samples, Dr. David Whitehouse for the Caucasian samples, and the Hong Kong Red Cross Blood Transfusion Service for the anonymous Chinese blood donor samples.

References

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Aminoglycoside Interference in the Pyrogallol Red-Molybdate Protein Assay Is Increased by the Addition of Sodium Dodecyl Sulfate to the Dye Reagent

To the Editor:
The pyrogallol red-molybdate (PRM) protein dye-binding assay (1) is commonly used for urinary protein determination (2). The addition of sodium dodecyl sulfate (SDS) to the dye reagent is recommended to improve the uniformity of response to different proteins (3). The assay is prone to interference from aminoglycoside antibiotics, particularly gentamicin, neomycin, tobramycin, paromomycin, genixin, and kanamycin (1, 2, 4, 5), but the degree of interference varies with different PRM methods. Thus, the DADE Behring PRM and the Sigma PRM assays are sensitive to aminoglycoside interference (4, 5), but the Cobas Farab and Roche Integra 700 PRM assays are not (4).

The present study indicates that the addition of SDS to the dye reagent increases the susceptibility of the PRM assay to aminoglycoside interference. Thus, the concentration of SDS in commercial PRM reagents may be a contributory factor in determining the differential responses of the assays to aminoglycosides.

We purchased amikacin (cat. no. A1774), dihydrostreptomycin (cat. no. D7253), gentamicin (cat. no. G5013), kanamycin (cat. no. G1914), neomycin (cat. no. N5285), paromomycin (cat. no. P9297), streptomycin (cat. no. S6501), and tobramycin (cat. no. T1783) from Sigma-Aldrich Co. Ltd. Aqueous aminoglycoside solutions (10 g/L) were prepared gravimetrically. Gentamicin was further diluted to 5 g/L and neomycin to 2 g/L. Pierce bovine serum albumin (BSA; cat. no. 23208) and bovine γ-globulin (BGG; cat. no. 23213) Prediluted Protein Assay Standards (0.5–2.0 g/L) were purchased from Perbio Science UK Ltd. Commercial urine control (cat. no. AU2353) was purchased from Randox Laboratories Ltd. and reconstituted in either the absence or presence of aminoglycoside (final concentration, 0.2 g/L).

The PRM reagent was prepared as described by Watanabe et al. (2) and modified by the addition of 25 mg/L SDS as recommended by Orsonneau et al. (3). For further investigations, the SDS concentration was increased to 50 or 100 mg/L.

For protein assays, 5–20 μL of sample (protein calibrator, aminoglycoside, or urine control with or without aminoglycoside), adjusted to 20 μL with water, was mixed with 1 mL of dye reagent. After 30 min, the absorbance (600 nm) was measured on a Jenway 6100 spectrophotometer zeroed against a water/reagent blank.

The PRM assay using dye reagent without SDS [as recommended by Watanabe et al. (2)] showed strong interference from neomycin, gentamicin, tobramycin, and paromomycin but relatively little interference from kanamycin, genixin, strepto-

### Table 1. Effect of SDS on aminoglycoside interference in the PRM assay as indicated by absorbance at 600 nm.

<table>
<thead>
<tr>
<th>Aminoglycoside</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin (2 g/L)</td>
<td>0.305</td>
<td>0.350</td>
<td>0.540</td>
<td>0.640</td>
</tr>
<tr>
<td>Gentamicin (5 g/L)</td>
<td>0.318</td>
<td>0.367</td>
<td>0.580</td>
<td>0.837</td>
</tr>
<tr>
<td>Tobramycin (10 g/L)</td>
<td>0.292</td>
<td>0.342</td>
<td>0.575</td>
<td>0.890</td>
</tr>
<tr>
<td>Paromomycin (10 g/L)</td>
<td>0.140</td>
<td>0.172</td>
<td>0.275</td>
<td>0.523</td>
</tr>
<tr>
<td>Kanamycin (10 g/L)</td>
<td>0.060</td>
<td>0.062</td>
<td>0.068</td>
<td>0.295</td>
</tr>
<tr>
<td>Geneticin (10 g/L)</td>
<td>0.033</td>
<td>0.045</td>
<td>0.051</td>
<td>0.286</td>
</tr>
<tr>
<td>Streptomycin (10 g/L)</td>
<td>0.030</td>
<td>0.031</td>
<td>0.032</td>
<td>0.030</td>
</tr>
<tr>
<td>Amikacin (10 g/L)</td>
<td>0.020</td>
<td>0.023</td>
<td>0.021</td>
<td>0.023</td>
</tr>
<tr>
<td>Dihydrostreptomycin (10 g/L)</td>
<td>0.020</td>
<td>0.024</td>
<td>0.023</td>
<td>0.024</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein calibrator</th>
<th>SDS, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (2 g/L)</td>
<td>0.712</td>
</tr>
<tr>
<td>BGG (2 g/L)</td>
<td>0.393</td>
</tr>
</tbody>
</table>

* The values correspond to the mean absorbance (A600 nm) obtained in the assay for 20 μL of sample (at the concentration indicated) with 1 mL of dye reagent (n = 4; CV < 8%).
mycin, amikacin, and dihydrostreptomycin (Table 1). The addition of SDS to the dye reagent [as recommended by Orsonneau et al. (3)] produced progressively increasing interference such that the color yields from kanamycin and gentamicin at 100 mg/L SDS were almost equivalent to those of neomycin, gentamicin, and tobramycin in the absence of SDS (Table 1). The effect of the SDS was compounded by a decrease in the color yields of the BSA and BGG when used as an additive in the Coomassie Brilliant Blue protein dye-binding assay (6). We confirmed the effect of SDS on the PRM assay by use of urine control containing 0.2 g/L amikacin, gentamicin, kanamycin, neomycin, streptomycin, or tobramycin. These aminoglycosides are excreted unmodified in patients’ urine at concentrations ≥0.2 g/L (4, 7, 8). When we used PRM reagent without SDS (2), the mean (SD) protein value of the urine control [0.189 (0.006) g/L] increased 149% with neomycin, 65% with gentamicin, 40% with tobramycin, 11% with kanamycin, 8% with amikacin, and 6% with streptomycin (n = 5; CV <5.0%). When we used PRM reagent plus 25 mg/L SDS (3), the respective values increased to 367%, 153%, 94%, 17%, 9%, and 7% (n = 5; CV <3.0%).

In conclusion, the protein concentration values of urine containing aminoglycosides will vary with the PRM assay depending on the concentration of SDS in the dye reagent. Consequently, caution is required when the PRM assay is used to monitor urinary protein in acute care situations. Suppliers of commercial PRM reagents usually note the presence of a “surfactant” without revealing its identity or concentration. A clearer declaration of the composition of such reagents is warranted.

References

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Quantification and Integrity Analysis of DNA in the Stool of Colorectal Cancer Patients May Represent a Complex Alternative to Fecal Occult Blood Testing

To the Editor:
I read with interest the reports by Klaassen et al. (1) and Boynton et al. (2) published in the July issue of Clinical Chemistry. The first study demonstrated increased amounts of human DNA in the feces of patients with colorectal tumors compared with healthy persons (1), and the second study showed that the majority of DNA isolated from the stools of patients with colorectal tumors was of high molecular weight, in contrast to the fragmented apoptotic DNA found in stools from colonscopy-negative patients (2). The authors hypothesized that this intact DNA originated from tumor cells because physiologically shed healthy mucosa cells are apoptotic, leading to nucleosomal DNA fragmentation.

The authors of both studies, however, did not discuss a frequently occurring phenomenon in patients with colorectal cancer, i.e., bleeding from the tumor into the lumen of the gut. This bleeding is the basis of the fecal occult blood test, a very simple and inexpensive method used as an indirect (but not very specific) tumor marker. During bleeding, leukocytes with high-molecular-weight DNA are also introduced into the feces. This DNA may still be intact in stool, especially when the site of the bleeding is in the distal part of the colon. The sensitivities and specificities reported in both studies closely resembled those of the immunochemical fecal occult blood tests (50% and 95%, respectively) that were also performed in patients with adenomas >1 cm (3). Increased concentrations of stool DNA were detected in patients with tumors in the distal part of the colon, but not in patients with tumors in the proximal part (1). This supports the suggestion that the increased amounts of (intact) DNA in stool originate mainly from blood and not from nuclease-resistant tumor cells. Even in the one patient with a tumor in the ileum (2), the presence of other sites of bleeding as the origin of intact fecal DNA (e.g., hemorrhoids) cannot be excluded.

In my opinion, both studies present a complex and expensive method to demonstrate bleeding in colorectal cancer patients. Any bleeding in the distal part of the colon obscures the specificity of the presented methods, and the only specific method to detect the presence of malignant cells (i.e., DNA) in stool is the demonstration of tumor-derived DNA alterations, which was unfortunately not performed in these studies.

References