Correlation Between Rapid Strip Test and the Quality of Sputum*

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Study objectives: To evaluate the use of a rapid strip test for the quick evaluation of sputum quality.

Design: Prospective, double-blind study. Sputum and saliva samples were collected. Sputum quality was assessed by the presence of polymorphonuclear neutrophils (PMNs) and squamous epithelial cells (SECs) per low-power (microscopic) field (LPF) [×10 objective]. Sputum was defined as follows: (1) informative (ie, > 25 PMNs and < 10 SECs per LPF); (2) semi-informative (ie, > 25 PMNs or < 10 SECs per LPF, but not both); or (3) uninformative (ie, < 25 PMNs and > 10 SECs per LPF). The first two levels were considered to be “sputum” and the third one was considered to be “nonsputum.” The quality of the sputum was compared to results obtained using a rapid strip test (Combur-Test; Roche Diagnostics; Basel, Switzerland) for specific gravity (SG), pH, leukocyte esterase (LE) activity, and levels of nitrites, protein, glucose, and erythrocytes. A Kruskal-Wallis test was used to compare the three levels of sputum quality and the rapid strip test. A Mann-Whitney test compared sputum and nonsputum to the rapid strip reagents. Pearson correlation and k tests were used to assess correlation. Receiver operating characteristic was used to calculate the best cut-point values, and the sensitivity and specificity of these values were calculated.

Results: Eighty-two samples were included, with 61 samples from hospitalized patients and 21 samples from healthy volunteers. The best predictor of sputum quality was the SG of the reagent. Using an SG threshold definition of > 1.01, the sensitivity was 86.8% and the specificity was 75.9%. The specificity of protein, glucose ≥ +1, and LE levels were relatively low. No relationship was found between the results of the reagent strip test for pH, nitrites, and erythrocytes, and the sputum quality.

Conclusion: Using an SG threshold definition of > 1.01, the rapid reagent strip test has been shown to be a sensitive test for the evaluation of sputum quality, which can be useful when facilities for sputum cytology are not available.

Key words: pneumonia; polymorphonuclear neutrophils; rapid strip test; reagent strip; sputum

Abbreviations: LE = leukocyte esterase; LPF = low-power field; LRT = lower respiratory tract; PMN = polymorphonuclear neutrophil; SEC = squamous epithelial cell; SG = specific gravity; URT = upper respiratory tract

The examination of sputum by Gram stain and culture is routine procedure for evaluating patients with acute pneumonia. An empiric treatment is often prescribed until microbiological results are available. Yet, up to 20% of patients with lower respiratory tract (LRT) infection reconsulted their physician within several days,1 and in 67% of patients the antibiotic treatment recommended on the basis of the results of the Gram stain differed significantly from the original treatment.2 It has been shown that early diagnosis of

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the etiology based on Gram stain of the sputum and appropriate antibiotic treatment correlate with more rapid resolution of the fever.3

The value of Gram staining for evaluating expectorated sputum has been debated.3–7 Nevertheless, in its guidelines,8 the Infectious Diseases Society of America recommends performing this simple, inexpensive procedure as an indicator for the initial selection of antimicrobial therapy. Accordingly, routine laboratory tests should include Gram staining, cytology screening, and aerobic culture of specimens that satisfy cytologic criteria.8

A major problem related to evaluating sputum samples is that of contamination by upper respiratory tract (URT) secretions. Most sputum secretions arise from the tracheobronchial tree. The presence of alveolar macrophages and other inflammatory cells suggests that the origin of the secretions is the LRT, while the presence of squamous epithelial cells (SECs) suggests contamination in the URT.8

The following criteria are useful for evaluating sputum samples: (1) informative (ie, originating in the LRT) in patients with a normal or elevated WBC count, with <10 SEC per low-power field (LPF) [×10 objective] and >25 polymorphonuclear neutrophils (PMNs) per LPF [×10 objective]; (2) semi-informative (ie, >25 PMNs or <10 SECs per LPF, but not both); and (3) uninformative (ie, <25 PMNs and >10 SECs per LPF). For the purpose of bacteriologic evaluation, the first two quality levels were considered to be “sputum” and the third one was considered to be “non-sputum.” In order to decrease the possibility of contamination with URT secretions, more invasive methods for sampling pulmonary material are used, such as a hypertonic saline solution-induced specimen, BAL, thin-needle aspiration, transbronchial aspiration, and open-lung biopsy.

The rapid strip test was originally designed as a semi-quantitative test for the presence of PMNs in urine through the detection of leukocyte esterase (LE) enzyme activity. In addition to LE, other indicators available in test strips include specific gravity (SG), pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin, and erythrocytes.

Beyond its utility for urinary tract infection screening, the LE test has been evaluated for the diagnosis of bacterial meningitis and peritonitis,11–16 peritoneal lavage, the LE test has been evaluated for the diagnosis of bacterial meningitis and peritonitis,11–16 peritoneal

In most settings, facilities for sputum cytology are not available on a 24-h basis. Thus, initial antibiotic therapy may be started before microbiology processing validates sputum quality. Obtaining a second sample for bacteriologic culture after antibiotics are administered is of lesser value as eradication may occur within 3 days.24 Consequently, we were interested in evaluating a simple, quick, and readily available method for determining sputum quality. Therefore, we examined a commercially available reagent strip for its ability to distinguish between samples originating in the LRT and those originating in the URT.

**Materials and Methods**

**Study Population**

During a 3-month period (March to May 2002), sputum samples, which were obtained from patients at the Rambam Medical Center for microbiological processing, were randomly tested. Healthy individuals with no active pulmonary disease, who therefore were unable to produce sputum, gave saliva samples in order to establish the difference between secretions from the URT and the LRT. The Rambam Medical Center Ethics Committee approved the study.

**Sampling Technique**

The samples were collected in sterile containers and were preserved at room temperature. All the samples were examined within 10 h from their arrival in the laboratory. Samples that contained an insufficient amount of sputum (ie, approximately <0.5 mL) for evaluation using a stick were not included.

All the sputum samples were Gram-stained and examined microscopically for the quantification of SECs and PMNs in LPF by the same highly qualified and experienced laboratory technician, according to the hospital’s protocol for processing biological material from the respiratory tract. The quality of the sputum samples was evaluated as informative (ie, >25 PMNs per LPF and <10 SECs per LPF), semi-informative (ie, >25 PMNs per LPF or <10 SECs per LPF, but not both), or uninformative (ie, <25 PMNs per LPF and >10 SECs per LPF). The first two levels were considered to be sputum, and therefore appropriate for culture, while the third level was considered to be non-sputum. The laboratory worker was not informed of the rapid strip test results.

A 10-patch reagent strip (Combust-Test; Roche Diagnostics; Basel, Switzerland), which was designed to test urine for SG, pH, leukocytes, levels of nitrites, proteins, glucose, ketone bodies, and urobilinogen, and erythrocytes, was also used to analyze each sample. The reagent strips were read with a urine chemistry analyzer (Urilux; Roche Diagnostics). The performance of the reagent strips was checked against positive and negative control sticks when using a new package. The procedures were performed according to the manufacturer’s instructions and the urine chemistry analyzer manual.
To compare the instrumental readings with the visual readings, each stick also was read against the scale provided on the label, immediately following the automatic reading by the urine chemistry analyzer, and up to 120 s after dipping the stick into the sputum. The variants that were recorded are shown in Table 1. Since it was impossible to dip the stick into thick sputum, all the samples also were tested after dilution with 1 mL normal saline solution.

**Statistical Analysis**

A Pearson correlation coefficient assessed the correlation between visual reading and the automatic reading by the urine chemistry analyzer, and between the readings performed before and after dilution for the variants SG and pH. Assessments for the variants (ie, nitrite, leukocyte, protein, glucose, and erythrocyte) were done using the $\kappa$ test, while a $\kappa$ value of $> 0.8$ was considered to be good concordance.

The comparison between the results of the rapid strip test and the three quality levels of the sputum (ie, informative, semi-informative, and uninformative) for the variants SG, pH, protein, glucose, and erythrocytes was done using the Kruskal-Wallis test. The relationship between the variants LE and nitrite and the three quality levels of the sputum was assessed using the $\chi^2$ test. A Mann-Whitney test evaluated the use of the rapid strip to define sputum and nonsputum. The receiver operating characteristic was used to calculate the best cut-point values, and the sensitivity and specificity of these values were calculated and compared to the cytologic results.

**RESULTS**

During the study period, 83 samples were obtained. Twenty-one of the samples were obtained from healthy people, and 62 samples were obtained from the bacteriology laboratory at the Rambam Medical Center. One of the samples was excluded, since it was found to be endotracheal aspirate from a ventilator-dependent patient.

The urine chemistry analyzer read only 47 of all the sputum samples, and it rejected 36 of 62 thick sputum samples. These samples (82 samples) were read visually and by the analyzer after dilution.

A total of 82 sputum samples (patients, 61 sputum samples; healthy volunteers, 21 sputum samples) were analyzed in the microbiology laboratory, of which 30 were considered to be informative, 29 were considered to be uninformative, and 23 were considered to be semi-informative. Of the 21 sputum samples from the control group, 19 were found to be uninformative (saliva), and 2 were found to be semi-informative. Of the 61 sputum samples from patients, 30 samples were found to be informative, 21 were found to be semi-informative, and 10 were found to be uninformative. The results obtained visually and by the urine chemistry analyzer (47 results) were compared using the Pearson test and $\kappa$ test. Good correlation was found for all of the variants of the visual reading and the reading of the urine chemistry analyzer ($p < 0.001$ [for SG and pH]; $\kappa$ range, 0.934 to 1.00 [for the other variables]). No correlation was found between the visual results and the results obtained after dilution, except for nitrites ($\kappa = 0.911$) and glucose ($\kappa = 0.768$).

Using the Kruskal-Wallis test, differences were found between the three quality levels of the 82 sputum samples (ie, informative, semi-informative, and uninformative) for the variants SG ($p < 0.0001$), leukocyte ($p = 0.047$), protein ($p = 0.022$), and glucose ($p = 0.025$), but no association was found between the variant erythrocytes or nitrite levels and the quality of sputum. A Mann-Whitney evaluation of sputum and nonsputum revealed $p < 0.0001$ for SG, $p < 0.014$ for LE, $p < 0.008$ for protein, and $p < 0.007$ for glucose (Table 2).

The best cut-point values and specificity and sensitivity of each reagent compared to cytological definition of “sputum,” and “nonsputum” were calculated using the receiver operating characteristic. The best validity was found between SG $> 1.01$ and samples that were defined as sputum (sensitivity, 86.8%; specificity, 75.9%; area, 0.821). The reagents of protein, glucose, and LE were of relatively low specificity. Using a combination of reagents did not yield higher validity. No validity was found for the use of erythrocytes, pH, or nitrites to evaluate sputum quality.

### Table 1—Variables Recorded by Urine Chemistry Analyzer*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG</td>
<td>1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030</td>
</tr>
<tr>
<td>LE enzyme activity</td>
<td>Negative, +, +++, +++</td>
</tr>
<tr>
<td>pH level</td>
<td>5, 6, 7, 8, 9</td>
</tr>
<tr>
<td>Nitrites</td>
<td>Negative or positive</td>
</tr>
<tr>
<td>Protein</td>
<td>Negative, +, +++, +++</td>
</tr>
<tr>
<td>Glucose</td>
<td>Normal, +, +++, +++, +++++</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Negative, +, +++, +++, +++++</td>
</tr>
</tbody>
</table>

*Plus-scale of magnitude ranging from + (minimal) to ++++ (significant).

### Table 2—Specificity and Sensitivity of the Reagents Compared to Cytologic Definition of Sputum as Informative or Uninformative

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Cut-Point Value*</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG</td>
<td>$&gt; 1.01$</td>
<td>96.8</td>
<td>75.9</td>
</tr>
<tr>
<td>Protein</td>
<td>Positive (≥+)</td>
<td>90.6</td>
<td>41.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>$\geq +1$</td>
<td>71.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>$\geq +2$</td>
<td>56.6</td>
<td>17.2</td>
</tr>
</tbody>
</table>

*Calculated by receiver operating characteristic curves (see “Materials and Methods” section).
Urine reagent strip tests have been implemented for testing body fluids with varying results.\textsuperscript{11–23} To our knowledge, they have not yet been evaluated for testing the quality of sputum samples before bacteriologic processing. The present prospective study demonstrated that the rapid strip could be used as a rapid method for evaluating the quality of sputum as informative or uninformative using the SG reagent. Applying the cut-point value of $\text{SG} > 1.01$, the sensitivity was 86.8% and the specificity was 75.9%.

The presence of protein in the sample was found to define a sample as informative with high sensitivity (90.6%). The origin of the protein is probably the inflammation reaction in the sputum, and the low specificity probably reflects cross-reactivity with saliva glycoproteins. Surprisingly, the presence of LE was found to be ineffective for the purpose of evaluating the quality of sputum. Apparently, the reason for this is the presence of enzymes such as amylase, glycoproteins, mucins, immunoglobulins, lysozymes, and other proteins that are found in saliva, which may cross-react with the LE reagent. Nitrite level, which is an important indicator of urinary tract infection, also was not helpful here, probably because the oral flora contain multiple organisms. Glucose level is expected to be lower in inflammatory processes, however, food was not avoided before obtaining sputum, hence glucose level was not a reliable indicator.

The population of this study was heterogeneous and included healthy people, however, the aim of the study was to test the ability of the rapid strip reagents to predict sputum quality, unrelated to the patient population. The utility of rapid strip sticks may be limited when the quantity of the sample is $< 0.5 \text{ mL}$, when the sputum is extremely thick, and in neutropenic patients.

The rapid evaluation of the quality of the sputum is very important. The evaluation of sputum quality using cytologic parameters requires experience and qualification,\textsuperscript{4,8} and is also time-consuming and resource-consuming. Usually, after obtaining the sample, antibiotic therapy is established. Hence, a second sample is less informative, and the identification of the infective agent is less likely. Using the rapid strip test might reduce the percentage of uninformative samples and enable a higher percentage of identification of the infecting organism than that achieved today. The association of the SG reagent of the rapid strip with sputum quality enables this test to be used as a reliable, simple, quick, safe, and inexpensive method for evaluating the quality of sputum.

**Discussion**

The rapid strip test, which was originally designed to analyze urine, can be used as a rapid screening test for evaluating the quality of sputum. Using an SG threshold definition of $> 1.01$, the rapid reagent strip has been shown to be a sensitive test for the evaluation of sputum quality, which can be highly useful when facilities for sputum cytology are not available on a 24-h basis.

**References**


**Conclusions**

ACKNOWLEDGMENT: The authors acknowledge the contribution of Ada Tamir for the statistical analysis of the correlation of rapid strip test and the quality of sputum.