Supplementary information

Determination of cerebrospinal fluid leakage by selective deletion of transferrin glycoform using an immunochromatographic assay

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Keywords: cerebrospinal fluid leakage, transferrin, sialic acid, immunochromatographic assay, point of care testing

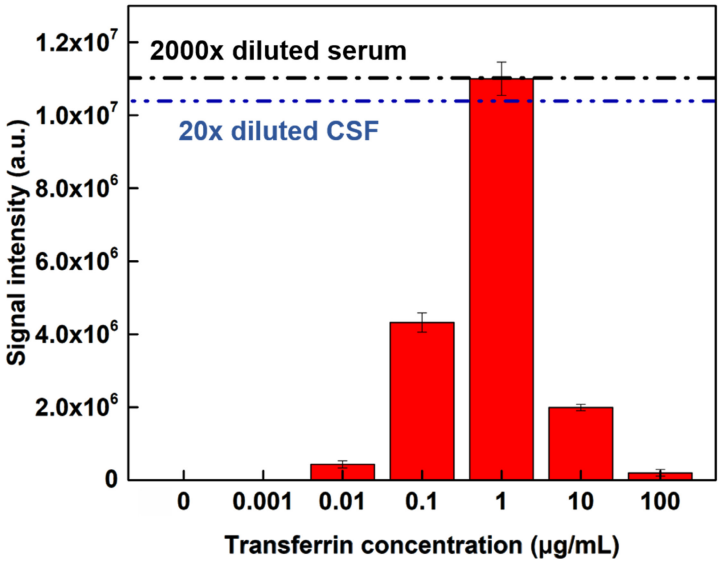

Figure S1. Determination of concentration of total transferrin in pooled serum and cerebrospinal (CSF) compared with buffer solution spiked with transferrin. The signal intensity (a.u.) of test line in the nitrocellulose (NC) membrane was quantified by image analysis (ChemiDoc TM XRS+ imaging system).

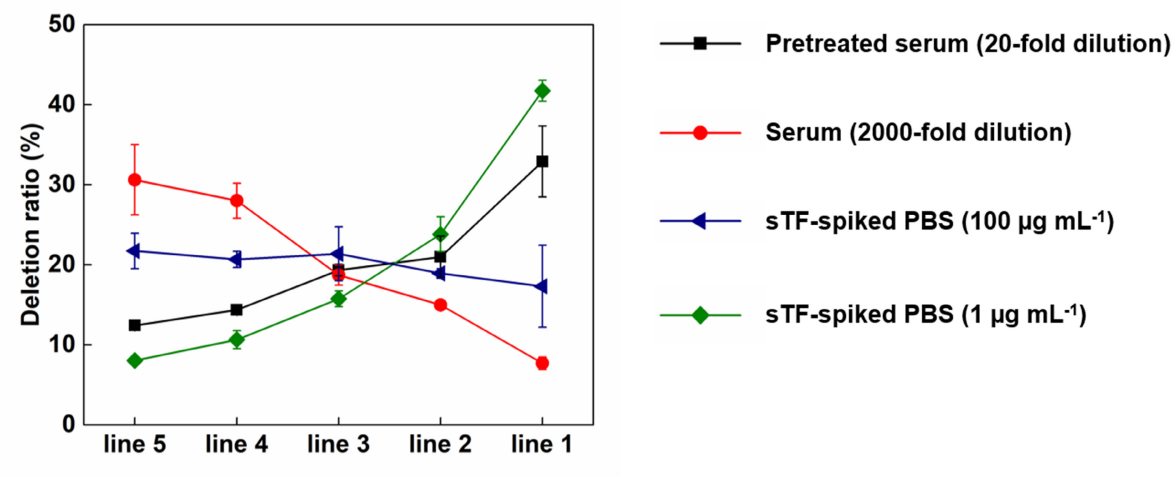

Figure S2. Comparison of pattern of deletion lines in several cases (from front to rear; lines 1 to 5)

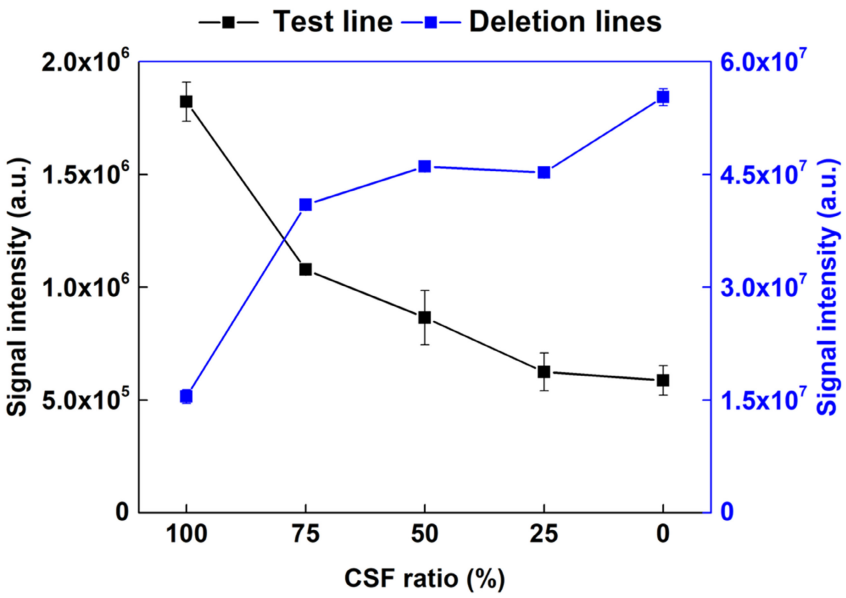

Figure S3. Evaluation of signal intensity of test line (black line; left axis) and sum of signal intensity of deletion lines (blue line; right axis) depending on percentage of cerebrospinal fluid (CSF) solution.

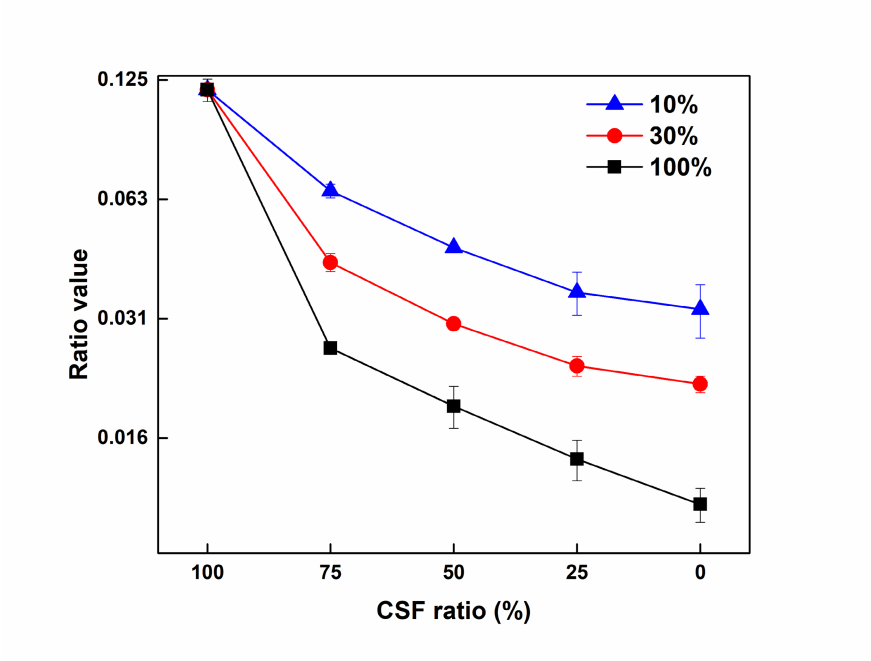

Figure S4. Comparison of the ratio value (the signals of a test line divided by the signals of the sum of deletion lines) based on dilution ratio of serum

Table S1. Comparison of evaluation with clinical sample and artificial mixture sample (AMS[a]) by conventional method (immunofixation) and results of immunochromatographic assay (ICA).

Sample No	Leaking place	Immuno -fixation	Results of ICA	
			Test line	Ratio value
1	Lumbar drain	Positive	Negative	Positive
2	Ventriculostomy	Positive	Positive	Positive
3	Post op lumbar drain	Negative	Negative	Negative
4	Lumbar drain	Positive	Positive	Positive
5	Post op lumbar drain	Negative	Negative	Negative
6	Brain	Positive	Positive	Positive
7	Post op lumbar drain	Negative	Negative	Negative
8	Post op drain	Negative	Negative	Negative
9	Post op drain	Negative	Negative	Negative
10	Post op drain	Negative	Negative	Negative
11	Post op drain	Negative	Negative	Negative
12	Post op drain	Negative	Negative	Negative
13	Post op drain	Negative	Negative	Negative
14	Post op drain	Negative	Negative	Negative
15	Ventriculostomy	Positive	Positive	Positive
16	Post op drain	Negative	Negative	Negative
17	Post op drain	Negative	Negative	Negative
18	Post op drain	Negative	Negative	Negative
19	Post op drain	Negative	Negative	Negative
20	Post op drain	Negative	Negative	Negative
21	Post op drain	Negative	Negative	Negative
22	Post op drain	Negative	Negative	Negative
23	Post op drain	Negative	Positive	Negative
24	Post op drain	Negative	Negative	Negative
25	Lumbar wound	Negative	Negative	Negative
26	Lumbar wound	Negative	Negative	Negative
27	Lumbar wound	Negative	Negative	Negative
28	Lumbar wound	Negative	Negative	Negative
29	Lumbar wound	Negative	Negative	Negative
30	Cervical wound	Negative	Negative	Negative
31	Brain ventric	Positive	Negative	Positive
32	Lumbar wound	Negative	Negative	Negative
33	Lumbar drain	Positive	Positive	Positive
34	Lumbar wound	Negative	Negative	Negative
35	Lumbar wound	Negative	Negative	Negative

36	Lumbar wound	Negative	Negative	Negative
37	Brain ventric	Positive	Positive	Positive
38	Brain ventric	Positive	Positive	Positive
39	Brain ventric	Positive	Positive	Positive
40	Brain ventric	Positive	Positive	Positive
41	Lumbar CSF drain	Positive	Positive	Positive
42	Lumbar wound	Negative	Negative	Negative
43	Lumbar wound	Negative	Negative	Negative
44	Lumbar wound	Negative	Positive	Negative
45	Brain ventric	Positive	Positive	Positive
46	Lumbar wound	Negative	Positive	Negative
47	Lumbar wound	Negative	Positive	Positive
AMS 1	Sample 1 (positive) + Sample 3		Positive	Positive
AMS 2	Sample 2 (positive) + Sample 5		Positive	Positive
AMS 3	Sample 4 (positive) + Sample7		Positive	Positive
AMS 4	Sample 6 (positive) + Sample 8		Positive	Positive
AMS 5	Sample 15 (positive) + Sample 9		Positive	Positive
AMS 6	Sample 31 (positive) + Sample 18		Positive	Positive
AMS 7	Sample 33 (positive) + Sample 20		Positive	Positive
AMS 8	Sample 37 (positive) + Sample 26		Positive	Positive
AMS 9	Sample 38 (positive) + Sample 28		Negative	Negative
AMS 10	Sample 39 (positive) + Sample 32		Positive	Positive
AMS 11	Sample 40 (positive) + Sample 35		Positive	Positive
AMS 12	Sample 41 (positive) + Sample 43		Positive	Positive
AMS 13	Sample 45 (positive) + Sample 47		Positive	Positive

[a]AMS indicate the sample artificially mixed with same volume of positive and negative clinical sample.

Table S2. Comparison of specification of the immunochromatographic assay (ICA) based on parameter for determination of cerebrospinal fluid (CSF) leakage

Determining parameter of CSF leakage	Area under the curve	Sensitivity	Specificity	Youden's index (J)
Ratio value	0.9729	96.2 %	97.1 %	0.9321
Signal intensity of test line	0.9333	88.2 %	88.5 %	0.7669

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1-2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	3-4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	7-8
Participants	**6**	Eligibility criteria	None
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	None
	8	Where and when potentially eligible participants were identified (setting, location and dates)	None
	9	Whether participants formed a consecutive, random or convenience series	None
Test methods	**10a**	Index test, in sufficient detail to allow replication	4-8
	10b	Reference standard, in sufficient detail to allow replication	7-8
	11	Rationale for choosing the reference standard (if alternatives exist)	7-8
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	11-12
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	None
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	None
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	None
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	11-13

	15	How indeterminate index test or reference standard results were handled	11-13
	16	How missing data on the index test and reference standard were handled	None
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	11-13
	18	Intended sample size and how it was determined	None
RESULTS			
Participants	**19**	Flow of participants, using a diagram	None
	20	Baseline demographic and clinical characteristics of participants	None
	21 a	Distribution of severity of disease in those with the target condition	None
	21 b	Distribution of alternative diagnoses in those without the target condition	None
	22	Time interval and any clinical interventions between index test and reference stan dard	None
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	11-12 Table S1
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence inte rvals)	12-13
	25	Any adverse events from performing the index test or the reference standard	11-12 Table S1
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and ge neralisability	11-13
	27	Implications for practice, including the intended use and clinical role of the index test	13
OTHER INFORM ATION			
	28	Registration number and name of registry	None
	29	Where the full study protocol can be accessed	4-8
	30	Sources of funding and other support; role of funders	13