

# Diagnostic accuracy of urinary reagent strip to determine cerebrospinal fluid chemistry and cellularity

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## ABSTRACT

**Background:** The gold standard for diagnosis of meningitis depends on cerebrospinal fluid (CSF) examination by microscopy, biochemistry, and culture, which require an experienced microscopist and laboratory support. We conducted this study to determine if urinary reagent strip is useful to make a semi-quantitative assessment of protein, glucose, and presence of leukocyte esterase in CSF. **Materials and Methods:** All consecutive CSF samples were evaluated in a blinded fashion. CSF was tested using Combur-10 urinary reagent strip as an index test, and CSF microscopy and biochemistry as reference standards. Combur-10 (Boehringer Mannheim) is a urinary reagent strip used to estimate ten parameters including protein, glucose, and leukocytes. We estimated diagnostic accuracy of each index test using corresponding cut-off levels (glucose 1+ vs. CSF glucose >50 mg/dL; protein 1+ and 2+ vs. CSF protein >30 mg/dL and >100 mg/dL; leukocyte esterase positivity vs. >10 granulocytes in CSF sample). We constructed receiver operating curves (ROC) to evaluate overall performance of index tests and estimated area under the curve (AUC). **Results:** CSF samples of 75 patients were included in the study. All the three indicator tests (CSF cells, protein, and glucose) were normal in 17 (22.6%) samples. Of the three tests, diagnostic accuracy of protein estimation (1+ or more on reagent strip) was best for detection of CSF proteins greater than 30 mg/dL [sensitivity 98.1% (95% CI 90.1-100%); specificity 57.1% (95% CI 34-78.2%)], with AUC of 0.97. Sensitivity and specificity for 2+ on reagent strip and CSF protein > 100 mg/dL were 92.6% (95% CI 75.1-99.1) and 87.5% (95% CI 74.8-95.3), respectively, with AUC of 0.96 (95% CI 0.92-1.01). Leukocyte esterase positivity by test strip had a sensitivity of 85.2 (95% CI 66.3-95.8%) and specificity of 89.6 (95% CI 77.3-96.5%) for detection of CSF granulocytes of more than 10/mm<sup>3</sup>. **Conclusion:** Existing urinary reagent strips can be used to diagnose meningitis in low-resource settings.

**Key words:** Cerebrospinal fluid, meningitis, urinary reagent strip

## Introduction

Cerebrospinal fluid (CSF) microscopy, CSF chemistry, and microbiological studies are required to make the diagnosis of meningitis.<sup>[1]</sup> An experienced microscopist is required to estimate CSF cell count, while reasonable laboratory support is required to estimate sugar and protein levels. These facilities are often not available in

resource-limited settings, and even in settings where these are available, turnaround times are long. Currently, no rapid point-of-care tests are available to detect meningitis.

The presence of glucose, protein, leukocytes, erythrocytes, and pH of a body fluid can be estimated using urinary reagent strips. CSF cellularity and chemistry have been determined in the past with help of reagent strips, but the results have been variable and the method has not gained popularity.<sup>[2-11]</sup> If proven useful, these strips can be an excellent test to help clinicians make a bedside rapid diagnosis of meningitis and initiate treatment. This would greatly benefit health professionals working in resource-limited settings.

We designed this study to determine if the use of urinary reagent strip to make a semi-quantitative assessment of

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protein, glucose, and presence of leukocyte esterase (as compared to CSF glucose, protein values, and cell count) is accurate to distinguish between a normal and an abnormal CSF sample.

## Materials and Methods

We conducted this study at Mahatma Gandhi Institute of Medical Sciences, a tertiary care rural medical school in Central India. The cytology laboratory of the hospital receives about 20-30 CSF samples every month. We included all consecutive CSF samples received in the cytology laboratory between 1<sup>st</sup> July and 30<sup>th</sup> September 2011. We excluded samples from the study if the quantity was insufficient for performing the index test. An independent researcher (DJ) performed microscopy (total cell count and differential count) immediately after receiving the sample. Other reference standard tests like CSF protein and glucose were performed in the biochemistry laboratory.

Combur-10 (Roche Diagnostics, Basel, Switzerland) is a 10-patch strip used to test urine for specific gravity, protein, glucose, leukocytes, nitrites, pH, hemoglobin, ketones, bilirubin, and urobilinogen. We used this strip as an index test to detect CSF cellularity (leukocyte esterase estimation), glucose (glucose oxidase-peroxidase method), and protein levels. A medical student (KK) performed all index tests, blinded to the results of the reference standard. The medical student used one to two drops of CSF, drawn in a pipette, and poured these on glucose, protein, and leukocyte patches to note the color changes.

### Index tests (using urinary reagent strip)

#### *Cerebrospinal fluid leukocytes*

A normal CSF WBC count is <5 cells/mm<sup>3</sup>, and the reagent strip needs a minimum of 10 cells for detection and has an upper detection limit of 500 cells/mm<sup>3</sup>. However, the reaction detects the presence of esterase on granulocytes. We used undiluted CSF and interpreted the test result using the manufacturer-provided color grading (less than 10 granulocytes/mm<sup>3</sup>, no color; 10-75 granulocytes/mm<sup>3</sup>, 1+; 75-500 granulocytes/mm<sup>3</sup>, 2+; >500 granulocytes/mm<sup>3</sup>, 3+).

#### *CSF glucose*

A normal CSF glucose level is two-thirds of the plasma glucose. The levels of CSF glucose fall in bacterial and fungal meningitis. Using the reagent strip, we determined if CSF glucose was below or above 50 mg/dL. The interpretation of results on reagent strip was no change in color as less than 50 mg/dL and any change in color as more than 50 mg/dL.

#### *CSF protein*

Normal CSF proteins range between 15 and 45 mg/dL; the range for protein detection of the strip is between less than 30 mg/dL to 500 mg/dL. We determined if CSF protein was <30 mg/dL (normal), between 30 and 100 mg/dL, or >100 mg/dL. The interpretation of maximum color on reagent strip was as follows: less than 30 mg/dL, no change in color; between 30 and 100 mg/dL, 1+; between 100 and 500 mg/dL, 2+; and more than 500 mg/dL, 3+.

#### Reference standards

We performed total CSF cell counts by microscopy (using a Neubauer's chamber), within 1 h of collection of a CSF sample. We also performed differential cell counts on Giemsa-stained, cytocentrifuged CSF sample. CSF sugar and protein levels were analyzed by conventional biochemistry. We have presented cut-off levels of reference standards in Table 1.

#### Statistical analysis

We estimated estimates of diagnostic accuracy (sensitivity, specificity, positive and negative predictive values and likelihood ratios) and expressed the precision of estimates by calculating 95% confidence intervals (CI). Furthermore, we used cut-off levels of CSF protein of >30 mg/dL and >100 mg/dL, CSF sugar less than 50 mg/dL and 40 mg/dL, and CSF neutrophils >10 cells/mm<sup>3</sup> and constructed receiver operating curves (ROC) to estimate the overall performance of the respective index tests, and to determine which index test cut-off levels correspond to them. The statistical analysis was performed using statistical software STATA (version 12.0, Stata Corp LP, College Station, TX, USA).

**Table 1: Cut-offs for index tests and reference standards used for estimation of diagnostic accuracy**

Index test	Reference standard
Leukocyte reagent strip	CSF microscopy
No color vs.	Less than 10 granulocytes/mm <sup>3</sup> vs.
Any color	10 or more granulocytes/mm <sup>3</sup>
No color vs. 1+/more color on color scale	>75 granulocytes/mm <sup>3</sup>
Glucose reagent strip	CSF biochemistry glucose
No color vs.	Less than 40, and 50 mg/dL vs.
Any color	More than 40, 50 mg/dL
Protein reagent strip	CSF biochemistry protein
Interpretation of less than 30 mg/dL vs.	Less than 30 mg/dL vs.
More than 30 mg/dL	More than 30 mg/dL
Protein reagent strip	CSF biochemistry protein
Index test interpretation of less than 100 mg/dL vs. more than 100 mg/dL	Less than 100 mg/dL vs. More than 100 mg/dL

CSF=Cerebrospinal fluid

## Results

CSF samples of 75 patients were included in the study. About a quarter of all patients were either less than 1 year of age (18/75; 24%) or between the ages of 1 and 12 years (19/75; 25%). Median age was 13 years (range 1 day to 75 years). Two-thirds of all patients were men. All the three indicator tests (CSF cells, protein, and glucose) were normal in 17 (22.6%) and abnormal in 16 (21.3%) patients [Figure 1].

We estimated the diagnostic accuracy of key indicator tests alone, and in combination, with rapid strip-based testing as index tests and microscopy, or laboratory-based measurements, as reference standards. Leukocyte esterase positivity by test strip had a sensitivity of 85.2 (95% CI 66.3-95.8%) and specificity of 89.6 (95% CI 77.3-96.5%) for detection of CSF granulocytes of more than 10/mm<sup>3</sup>. While protein reagent strip positivity had a high sensitivity for detection of CSF proteins greater than 30 mg/dL [98.1 (95% CI 90.1-100%)], the specificity was low [57.1 (95% CI 34-78.2%)] due to a higher proportion of false positives detected with the strip test. Specificity improved when we used a higher cut-off detection of 2+ or higher and CSF proteins (greater than 100 mg/dL). Using tests in combination did not result in higher accuracy estimates [Table 2].

Higher levels on the leukocyte esterase and protein patches of the test strip were associated with higher levels of CSF leukocytes and protein levels, respectively [Figure 2]. ROC analysis showed that the performance of urinary protein strip was best at a cut-off of 1+ to estimate CSF protein of 30 mg/dL or more [area under the curve 0.97 (95% CI 0.87-1.07%)] and at 2+ to estimate CSF protein of 100 mg/dL or more [area under the curve 0.96 (95% CI 0.92-1.01%)] [Figure 3].

## Discussion

The results of our study suggest that using ROC derived cut-offs as 2+ for protein, 1+ for neutrophils, and normal for glucose, Combur-10 strips can determine CSF protein levels more than 100 mg/dL, neutrophil count more than 10/mm<sup>3</sup>, and glucose levels less than 40 mg/dL with reasonable accuracy.

In the current study, using the protein strip test cut-off of 2+ and CSF protein > 100 mg/dL, 67 cases were correctly classified and 8 were either false positives or negatives. All six false positives had CSF proteins above 45 mg/dL, biochemical cut-off level used for interpretation of CSF

**Table 2: Diagnostic accuracy of test strip for detection of cerebrospinal fluid abnormalities**

Index test (test strip)	Reference standard (CSF testing)	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUC
<b>Tests used alone</b>												
Positive leukocyte esterase	Leukocyte pleocytosis (>10 cells/mm <sup>3</sup> )	23	5	4	43	<b>85.2 (66.3-95.8)</b>	89.6 (77.3-96.5)	82.1 (63.1-93.9)	91.5 (79.6-97.6)	8.2 (3.5-19)	0.16 (0.06-0.41)	0.87 (0.79-0.95)
Positive protein (1+ or more)	Protein>30 mg/dL	53	Q	1	12	98.1 (90.1-100)	57.1 (34-78.2)	85.5 (74.2-93.1)	92.3 (64-99.3)	2.3 (1.4-3.7)	0.03 (0.01-0.23)	0.77 (0.66-0.88)
Positive protein (2+ or more)	Protein>100 mg/dL	25	6	2	42	92.6 (75.1-99.1)	87.5 (74.8-95.3)	80.6 (62.5-92.5)	95.5 (84.5-99.4)	7.4 (3.4-15.8)	0.08 (0.02-0.32)	0.90 (0.83-0.97)
Negative glucose	Glucose<50 mg/dL	12	1	14	48	46.2 (26.6-66.6)	98 (89.1-99.9)	92.3 (64-99.8)	77.4 (65-87.1)	22.6 (3.1-164)	0.55 (0.38-0.78)	0.72 (0.62-0.82)
Negative glucose	Glucose<40 mg/dL	11	2	7	55	61.1 (35.5-82.7)	96.5 (87.9-99.9)	84.6 (54.6-98.1)	88.7 (78.1-95.3)	17.4 (4.2-71.4)	0.40 (0.22-0.72)	0.78 (0.67-0.90)
<b>Tests in combination</b>												
Both leukocyte esterase and protein test positive (1+)	Leukocyte pleocytosis (>10 cells/mm <sup>3</sup> ) and Protein>30 mg/dL	54	10	2	9	96.4 (87.7-99.6)	47.4 (24.4-71.1)	84.4 (73.1-92.2)	81.8 (48.2-97.7)	1.8 (1.2-2.8)	0.07 (0.01-0.31)	0.71 (0.60-0.83)
Both leukocyte esterase and protein test positive (2+)	Leukocyte pleocytosis (>10 cells/mm <sup>3</sup> ) and protein>100 mg/dL	31	8	4	32	88.6 (73.3-96.8)	80.0 (64.4-90.9)	79.5 (63.5-90.7)	88.9 (73.9-96.9)	4.4 (2.3-8.3)	0.14 (0.05-0.36)	0.84 (0.76-0.92)

AUC=Area under the curve, CSF= Cerebrospinal fluid

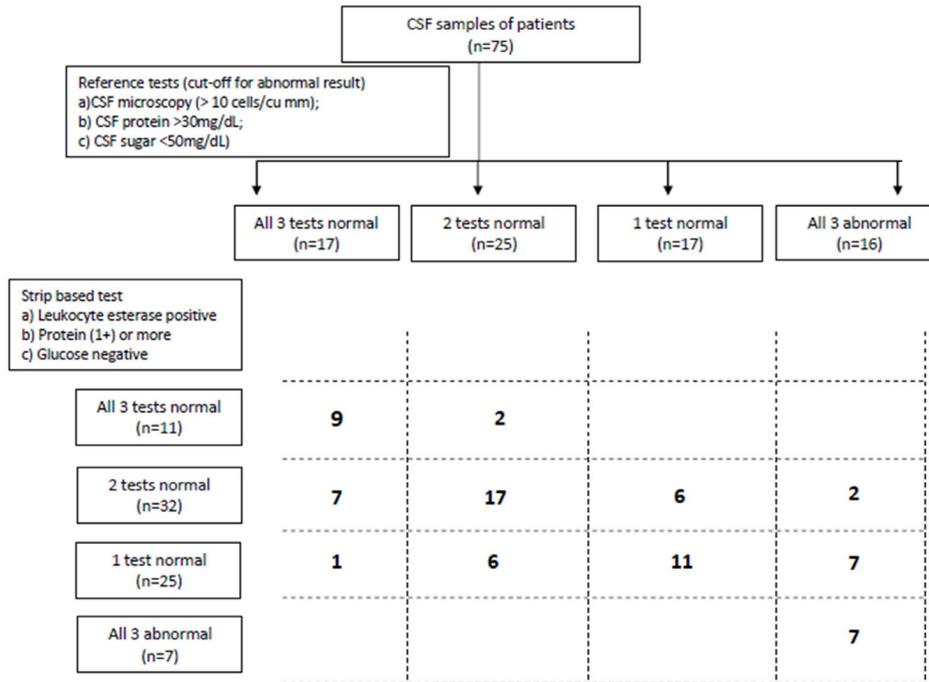


Figure 1: The study flow

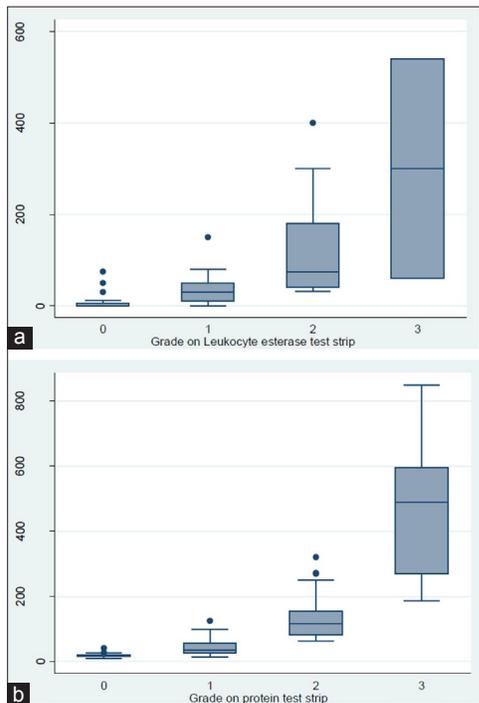
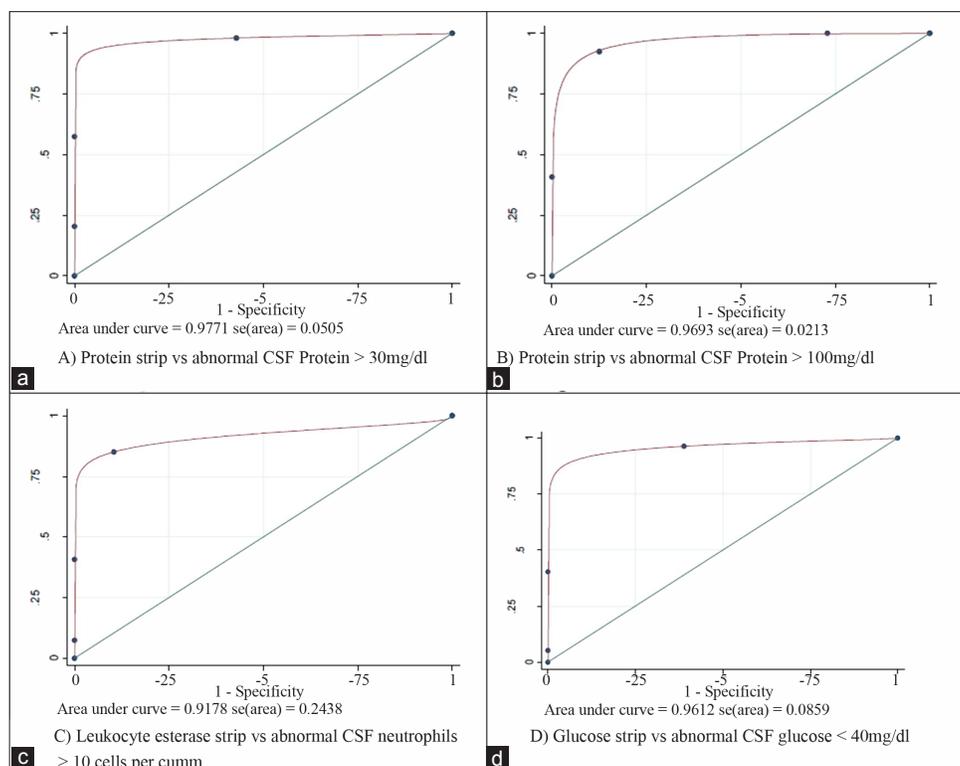


Figure 2: Cerebrospinal fluid leukocyte count and protein level by grade on test strip (a) Leukocyte count by leukocyte esterase test strip. Median leukocyte count on microscopy for test strip grades 0, 1, 2 and 3 are 0,30,74 and 300 per cu mm respectively.(b) CSF protein levels by grades on protein test strip. Median laboratory measured CSF protein level for test strip grades 0,1,2 and 3 are 18, 36, 115 and 490 mg/dl respectively. central line in each box depicts the median values for the grade, and the box represents interquartile (25 to 75%) range. Whiskers extend upto 5 and 90% range. and dots represents outliers

proteins. Thus, if a test strip is designed for this cut-off level, higher accuracy will be obtained. Similarly, a positive leukocyte esterase test correctly classified 66 of 75 cases, and 9 as either false positives or false negatives. The likely reason for false positives was presence of RBCs on cytology examination. Since the leukocyte esterase test is specific for granulocytes, we need to design a better pan-leukocyte marker, which will be more useful.

Results of our study and those of a few other published studies suggest that there is good agreement between the strip method and laboratory methods of determining CSF protein, glucose, and neutrophils. Using the same strips, Parmar *et al.*<sup>[3]</sup> reported the sensitivity and specificity of the reagent strips for the diagnosis of meningitis as 97.14% and 96.42%, respectively. The sensitivity and specificity for tuberculous meningitis and bacterial meningitis were reported as 100% and 96.55%, respectively, and the values for aseptic meningitis as 70% and 96.55%, respectively. Accuracy for the diagnosis of meningitis as a whole, bacterial meningitis, tuberculous meningitis, and aseptic meningitis was reported as 96.78%, 98.2%, 98.27%, and 83.0%, respectively. Romanelli *et al.*<sup>[11]</sup> compared the results of reagent strips and those of the cytological and biochemical assay, and obtained values for sensitivity, specificity, positive and negative predictive values, and accuracy (90.7%, 98.1%, 95.1%, 96.4%, and 96.1%, respectively). Moosa *et al.*<sup>[8]</sup> and Salvador *et al.*<sup>[9]</sup> used Combur-9 strips and



**Figure 3:** Receiver operator curves for performance of test strips at different cut-off levels for cerebrospinal fluid protein, sugar, and neutrophil count Area under the curves and their 95% confidence intervals are as follows. (a) 0.97 (0.87-1.07); (b) 0.96 (95% CI 0.92-1.01); (c) 0.91 (95% CI 0.43-1.39); (d) 0.96(95% CI 0.79-1.12); The best cut off for CSF protein >30mg/dL was at 1+, for protein >100mg/dL at 2+ on the protein strip. Best cut-off for CSF neutrophil count >10 cell per cumm was at 1+ on leukocyte esterase strip, and CSF glucose <40mg/dL for 1+ grade on sugar strip

found this method useful in making a rapid bedside diagnosis of meningitis.

Despite these and our study, physicians and laboratories in resource-constrained settings do not use this method for making a rapid diagnosis of meningitis. An issue in using these strips is the different cut-offs for protein and glucose values for CSF and urine analysis. These strips can be designed to indicate clinically meaningful cut-offs for CSF analysis. The cut-offs for urine analysis are 30, 100, and 500 mg/dL. These can be modified to 45, 100, and 500 mg/dL, as a CSF protein level of more than 45 mg/dL is considered abnormal. Similarly, since a low CSF glucose is clinically more meaningful, the lowest cut-off for CSF sugar detection can be made to be 40 mg/dL rather than 50 mg/dL in the current strip. Since this strip detects only granulocytes, and even a single granulocyte in CSF is considered as abnormal, this patch may not be changed. We used a cut-off of 10 granulocytes rather than 1 as even slight trauma during lumbar puncture may introduce a few granulocytes into the CSF. The strips may be designed so as to include only three parameters for proteins, glucose, and granulocytes, rather than the 10 for urinary analysis, thereby cutting down the cost of these tests and making the CSF strip analysis more cost effective.

Our study has several strengths. We carried out this study in a blinded fashion and we performed both CSF microscopy and the index tests within 30 min of receiving the sample. Small sample size is one of the limitations of our study. As already mentioned, another limitation is the different clinically significant cut-offs for urine and CSF analysis. However, despite these limitations, these strips can be a useful aid in guiding the treating physician to make a quick bedside diagnosis of an abnormal CSF. Main problem lies in distinguishing between a 1+ CSF protein as normal or abnormal. A simple solution to this problem can be repeat testing of a 1+ CSF in double dilution. By this method, protein levels between 60 and 100 mg/dL can be identified. In addition, the blood patch of the strip may be used to indicate whether the CSF tap is traumatic or not; in case of a positive traumatic tap, 1+ neutrophils may not be considered as significant.

## Conclusion

The results of our study demonstrate that urinary reagent strips can reliably predict raised CSF protein (>100 mg/dL), decreased glucose (<40 mg/dL), and increased neutrophil count (>10/mm<sup>3</sup>). Hence, these strips can be of value to

clinicians working in resource-constrained settings to reliably make a rapid diagnosis of meningitis and initiate appropriate treatment.

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