Urine Total Protein Measurement with the Vitros Dry Reagent Technology: Modification of Diluent to Resolve Positive Bias of Diluted Samples, Patrick Lionel Mark Lynch,1* John Savory,1,2 and Doris Mather Haverstick1
(Depts. of 1Pathol. and 2Biochem., Univ. of Virginia, Charlottesville, VA 22908; *author for correspondence: fax 804-924-2574, e-mail pll8n@avery.med.virginia.edu)

The measurement of urinary total protein continues to have a place in the repertoire of clinical laboratory tests, particularly in the clinical assessment of patients with renal disease. Although the test volume is often modest, there exists the need to perform the assay on the major automated chemistry analyzers currently in use in most hospital laboratories. We have evaluated the performance of a new urine total protein procedure developed for dry reagent slide technology for use on the Vitros 950 analyzer (Johnson & Johnson). In this method, protein in the sample binds to a pyrocatechol violet–molybdate complex, resulting in an absorption shift. The method is calibrated with aqueous solutions of bovine serum albumin supplied by Johnson & Johnson. We compared the new method with that in current use on the Cobas Fara analyzer (Hoffmann–La Roche), which involves pyrogallol red dye binding of urine total protein [1] calibrated with human serum albumin (Biotrol). For this comparison we analyzed 53 patient samples (median 0.65 g/L, range 0.02–5.44 g/L, pyrogallol red results) and obtained the following Deming linear regression analysis:

\[ r = 0.807, \text{ slope } = 3.01, \text{ intercept } = 0.72 \text{ g/L} \]

These results showed a strong positive bias with the Vitros method with respect to the pyrogallol red method. The differences between the procedures appeared greatest in samples that required dilution, i.e., those >2.00 g/L, the linear range of both methods. Dilutions, when required, were carried out with deionized water in accordance with both manufacturers’ instructions. Deming linear regression analysis of the samples that did not require dilution (n = 36, median 0.23 g/L, range 0.02–1.51 g/L, pyrogallol red results) gave the following:

\[ r = 0.980, \text{ slope } = 1.16, \text{ intercept } = 0.06 \text{ g/L}, S_{\text{y|x}} = 0.06 \text{ g/L}. \]

Thus the discrepancies between the two methods were markedly increased when dilutions were involved.

At this point, three specimens were analyzed with Vitros methodology with various dilutions in deionized water; the results are given in Table 1. Clearly, urine total protein measurements with Vitros methodology are dilution dependent, showing an increasing positive bias the greater the dilution. Similar results were obtained with human serum albumin/deionized water solutions (data not shown). We hypothesize that the denaturing conditions of a deionized water dilution enhances the reaction of the proteins.

Because urine contains a relatively high concentration of potassium, the three specimens were then diluted with 150 and 75 mmol/L KCl solutions and analyzed as before. These results are also shown in Table 1. The KCl concentrations were chosen to reflect the normal range of potassium excretion in urine (25–125 mmol per day [2]). They show that the use of a KCl solution effectively reduces the large positive bias observed when deionized water alone is used as the diluent. The same response to KCl was observed when human serum albumin solutions were analyzed (data not shown).

To validate further the modified dilution protocol we compared the pyrogallol red and Vitros methods to a manual biuret reference procedure. This method provides essentially equal sensitivity for different proteins and is claimed to be the most accurate procedure for urine total

### Table 1. Urine total protein results (g/L) with Vitros methodology with deionized water and 75 and 150 mmol/L KCl solutions as diluents.

<table>
<thead>
<tr>
<th>Urine</th>
<th>Deionized water dilution</th>
<th>75 mmol/L KCl dilution</th>
<th>150 mmol/L KCl dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 in 3</td>
<td>1 in 5</td>
<td>1 in 10</td>
</tr>
<tr>
<td>1</td>
<td>4.89</td>
<td>4.95</td>
<td>6.10</td>
</tr>
<tr>
<td>2</td>
<td>4.71</td>
<td>5.20</td>
<td>6.20</td>
</tr>
<tr>
<td>3</td>
<td>6.06</td>
<td>6.65</td>
<td>7.90</td>
</tr>
</tbody>
</table>

Values are corrected for dilution.

### Table 2. Deming linear regression analysis data for pyrogallol red and Vitros methods with different diluents compared with the reference biuret procedure.

| x     | y         | r      | Slope  | Intercept, g/L | S_{\text{y|x}}, g/L |
|-------|-----------|--------|--------|----------------|---------------------|
| Biuret| Pyrogallol red | 0.991  | 0.85   | 0.07           | 0.18                |
| Biuret| Vitros: water    | 0.994  | 1.27   | −0.17          | 0.17                |
| Biuret| Vitros: KCl, 75 mmol/L | 0.991  | 1.00   | 0.02           | 0.19                |
| Biuret| Vitros: KCl, 150 mmol/L | 0.986  | 0.91   | 0.05           | 0.24                |
important role in the metabolism of ingested ethanol. ADH2, and ADH3 genes, respectively, and play an im-
zymes, the subunits of which are encoded by the ADH1, ADH2, and ADH3 genes are closely related, and share
94% homology in their coding nucleotide sequences [7]. Researchers have reported methods for the detection of
ADH2 and ADH22 genotypes with genomic DNA with allele-specific oligonucleotides (ASO) [8], the dot-
blot hybridization of PCR product with ASO [9], PCR coupled with restriction fragment length polymorphism
(RFLP) [9, 10], and direct sequencing of the PCR product [6]. The present communication reports a new rapid
inexpensive and reliable method for genotype determination of ADH21 and ADH22 involving nail clippings.

Detection of ADH21 and ADH22 Alleles in Fingernails from Japanese, Atsushi Ninshiyori,1,2* Katsushihiro Fukuda,1
Itsuro Ogimoto,1 and Hirohisa Kato2 (Dept. 1Public Health and 2Pediatrics, Kurume Univ. School of Med., 67
Asahi-Machi, Kurume, 830, Japan; *author for correspondence: fax 81-942-31-7698)

Alcohol dehydrogenase (ADH; EC 1.1.1.1.) is a zinc me-
talloenzyme with at least five classes. Class I enzymes (α, β, and γ) form homodimeric and heterodimeric isoen-
yzmes, the subunits of which are encoded by the ADH1, ADH2, and ADH3 genes, respectively, and play an im-
portant role in the metabolism of ingested ethanol. Among the α, β, and γ enzymes, the largest kinetic differ-
ces occur in the three polymorphic variants at ADH2. A G-to-A transition that resulted in conversion of arginine 47 (β1, encoded by ADH21) to histidine (β2, encoded by ADH22) in exon 3, and a substitution of an arginine 369 to cystine (β3, encoded by ADH23) of the
ADH2 gene have been observed. We have focused on
ADH21 and ADH22, as β3 has been identified only in Africans [1]. The atypical enzymes involving the β2
subunit(s) have a high activity as indicated by Vmax values [2,3], but the significance of ADH2 genotypes in patients
with alcohol-related problems is still controversial: Takeshita et al. reported that drinking habits were not
significantly associated with the ADH2 genotype [4]; Muramatsu et al. reported that the ADH22/22 homozy-
gote appeared less frequently in nonalcoholic subjects (though not statistically significantly) and that the ADH22
allele was present with significantly lower incidence among alcoholics than nonalcoholics [5]; and Tanaka et al.
reported that the ADH2 genotype played important roles in habitual alcohol-intake behavior [6]. The ADH1,
ADH2, and ADH3 genes are closely related, and share
94% homology in their coding nucleotide sequences [7]. Researchers have reported methods for the detection of
the ADH21 and ADH22 genotypes with genomic DNA
with allele-specific oligonucleotides (ASO) [8], the dot-
blot hybridization of PCR product with ASO [9], PCR coupled with restriction fragment length polymorphism
(RFLP) [9, 10], and direct sequencing of the PCR product [6]. The present communication reports a new rapid
inexpensive and reliable method for genotype determination of ADH21 and ADH22 involving nail clippings.

After informed consent was obtained from 120 healthy Japanese, nails were collected with an ordinary nail
clipper cleaned with 700 mL/L ethanolic cotton pad. Blood samples were collected from 20 of these. Between
20 and 30 mg of nail clippings, or the sediment from a
100-μL blood sample, in a 1.5-mL microtest tube was
amplified only the ADH2 allele. The PCR reaction was
conducted in a final volume of 25 μL containing 6
μL of 4 mol/L guanidine thiocyanate, 5
g/L sodium N-lauroyl salcinlate, 25 mmol/L sodium citrate, and 0.1 mol/L 2-mercaptoethanol [11], and was
then incubated for 30 min at room temperature. Nucleic
aids were extracted with phenol/chloroform [TE buffer
[10mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0)]
saturated], and then precipitated with ethanol and so-
dium chloride. After centrifugation, the pellet was dis-
solved in 30 μL of TE buffer diluted 1:10. One-fifth of the
genomic DNA from nail, or one-tenth of that from a blood
sample was subjected to 30 cycles of amplification with
the following primers respectively; primer MF3: 5’-CTG-
TAGGAATCTGTC-3’; MR3: 5’-CCTTCTCACAACACTA-3’
(Fig. 1A). These primers were designed as mismatched
3’-end nucleotides of the ADH1 or ADH3 alleles for
amplifying only the ADH2 allele. The PCR reaction was
conducted in a final volume of 25 μL containing 6 μL of
genomic DNA, 200 μmol/L dNTP mix, 1× PCR buffer
(Nippon Gene), 0.4 μmol/L of each primer, and 0.625 U of
Taq DNA polymerase, an N-terminal truncated 68-kDa
polymerase (Nippon Gene). PCR variables were; 94 °C for
the first 4 min for denaturation, followed by 30 cycles of
denaturation at 94 °C for 1 min, annealing at 52 °C for
30 s, and extension at 72 °C for 30 s. The amplified DNA
fragments were digested with Msll and underwent elec-
trophoresis in an 8% polyacrylamide gel, and were then
visualized by ethidium bromide staining. The ADH21

References
improved pyrogalarol red-molybdate method for determining total urinary
Tietz textbook of clinical chemistry, 2nd ed. Philadelphia: WB Saunders;
Ashwood ER, eds. Tietz textbook of clinical chemistry, 2nd ed. Philadelphia;
5. Savory J, Pu PH, Sunderman FW Jr. A biuret method for the determination of
6. Rice EW. Improved biuret procedure for routine determination of urinary total