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**Original Research Article**

# Determining the degree of accuracy of Combar-10 reagent strip in diagnosing bacterial meningitis

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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 experienced personnel 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.
**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 an 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 2 + and 3 + 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 150 patients were included in the study. Of the three tests, diagnostic accuracy of protein estimation (2+ or more on reagent strip) was best for detection of CSF proteins greater than 50 mg/dL [sensitivity 93.2% (95% CI 86.63-96.67); specificity 91.49% (95% CI 80.07, 96.64)], with AUC of 0.97. Leukocyte esterase positivity by test strip had a sensitivity of 90% (95% CI 82.08, 94.65) and specificity of 91.6% (95% CI 81.93, 96.39) for detection of CSF granulocytes of more than 10/mm$^3$.
**Conclusions:** The degree of accuracy of Combar-10 reagent strip in diagnosing bacterial meningitis is very high similar to CSF microscopy and biochemistry.

**Keywords:** Cerebrospinal fluid, Meningitis, Urinary reagent strip

## INTRODUCTION

Meningitis is a dangerous infection because of the very delicate nature of the brain. Brain cells once killed, will not revitalize themselves. Therefore, if enough brain tissue is damaged by an infection, serious lifelong handicaps will remain.[1] Incidence of meningitis is more frequent nowadays in childhood, where signs and symptoms are usually nonspecific. Early diagnosis and timely treatment are imperative in the management of children with meningitis.[2] Several times, patients are submitted to lumbar puncture, but technical difficulty in obtaining enough material or a delay in the cytological and biochemical assay usually get in the way of promptly deciding on antibiotic therapy.[3]

Given the restrictions and non-availability of culture methods, many of the treating pediatricians have to rely upon the clinical evaluation and results of the biochemical and cell analysis of the CSF for making the

diagnosis of meningitis.[4,5] Unfortunately even these services are not available in many parts of the world. The use of quick cerebrospinal fluid (CSF) tests by means of urine reagent strips has been described in few studies, in which sensitivity and specificity reached respectively 97% and 100%.[5] Reagent strips that measures glucose and protein in blood and urine have been used with varying results to evaluate CSF in the past. If established useful, it can serve as an important diagnostic aid in the management of these children.[6] Combur-10 (Boehringer - Mannheim) is a 10-patch strip used to test urine for specific gravity, protein, glucose, leukocytes, nitrites, pH, haemoglobin, ketones, bilirubin, and urobilinogen. Here we used this test strip to measure the CSF protein, glucose and leukocytes with the aim to calculate the utility of Combur-10 urine reagent strips for the diagnosis of meningitis and its subtypes and also to correlate its accuracy with the laboratory method for CSF analysis.

## METHODS

A Single blinded Prospective study was conducted over one-year duration, from September 2017 to August 2018 at Department of Pediatrics, Coimbatore Medical College Hospital. 150 samples from children suspected to have meningitis constituted study material. All patients underwent a lumbar puncture following thorough clinical evaluation and fundus examination.

Each CSF sample was divided into two parts which were utilized for routine diagnostic tests and reagent strip analysis of glucose, protein and leukocyte. Neuroimaging was carried out whenever clinically warranted. An independent researcher performed microscopy (total cell 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 as an index test to detect CSF cellularity (leukocyte esterase estimation), glucose (glucose oxidase-peroxidase method), and protein levels.

### *Data analysis*

Data were analyzed using SPSS 11.2 software version. The results obtained from reagent strip analysis were compared with the standard tests like estimation of CSF protein and glucose performed in the biochemistry laboratory. The sensitivity, specificity, positive predictive value and the negative predictive values of the reagent strips for the diagnosis of meningitis and its subtypes were calculated. Accuracy of the reagent strips as a screening tool was established using receiver operating curve.

## RESULTS

At the beginning, 164 patients were included in the study. Among the 164 CSF samples, 14 were lost due to reagent strip misreading and unanalyzed hemorrhagic CSF.

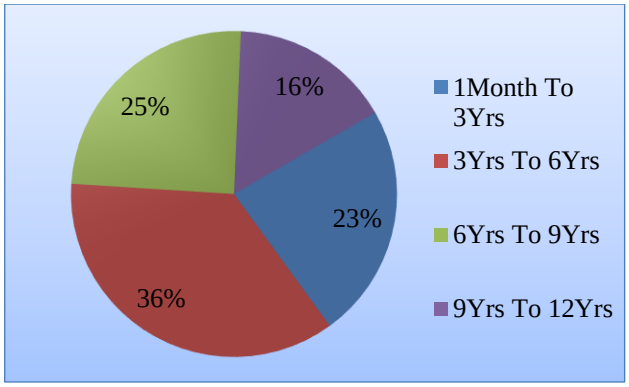


**Figure 1: Incidence of meningitis in various age groups.**

Therefore, 150 CSF samples were analyzed in the age group of one month to 12 years. Among 150 children, 35 children were between 1 month to 3 years, 54 children were 3 years to 6 years, 37 children were 6 years to 9 years, 24 children were 9 years to 12 years. In this study incidence of meningitis was more in the age group of 3 years to 6 years which contributes 36% of cases.

**Table 1: Presenting complaints of meningitis.**

| Parameter | | No. of patients 150 | % |
|---|---|---|---|
| Presenting manifestation in general | Fever | 150 | 100 |
| | Projectile vomiting | 70 | 47 |
| | Convulsion | 109 | 77 |
| | Headache | 84 | 56 |
| | Photophobia | 20 | 13 |
| | refusal of feeds | 54 | 36 |

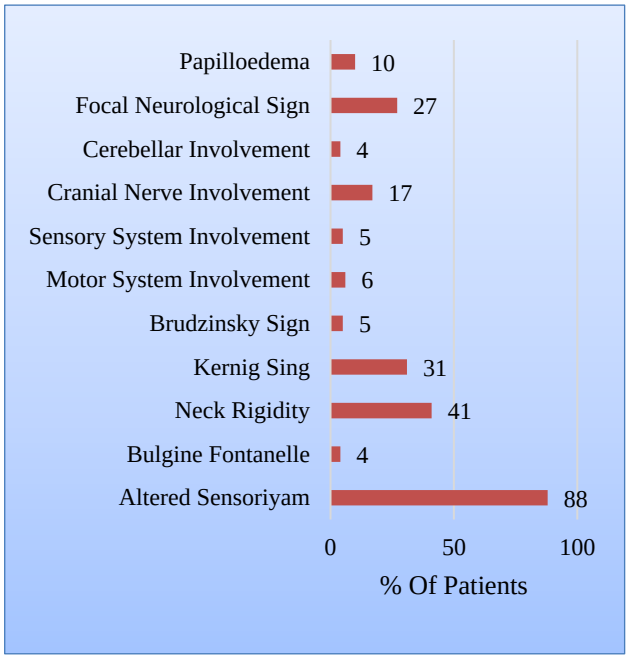


**Figure 2: Presenting signs of meningitis**

Among the number of positive cases shown both by lab and strip, fever was the predominant complaint followed by altered sensorium, convulsion and neck rigidity. Few cases admitted with altered sensorium and fever were not having classical laboratory picture suggestive of meningitis.

***Based on sugar value***

Among the cases of suspected bacterial meningitis, lab biochemistry analysis showed 90 cases had sugar value below 50 mg/dl which was considered as positive cases. Among 90 lab positive cases strip shown true positive were 81 and false negative were 9. In another 60 cases, lab biochemistry analysis showed sugar values more than 50 which was considered as negative cases. Among 60 lab negative cases strip shown true negative were 55 and false positive were 5.

Sensitivity of the index test for sugar to diagnose the meningitis by comparing lab standard were 90% with Upper and lower limit of 95% confidence interval (82.08, 94.65). Specificity of the index test for sugar to diagnose the meningitis by comparing lab standard were 91.67% with upper and lower limit of 95% confidence interval (81.93, 96.39).

**Table 2: Comparison of strip CSF sugar value vs lab CSF sugar value.**

| Parameter | | | Lab sugar | | Total | P-value |
|---|---|---|---|---|---|---|
| | | | < 50 Positive | > 50 Negative | | |
| Strip Sugar | <1+ Positive | Patients | 81 | 5 | 86 | .000 Sig |
| | | % | 90% | 8% | 57% | |
| | >1+ Negative | Patients | 9 | 55 | 64 | |
| | | % | 10% | 92% | 43% | |
| Total | | Patients | 90 | 60 | 150 | |
| | | % | 100.0% | 100.0% | 100.0% | |

**Table 3: Calculation of diagnostic accuracy based on strip sugar values.**

| Parameter | Estimate | Lower-upper 95% CIs |
|---|---|---|
| Sensitivity | 90% | (82.08, 94.65) |
| Specificity | 91.67% | (81.93, 96.39) |
| Positive predictive value | 94.19% | (87.1, 97.49) |
| Negative predictive value | 85.94% | (75.38, 92.42) |
| Diagnostic accuracy | 90.67% | (84.94, 94.36) |

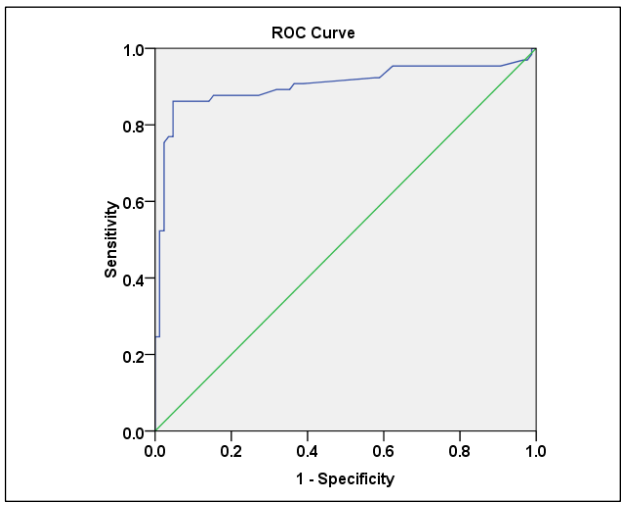


**Figure 3: Calculation of ROC curve based on strip sugar values.**

Positive predictive value of the index test for sugar to diagnose the meningitis by comparing lab standard were 94.19% with upper and lower limit of 95% confidence interval (87.1, 97.49). Negative predictive value of the index test for sugar to diagnose the meningitis by comparing lab standard were 85.94% with upper and lower limit of 95% confidence interval (75.38, 92.42). Diagnostic accuracy for this study based on sugar estimation 90.67%.

Receiver operator curves for performance of test strips at different cut-off levels for CSF sugar; area under the curves (A) 0.903; and 95% confidence intervals were (0.84-0.96).

***Based on protein value***

Among the cases of suspected bacterial meningitis lab biochemistry showed 103 cases had protein value more than 50mgs/dl which was considered as positive cases. Among 103 lab positive cases, strip shown true positive were 96 and false negative were 7. Among the cases of suspected bacterial meningitis lab biochemistry analysis showed 47 cases had protein value below 50. Among 47 lab negative cases, strip shown true negative are 43. Hence false positive are 4.

Sensitivity of the index test for protein to diagnose the meningitis by comparing lab standard were 93.20% with Upper and lower limit of 95% confidence interval (86.63,

96.67). Specificity of the index test for protein to diagnose the meningitis by comparing lab standard were 91.49% with Upper and lower limit of 95% confidence interval (80.07, 96.64)

**Table 4: Comparison of strip CSF protein value vs lab CSF protein value.**

| Parameter | | | Lab Protein | | Total | P-Value |
|---|---|---|---|---|---|---|
| | | | > 50 Positive | < 50 Negative | | |
| Strip protein | >1 Positive | Patients | 96 | 4 | 100 | .000 |
| | | % | 93% | 9% | 67% | |
| | <1 Negative | Patients | 7 | 43 | 50 | |
| | | % | 7% | 92% | 33% | |
| Total | | Patients | 103 | 47 | 150 | |
| | | % | 100.0% | 100.0% | 100.0% | |

Positive predictive value of the index test for protein to diagnose the meningitis by comparing lab standard were 96% with Upper limit and lower limit of 95% confidence interval (90.16, 98.43).

Negative predictive value of the index test for protein to diagnose the meningitis by comparing lab standard were 86% with Upper and lower limit of 95% confidence interval (73.81, 93.05). Diagnostic accuracy for this study based on protein estimation 92.67%.

**Table 5: Calculation of diagnostic accuracy based on strip protein values.**

| Parameter | Estimate | Lower-Upper 95% CI |
|---|---|---|
| Sensitivity | 93.20% | (86.63, 96.67) |
| Specificity | 91.49% | (80.07, 96.64) |
| Positive predictive Value | 96% | (90.16, 98.43) |
| Negative predictive value | 86% | (73.81, 93.05) |
| Diagnostic accuracy | 92.67% | (87.35, 95.86) |

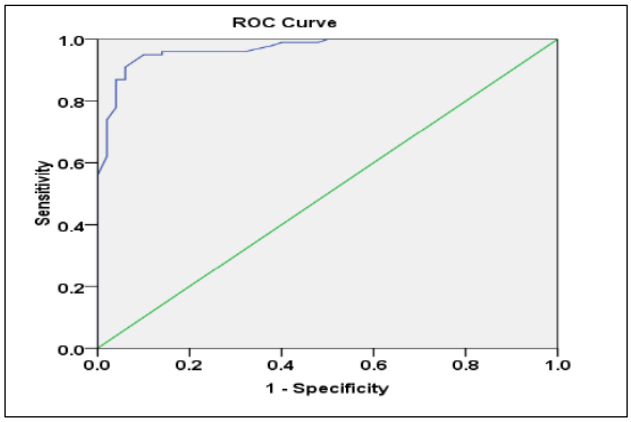


**Figure 4: Calculation of ROC curve based on strip protein values.**

Receiver operator curves for performance of test strips at different cut-off levels for CSF protein; Area under the curves (A) 0.969; and 95% confidence intervals were (0.94-0.99).

**Table 6: Comparison of strip CSF cell count versus lab CSF cell count.**

| Parameter | | | Lab Cell Count | | Total | P-Value |
|---|---|---|---|---|---|---|
| | | | > 10 Positive | < 10 Negative | | |
| Strip cell count | 1+ Positive | Patients | 81 | 5 | 86 | 0.000 Sig |
| | | % | 90% | 8% | 57% | |
| | 0 Negative | Patients | 9 | 55 | 64 | |
| | | % | 10 | 92% | 43% | |
| Total | | Patients | 90 | 60 | 150 | |
| | | % | 100.0% | 100.0% | 100.0% | |

***Based on cells value***

Among the cases of suspected bacterial meningitis lab cytological analysis showed 90 cases had cells more than 10. Among 90 lab positive cases, strip shown true positive were 81, hence false negative was 9. Among the cases of suspected bacterial meningitis lab cytological analysis showed 60 cases had cells less than 10. Among

60 lab negative cases, strip shown true negative were 55 and false positive were 5. Sensitivity of the index test for cells to diagnose the meningitis by comparing lab standard were 90.00% with Upper and lower limit of 95% confidence interval (82.08, 94.65) Specificity of the index test for cells to diagnose the meningitis by comparing lab standard were 91.67% with Upper and lower limit of 95% confidence interval (81.93, 96.39).

**Table 7: Calculation of diagnostic accuracy based on strip cell count.**

| Parameter | Estimate | Lower - Upper 95% CIs |
|---|---|---|
| Sensitivity | 90% | (82.08, 94.65) |
| Specificity | 91.67% | (81.93, 96.39) |
| Positive predictive value | 94.19% | (87.1, 97.49) |
| Negative predictive value | 85.94% | (75.38, 92.42) |
| Diagnostic accuracy | 90.67% | (84.94, 94.36) |

Positive Predictive Value of the index test for cells to diagnose the meningitis by comparing lab standard were 94.19% with Upper and lower limit of 95% of confidence interval (87.1, 97.49). Negative Predictive Value of the index test for cells to diagnose the meningitis by comparing lab standard were 85.94% with Upper and lower limit for 95% of confidence interval were (75.38, 92.42). Diagnostic accuracy for this study based on protein estimation 90.67%.

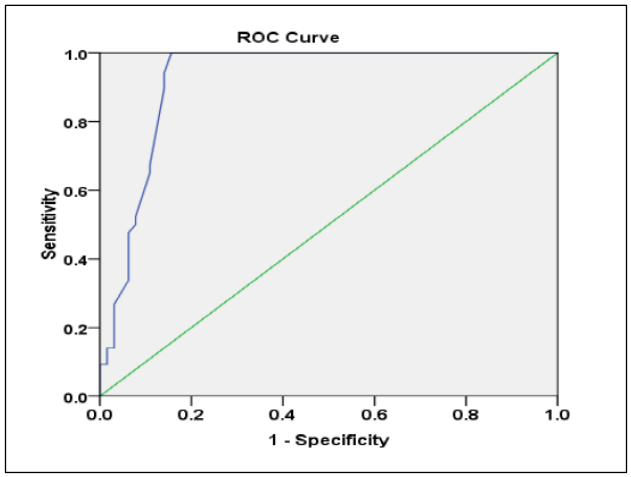


**Figure 5: Calculation of ROC curve based on strip cell count.**

Receiver operator curves for performance of test strips at different cut-off levels for CSF cytology, Area under the curves (A) 0.923; and 95% confidence intervals (0.87 - .97)

## DISCUSSION

The results of our study suggest that using ROC derived cut-offs as 2 + for protein, 1 + for neutrophils, and <1+ for glucose, Combur-10 strips can determine CSF protein levels more than 50 mg/dL, neutrophil count more than 10/mm3, and glucose levels less than 50 mg/dL with reasonable accuracy.

Many false negative values by strip were having CSF proteins between 50 to 100 mg/dL, if CSF protein range above 100 mgs% strip used to diagnose the case without any false negative. Thus, if a test strip is designed for this cut-off level, higher accuracy will be obtained.[7] Among 90 cases positive for CSF leukocytes by lab microscopy, 5 cases were false positive by strip method. 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 for more usefulness. Results of the present study and those of few other published studies suggest that there is good concurrence between the strip method and laboratory methods of determining CSF protein, glucose, and neutrophils.[8]

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, 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 & of value to clinicians working in resource constrained settings to reliably make a rapid diagnosis of meningitis.[9,10] Present 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).

An issue in using these strips is the different cut-offs for protein and glucose values for CSF and urine analysis

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, in case of a positive traumatic tap. Main problem lies in distinguishing between a 1 + CSF protein as normal or abnormal.

## CONCLUSION

The results of the present study demonstrate that urinary reagent strips can reliably predict raised CSF protein (>50 mg/dL), decreased glucose (<50 mg/dL), and increased neutrophil count (>10/mm$^3$).

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.

***Recommendations***

No rapid diagnostic test exists for the analysis of CSF. Our results show that the rapid diagnosis of meningitis can be made with the Combur-10 reagent strips with a high specificity and sensitivity.

This test can help in the making the rapid decision whether to use or withhold antibiotics in patients with meningitis. It would be of particular value in the parts of the world where no laboratory facilities exist and referral at higher centers is not possible. The test strip could be simplified to only the three patches of interest, which will help in cutting down the cost to about Rs. 15.00 per strip.

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