population to which the patient belongs. For inherently binary investigation results (*e.g.*, presence or absence of a clinical sign), or quantitative test results scored as positive or negative above or below a defined threshold, a standard 2 × 2 table summarizes disease status *versus* test result:

	Disease		
Result	Present	Absent	Total
Positive	True Positive (TP)	False Positive (FP)	TP+FP
Negative	False Negative (FN)	True Negative (TN)	FN+TN
Total	TP+FN	FP+TN	TP+FP+TN+FN

A test's *sensitivity* (Se, or the true-positive rate) is defined as TP/(TP+FP), and *specificity* (Sp, or the true-negative rate) as TN/(TN+FN). Both of these measures express test accuracy in terms of probability of a particular test result, given disease status. In clinical practice however, exactly the reverse is sought; *i.e.*, probability of disease status given a test result.

A more immediately applicable measure of test accuracy is the *likelihood ratio* (LR), defined as the ratio of probabilities for presence *versus* absence of disease, given a particular test result. It is customary to define a *positive LR* (LR+) as Se / (1–Sp), in other words, the ratio of the true-positive rate to the false-positive rate; and a *negative LR* (LR–) as (1–Se) / Sp, which is the ratio of the false-negative rate to the true-negative rate. LR+ values of 1–2, 2–5, 5–10 and > 10, or LR– values of 0.5–1, 0.2–0.5, 0.1–0.2 and < 0.1, represent clinically non-useful, low, moderate and high discriminatory power respectively. These scales facilitate comparisons of utility among tests, including those based on different underlying principles.

The key relationship linking these concepts to practical clinical decision-making is *Bayes' Theorem*, most simply stated as: Post-test Odds = Pre-test Odds × LR. To use the more intuitive language of probabilities (usually expressed as percentages or decimal fractions), odds and probabilities are readily interconvertible, using the formulas Odds = Probability / 1–Probability; and Probability = Odds / Odds +1. Bayes' Theorem can be applied sequentially as more information about a patient's condition becomes available.

Appendix to: Coulthart MB, Jansen GH, Cashman NR. Interpretation of cerebrospinal fluid protein tests in the diagnosis of sporadic Creutzfeldt–Jakob disease: an evidence-based approach. *CMAJ* 2013. DOI: 10.1503/cmaj.130720.

II. Surveillance case definitions for sporadic CJD

Definite:

Progressive neurological disorder + neuropathological confirmation of one or more of

- spongiform degeneration in cortical and/or subcortical grey matter
- positive immunocytochemistry for deposits of abnormal PrP
- positive immunoassay (Western blot) for protease-resistant PrP

Probable:

Progressive dementia of < 2 years' duration + two or more of I + one or more of II

Possible:

Progressive dementia of < 2 years' duration + two or more of I

I.
A. Myoclonus
B. Visual disturbance or cerebellar dysfunction (ataxia)
C. Pyramidal or extrapyramidal features
D. Akinetic mutism

II.
A. Periodic (ca. 1–2 Hz) sharp-wave complexes on EEG
B. Positive CSF 14-3-3 assay
C. High signal abnormalities in caudate nucleus and putamen in either DWI or FLAIR magnetic resonance imaging

III. Primary data, test performance characteristics and methodological features of reviewed studies[1-13]

The goal of the review was to assess published evidence on the diagnostic accuracies of 14-3-3, tau and S100B proteins in relation to sporadic CJD. The search was restricted to peer-reviewed journal articles published in English as of December 31, 2012. Initially, 339 literature citations were retrieved from the National Library of Medicine's online MEDLINE® database (PubMed), using the search profile: ("14-3-3"[All fields] OR "tau"[All fields] OR "S100B"[All fields]) AND ("CJD"[All fields] OR "Creutzfeldt-Jakob"[All Fields]) AND ("CSF"[All fields] OR "cerebrospinal"[All Fields]). Additional selection criteria were as follows:

- One or more of 14-3-3, tau and S100B were studied;
- The study was conducted on a prospectively recruited patient cohort, with intake based on a pre-test clinical suspicion of sporadic CJD;
- It was possible to retrieve or reconstruct 2×2 tables of disease status (present/absent) $\times$ test result (positive/negative) using a single intermediate test-scoring threshold – however defined, and with or without additional scoring methods;
- Overlap between the patient cohort on which the study was based with those of other published studies could be confidently excluded; in some cases this judgement required exclusion of earlier studies among several published by the same research group;
- Two smaller studies meeting the above criteria but including only 10 and 13 sporadic CJD patients respectively, were also excluded.

On this basis 13 articles were selected for review, comprising studies by expert centres in 15 different countries and data from a total of 15,814 CSF protein tests (10,131 for 14-3-3; 3,525 for tau; 2,158 for S100B). The data presented in these articles, estimates of basic test performance characteristics, and study characteristics are summarized below.

Table A1: Primary data and test performance characteristics[a,b,c]

Study[d]	Marker	sCJD	nCJD	N	DCJD	TP	FN	FP	TN	Se [95%CI]	Sp [95% CI]	LR+ [95% CI]	LR– [95% CI]
1	14-3-3	1457	1089	2546	NA	1240	217	169	920	0.85 [0.83–0.87]	0.84 [0.82–0.87]	5.5 [4.8–6.3]	0.18 [0.16–0.20]
	tau	819	220	1039	NA	704	115	26	194	0.86 [0.83–0.88]	0.88 [0.83–0.92]	7.3 [5.1–10.4]	0.16 [0.13–0.19]
	S100B	589	162	751	NA	483	106	39	123	0.82 [0.79–0.85]	0.76 [0.68–0.82]	3.4 [2.6–04.5]	0.24 [0.20–0.28]
2	14-3-3	30	41	71	30	26	4	9	32	0.97 [0.81–1.00]	0.78 [0.62–0.89]	4.4 [2.5–7.9]	0.04 [0.01–0.30]
	tau	30	41	71	30	27	3	2	39	0.90 [0.72–0.97]	0.95 [0.82–0.99]	18.5 [4.7–71.7]	0.11 [0.04–0.31]
	S100B	30	41	71	30	28	2	3	38	0.93 [0.76–0.99]	0.93 [0.79–0.98]	12.8 [4.3–38.1]	0.07 [0.02–0.28]
3	14-3-3	245	171	416	245	210	35	44	127	0.86 [0.81–0.90]	0.74 [0.67–0.80]	3.3 [2.6–4.3]	0.19 [0.14–0.26]
	tau	216	135	351	216	175	41	20	115	0.81 [0.75–0.86]	0.85 [0.78–0.91]	5.5 [3.6–8.2]	0.22 [0.17–0.29]
	S100B	243	169	412	243	158	85	17	152	0.65 [0.59–0.71]	0.90 [0.84–0.94]	6.5 [4.1–10.2]	0.39 [0.33–0.46]
4	14-3-3	127	873	1000	127	112	15	244	629	0.88 [0.81–0.93]	0.72 [0.69–0.75]	3.1 [2.8–3.6]	0.16 [0.10–0.26]
	tau	120	826	946	120	109	11	99	727	0.91 [0.84–0.95]	0.88 [0.85–0.90]	7.4 [6.9–7.8]	0.10 [0.06–0.20]
	S100B	122	802	924	122	106	16	104	698	0.87 [0.80–0.92]	0.87 [0.84–0.89]	6.5 [4.1–10.2]	0.15 [0.09–0.20]
5	14-3-3	52	198	250	47	49	3	7	191	0.94 [0.83–0.98]	0.96 [0.93–0.98]	26.7 [12.8–55.3]	0.06 [0.02–0.18]
	tau	52	198	250	47	45	7	5	193	0.87 [0.74–0.94]	0.97 [0.94–0.99]	34.7 [14.3–82.0]	0.14 [0.07–0.28]
6	14-3-3	40	135	175	0	31	9	24	111	0.78 [0.69–0.81]	0.82 [0.74–0.88]	4.4 [2.9–6.5]	0.27 [0.17–0.49]
	tau	40	135	175	0	36	4	8	127	0.90 [0.75–0.97]	0.94 [0.88–0.97]	15.2 [7.7–30.0]	0.11 [0.04–0.27]
7	14-3-3	53	417	470	0	41	12	70	347	0.77 [0.63–0.87]	0.83 [0.79–0.87]	4.6 [3.6–6.0]	0.27 [0.17–0.45]
	tau	30	243	273	0	25	5	17	226	0.83 [0.65–0.94]	0.93 [0.89–0.96]	11.9 [7.3–19.4]	0.18 [0.08–0.40]

Table A1 (continued)

Study	Marker	sCJD	nCJD	N	DCJD	TP	FN	FP	TN	Se [95%CI]	Sp [95% CI]	LR+ [95% CI]	LR– [95% CI]
8	14-3-3	245	175	420	245	221	24	105	70	0.58 [0.53–0.63]	0.40 [0.33–0.48]	1.5 [1.3–1.7]	0.24 [0.16–0.36]
	tau	245	175	420	245	213	32	57	118	0.87 [0.82–0.91]	0.67 [0.60–0.74]	2.7 [2.1–3.3]	0.19 [0.14–0.27]
9	14-3-3	33	77	110	25	32	1	10	67	0.97 [0.82–1.00]	0.87 [0.77–0.93]	7.5 [4.2–13.4]	0.03 [0.01–0.24]
10	14-3-3	30	68	98	21	28	2	5	63	0.93 [0.76–0.99]	0.93 [0.83–0.97]	12.7 [5.4–29.7]	0.07 [0.02–0.28]
11	14-3-3	63	84	147	41	59	4	2	82	0.94 [0.84–0.98]	0.98 [0.91–1.00]	39.3 [10.0–154.9]	0.06 [0.03–0.17]
12	14-3-3	365	3391	3756	365	315	50	254	3137	0.86 [0.82–0.90]	0.93 [0.92–0.93]	11.5 [10.2–13.1]	0.15 [0.11–0.19]
13	14-3-3	177	495	672	75	155	22	15	480	0.88 [0.82–0.92]	0.97 [0.95–0.98]	28.9 [17.5–47.7]	0.13 [0.09–0.19]

[a] **Abbreviations:**
sCJD : Number of sporadic CJD cases
nCJD : Number of non-CJD cases
N : Study size (sCJD + nCJD)
DCJD : Number of definite (neuropathologically confirmed) sporadic CJD cases
NA : Data not available
TP : Number of true positive test results
FN : Number of false negative test results
FP : Number of false positive test results
TN : Number of true negative test results
Se : Sensitivity [TP / (TP+FN)]
Sp : Specificity [TN / (FP+TN)]
LR+ : Positive likelihood ratio [Se / (1–Sp)]
LR– : Negative likelihood ratio [(1–Se) / Sp]
95% CI : 95% Confidence interval (2-sided)

[b] Data were analyzed using MedCalc® for Windows version 12.4.0.0 (MedCalc Software, Mariakerke, Belgium), and statistical calculators available on the Vassar Stats website.[14]
[c] Two smaller studies including 13 and 10 CJD patients respectively were excluded from further consideration.[15,16]
[d] Studies are denoted by their citation number in the reference list.

Table A2: Methodological features

Study	Marker	Cutoff	Assay materials	Method for selection of cutoff threshold
1	14-3-3	Not quantified; standards not specified	Primary antibody: primarily SC-629[a]	Empirical; weak positive results scored as negative
	tau	1300 pg/mL	hTau ELISA[b]	Maximization of Youden Index (= Sensitivity + Specificity – 1) for previously published data[17]
	S100B	4.2 ng/mL 0.5 ng/mL	Sangtec ELISA[c] In-house ELISA (UK data)	Not specified
2	14-3-3	Not quantified; CSF standards	Primary antibody: SC-1657[d]	Not specified
	tau	1203 pg/mL	hTau ELISA	Receiver Operating Characteristic (ROC) analysis of study data
	S100B	2.59 ng/mL	Sangtec ELISA	Receiver Operating Characteristic (ROC) analysis of study data
3	14-3-3	Not quantified; CSF standards	Primary antibody: not stated	Empirical; weak-positive results scored as negative
	tau	1260 pg/mL	hTau ELISA	Receiver Operating Characteristic (ROC) analysis of previously published data[15]
	S100B	0.5 ng/mL	In-house ELISA	Not stated
4	14-3-3	~ 1.5 ng/lane recombinant 14-3-3γ protein	Primary antibody: SC-1657	Empirical; results scored as positive or negative with respect to recombinant protein standard
	tau	976 pg/mL	hTau ELISA	Receiver Operating Characteristic (ROC) analysis of study data
	S100B	2.5 ng/mL	Sangtec ELISA	Receiver Operating Characteristic (ROC) analysis of study data
5	14-3-3	Not quantified; brain homogenate standard	Primary antibody: SC-718[e]	Empirical; different scoring thresholds used; weak-positive results scored as negative for present review
	tau	1300 pg/mL	hTau ELISA	Maximization of Youden Index (= Sensitivity + Specificity – 1) for previously published data[17]
6	14-3-3	Not quantified; brain homogenate standard	Primary antibody: SC-629	Not specified
	tau	1400 pg/mL	hTau ELISA	Receiver Operating Characteristic (ROC) analysis of study data

Table A2 (continued)

7	14-3-3	Not quantified; standards not specified	Primary antibody: SC-629	Empirical; different scoring thresholds used; weak positive results scored as negative
	tau	1000 pg/mL	hTau ELISA	Receiver Operating Characteristic (ROC) analysis of previously published data[15]
8	14-3-3	~100 ng/mL, ~ 200 ng/mL, ~ 300 ng/mL CSF standards	Primary antibody: SC-629	Empirical; different scoring thresholds used; weak positive results scored as negative for present review ("Single decision point" model in original publication)
	tau	1150 pg/mL	TAU ELISA[f]	Graphical method: tau level at which histograms of CJD and non-CJD results intersect
9	14-3-3	Not quantified; brain homogenate and CSF standards	Primary antibody: SC-629	Not specified
10	14-3-3	Not quantified; CSF standards	Primary antibody: SC-629	Not specified
11	14-3-3	Not quantified; CSF standards	Primary antibody: SC-629	Not specified
12	14-3-3	Not quantified; CSF standards	Primary antibody: SC-629	Not specified
13	14-3-3	Not quantified; CSF standards	Primary antibody: SC-629	Empirical; weak-positive results scored as negative

Notes

[a] Anti-14-3-3β rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA

[b] Innotest® hTau AG ELISA kit, Innogenetics, Ghent, Belgium

[c] Sangtec® 100 ELISA, Diasorin, Saluggia, Italy

[d] Anti-14-3-3β mouse monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA

[e] Anti-14-3-3γ rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA

[f] Novex® TAU (Total) Human ELISA, Life Technologies, Carlsbad, CA, USA

IV. Meta-analyses of sensitivity and specificity

Meta-analyses of sensitivity and specificity were performed with the software OpenMeta[Analyst],[18] using a random-effects method[19] to derive pooled point and interval estimates of sensitivity and specificity for 14-3-3 and tau proteins (13 and 8 included studies, respectively). Estimators of between-study heterogeneity (τ^2, Q and I^2) were also calculated. Based on these results, significant heterogeneity [p (Q) < 0.01] was observed between studies for estimates of specificity for both markers, but not for sensitivity. In addition, the proportion of between-study heterogeneity in specificity not attributable to sampling variation (I^2) was > 90% for both markers, suggesting underlying differences in composition of study populations, technical factors, or both.

Results of meta-analyses are shown below in Table A3, and in Figures A1 and A2. Note also that another recent meta-analysis of 9 studies of diagnostic accuracy for 14-3-3 yielded meta-estimates of 0.92 [0.90–0.94] for sensitivity and 0.80 [0.77–0.83] for specificity.[20]

Table A3: Meta-estimates of sensitivity and specificity for 14-3-3 and tau[a]

Marker	Metric	Estimate [95% CI]	τ^2	Q [df]	p (Q)	I^2
14-3-3	Sensitivity	0.87 [0.85–0.89]	0.04	20.45 [12]	0.06	0.41
	Specificity	0.87 [0.79–0.92]	0.87	531.14 [12]	< 0.01	0.98
tau	Sensitivity	0.86 [0.84–0.88]	0.01	7.81 [7]	0.34	0.11
	Specificity	0.90 [0.84–0.94]	0.54	84.10 [7]	< 0.01	0.92

[a] **Abbreviations**

τ^2 : Variance of study estimates
Q : Total weighted sum of squares of study estimates
df : Degrees of freedom (= number of studies – 1)
p (Q) : Significance level for rejection of H_0: Q = df – 1
(study samples all drawn from the same underlying population, and tested with identically performing methods)
I^2 : Proportion of between-study heterogeneity not attributable to sampling variation

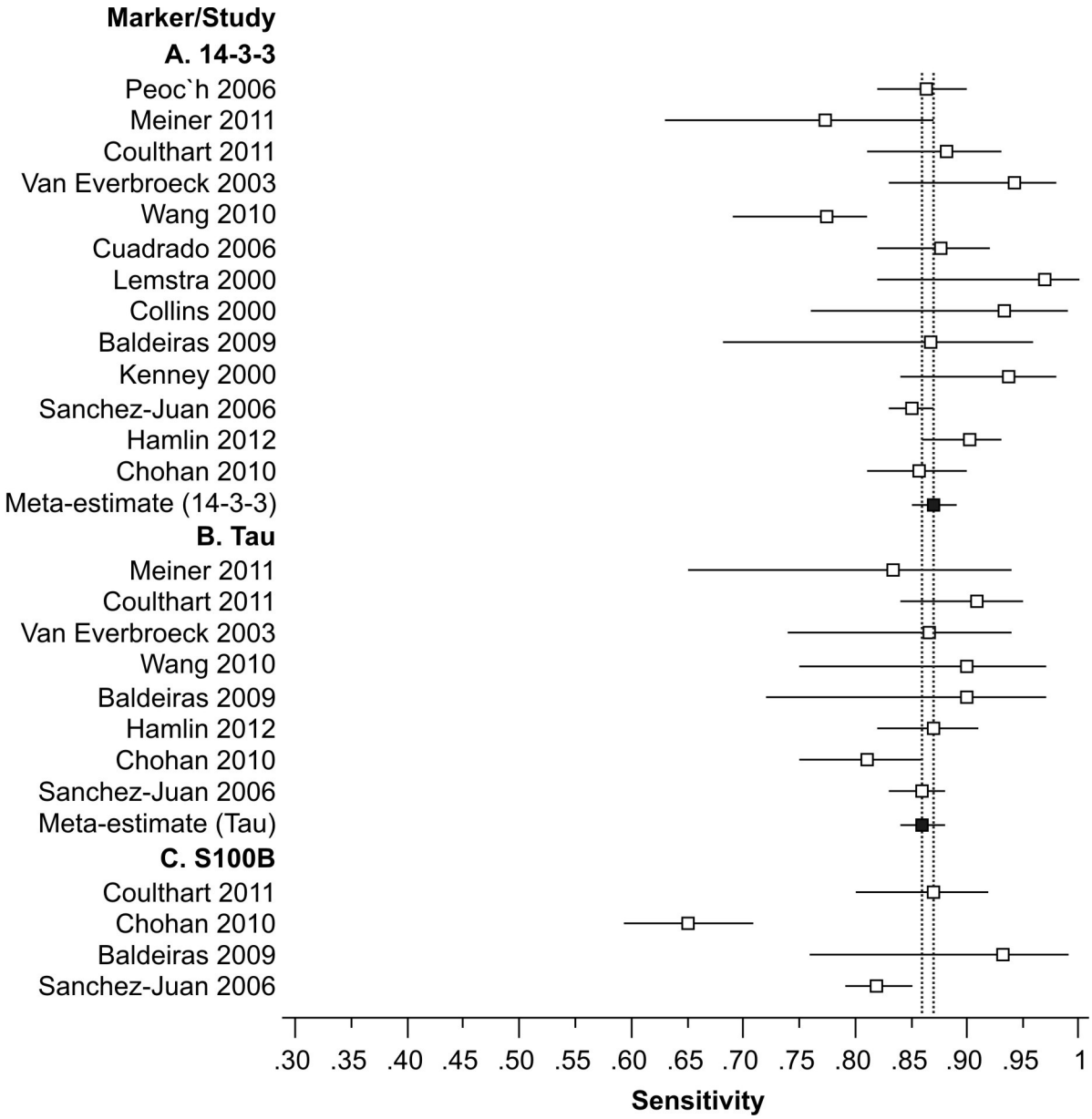

Figure A1: Diagnostic sensitivities of 14-3-3, tau and S100B proteins reported by 13 individual studies (open squares), and meta-estimates of sensitivity based on these data (closed squares and vertical dashed lines). Studies are listed by first author and year of publication at left, with results grouped by marker as labeled at right. 95% confidence intervals are indicated by horizontal bars.

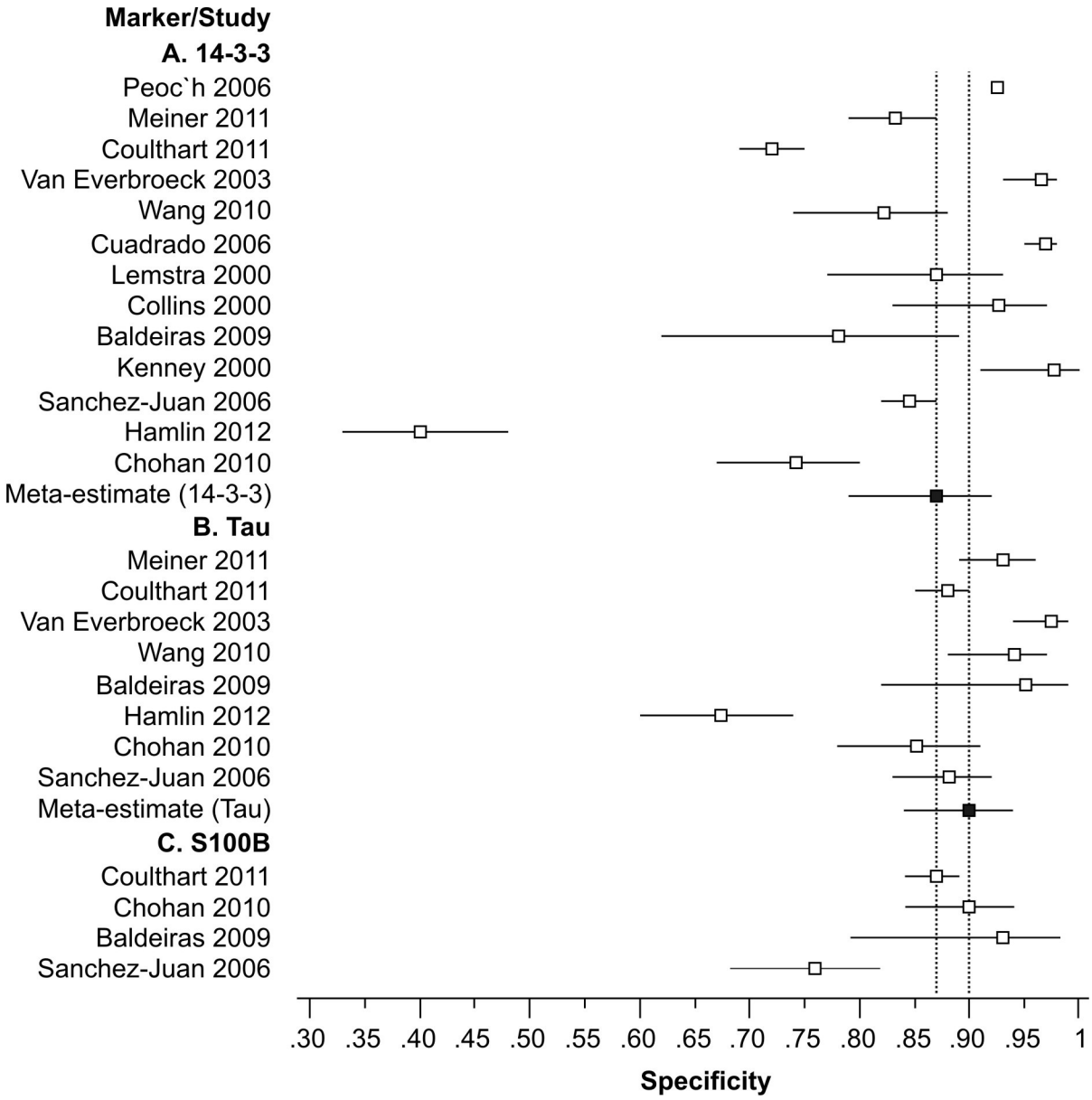

Figure A2: Diagnostic specificity of 14-3-3, tau and S100B proteins reported by 13 individual studies (open squares), and meta-estimates of specificity based on these data (closed squares and vertical dashed lines). Studies are listed by first author and year of publication at left, with results grouped by marker as labeled at right. 95% confidence intervals are indicated by horizontal bars.

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