Table 1. Effect of tHcy and other confounding factors on PON1 activity and concentration. 

<table>
<thead>
<tr>
<th>Percentage change</th>
<th>PON1 activity</th>
<th>PON1 concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μmol/L increase in tHcy</td>
<td>-2.3</td>
<td>-3.1</td>
</tr>
<tr>
<td>1 mmol/L increase in HDL</td>
<td>16.9</td>
<td>-16</td>
</tr>
<tr>
<td>PON1&lt;sub&gt;192&lt;/sub&gt; RR genotype</td>
<td>77</td>
<td>-2</td>
</tr>
<tr>
<td>PON1&lt;sub&gt;54&lt;/sub&gt; MM genotype</td>
<td>-33</td>
<td>5.7</td>
</tr>
<tr>
<td>PON&lt;sub&gt;107&lt;/sub&gt; TT genotype</td>
<td>-16</td>
<td>-2</td>
</tr>
<tr>
<td>F</td>
<td>48.475</td>
<td>1.002</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Multiple linear regression analysis. Models are adjusted for body mass index and sex. The table shows the percentage changes in PON1 activity and concentration for the indicated predictor. In the case of PON1 polymorphisms, the table shows the effect of being homozygotic.

b P<0.001.

*NS, not significant.

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References


Tumor M2 Pyruvate Kinase as a Stool Marker for Colorectal Cancer: Stability at Room Temperature and Implications for Application in the Screening Setting

Colorectal cancer (CRC) remains the third most common malignancy worldwide (1, 2). To improve noninvasive screening for CRC, various stool tests have been described based on tumor-associated markers (3). Among these is a test for fecal tumor M2 pyruvate kinase (M2-PK) activity.

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Tumor M2-PK, an isoform of PK, is found in proliferating tissues such as tumor cells (5). To date, the performance of this test has been evaluated only in small-scale investigations (4, 6). Regarding large-scale applications, the stability of tumor M2-PK, which would affect the handling of stool samples, is a critical issue. We investigated the average stability of tumor M2-PK in stool at room temperature to estimate the potential impact of its degradation on the sensitivity and specificity of the test.

We collected stool specimens before bowel preparation for surgery from 20 patients with histologically confirmed CRC. Samples were kept at room temperature for 5 days. Immediately after sample collection and on each of the following days, the amount needed for duplicate determinations of the tumor M2-PK activity was taken and stored at −20 °C until analysis. Samples were analyzed blinded to patient identity and day of sampling.

Tumor M2-PK was measured by ELISA (ScheBo® Biotech AG). The lower detection limit was 2 kU/L.

For each measurement point, the mean tumor M2-PK activity was calculated as the mean of duplicate determinations. For further analyses, only patients with an initial tumor M2-PK activity >4 kU/L, the proposed cutoff, were included. The values for each patient were expressed as a proportion of the initial tumor M2-PK activity. Linear regression, with log(tumor M2-PK activity) as dependent variable, was performed to obtain the relative activity for each day of storage at room temperature over all included patients.

To estimate the potential impact of degradation of tumor M2-PK on the diagnostic accuracy of the test, results of stability testing were used in combination with fecal tumor M2-PK activities measured in 65 CRC patients (26 with colon cancer and 39 with rectal cancer) and in 917 unselected older adults. Stool samples from CRC patients were frozen immediately after specimen collection, whereas stool samples from unselected older adults were collected by mail in the context of the ESTHER study (7) and frozen at −80 °C on receipt. The study was approved by local and state Ethics Committees.

The theoretical tumor M2-PK activity after n days of storage at room temperature was estimated as the relative activity after n days multiplied by the initial activity in CRC patients (to determine sensitivity) and of ESTHER study participants (to determine specificity). Given that stool samples from ESTHER study participants were mailed (i.e., stored at room temperature for 1–4 days), the equation was first transformed to calculate initial activities before proceeding as described. Finally, the sensitivity and specificity of the test were calculated for each day of storage at room temperature, using the cutoff of 4 kU/L.

Overall, 13 patients fulfilled the inclusion criteria (initial tumor M2-PK activity ≥4 kU/L). Their initial mean tumor M2-PK activity was 13.1 kU/L. Regression analysis as described yielded a degradation rate of 18% per day (95% confidence interval, 13%–23%). After 1, 2, 3, 4, and 5 days of storage at room temperature, the relative activities were 73%, 60%, 49%, 40%, and 32%, respectively.

The Spearman correlation coefficient, used as an indicator of test-retest repeatability (8), was 0.93 (P <0.001).

The potential impact of tumor M2-PK degradation on test performance characteristics is shown in Table 1. In CRC patients, the median (interquartile range) initial activity was 8.6 (2.8–18.0) kU/L, yielding a sensitivity of 68%. Initial specificity was 73% based on initial activities calculated for ESTHER study participants: median (interquartile range), <2 (<2 to 4.7) kU/L. After 1 day of storage at room temperature, sensitivity decreased to 62%, whereas specificity increased to 77%. During the following days, the loss of sensitivity also exceeded the gain in specificity.

We conclude that the handling of stool samples affects performance characteristics of the tumor M2-PK stool test. The size of the effect may vary in individuals, as our data allowed only indirect estimation of the impact of degradation by combining the results from different study samples. Another potential limitation concerns estimates of specificity. The group of unselected older adults serving as the control group did not undergo colonoscopy, and undiagnosed CRC among controls cannot definitely be excluded. However, their proportion would be expected to be very small (<1%) and unlikely to have led to appreciable bias (9, 10). Although assessed only for the tumor M2-PK stool test, the stability issues addressed in our study may also have important practical relevance for other stool tests intended to detect CRC in the screening setting, regarding the question of whether mailing of samples is possible without loss of information.

### Table 1. Estimated impact of degradation on performance characteristics of the tumor M2-PK stool test after 1–5 days of storage at room temperature. a

<table>
<thead>
<tr>
<th>Duration of storage at room temperature, days</th>
<th>Sensitivity, %</th>
<th>Loss of sensitivity (relative to day 0), %</th>
<th>Specificity, %</th>
<th>Gain in specificity (relative to day 0), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67.8</td>
<td>72.8</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>61.5</td>
<td>6.3</td>
<td>77.0</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>56.9</td>
<td>10.9</td>
<td>80.0</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>52.3</td>
<td>15.5</td>
<td>83.4</td>
<td>10.6</td>
</tr>
<tr>
<td>4</td>
<td>47.7</td>
<td>20.1</td>
<td>86.5</td>
<td>13.7</td>
</tr>
<tr>
<td>5</td>
<td>36.9</td>
<td>30.9</td>
<td>89.4</td>
<td>16.6</td>
</tr>
</tbody>
</table>

a Cutoff value for the assay, 4 kU/L.
References


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Potentially Inappropriate Repeat Laboratory Testing in Inpatients

To the Editor:

Information technology provides data that are useful for assessing potentially unnecessary repeat laboratory testing (1, 2). Rather than using these data to directly measure the appropriateness of laboratory testing, we identified an outcome that we believe is indicative of inappropriate testing the majority of times. This useful surrogate measure of potentially inappropriate test use within and between departments and institutions can help to standardize discussion of this problem. We extended the approach of Weydert et al. (3), who calculated an index of test overuse by counting inpatients with serum sodium results within reference intervals on ≥4 consecutive days over a 1-month period and presented the monthly volume of tests ordered on these patients as a proportion of the total sodium testing workload.

At Tan Tock Seng Hospital, a 1200-bed acute-care hospital in central Singapore, we examined repeat testing on inpatients for the top 14 analytes by volume and repeat testing rates in the different clinical departments over a 1-year period. All 2002 records for the top 14 analytes by inpatient volume [potassium, sodium, creatinine, glucose, urea, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), chloride, bicarbonate, albumin, bilirubin, total protein, and γ-glutamyltransferase (GGT)] were extracted from the laboratory information system into Microsoft Access, after which individual sample records were processed with Visual Basic for Applications (VBA) Access to determine repeat samples and the time differences between sequential samples. A single piece of VBA Access code retrieved the distinct patient identifiers from the data table and then looped through the individual samples in ascending order by sample date/time for each distinct patient identifier. With each iteration, the loop-counter value increased by 1 and was written to the sequence number field. If the sequence number was 1, then the time difference was set to null; otherwise the time difference was calculated as the difference between the present sample date/time and a date variable, PreviousSampleDateTime. After this “IF” statement, PreviousSampleDateTime was replaced by the present date/time, and the loop was restarted. We used 2 different definitions of potentially inappropriate repeat testing, both with intersample time intervals of ≤24 h (for sodium, potassium, urea, creatinine, chloride, bicarbonate, and glucose) or ≤50 h (for AST, ALT, GGT, ALP, bilirubin, total protein, and albumin) chosen to approximate daily or QOD (every second day) requesting with an added 2 h to avoid missing repeats falling strictly outside a 24-h interval. Definition A, which required 4 or more sequential samples with results all within the reference interval, was similar to that used by Weydert et al. (3), whereas definition B differed by considering sequences of 3 or more and counting only the third and subsequent samples as potentially inappropriate (vs all samples on that patient; Table 1).

The tests we analyzed represented 73.7% of the total biochemistry inpatient workload. Overall, 58% of all measurements were repeat tests. The cumulative potentially inappropriate repeat rates according to definitions A and B for the top 7 tests were 2.2% and 1.9%, respectively, of the entire annual inpatient biochemistry workload and for the top 14 tests were 2.3% and 2.1%, respectively. The top 6 department A and B rates were as follows: neurointensive care, 32.1% and 25%; surgical intensive care,