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LETTER TO THE EDITOR

Urine test strips vs. pyrogallol red-molybdate assays for proteinuria: a critical approach

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To the Editor

With interest we read the paper on the quantitative measurement of urinary protein [1] in which the pyrogallol red-molybdate (PRM) assay was considered as a reference standard for assaying proteinuria in a screening setting. The PRM protein dye-binding exploits a shift in the absorbance maximum of the dye, when it binds protein [2]. However, this type of assay (for which no standard recipe exists) is prone to numerous positive (e.g., aminoglycosides, antipsychotic drugs, ampholytes, detergents, phenothiazines, reducing agents) and negative interferences, e.g., sodium dodecyl sulfate (SDS), citric acid, dextran sulfate, EDTA, oxalic acid and tartaric acid. The level of interference varies in the presence of different proteins (albumin, gamma globulin, alpha-1-acid glycoprotein, or lysozyme) and increases when SDS is added to the dye reagents. As recipes of commercial PRM reagents differ, there is a variable response to these analytical interferences [3]. As the population investigated by Naruse [1] was administered a large variety of drugs, it is not surprising that, in particular at low protein levels, the relative error caused by interfering substances is important resulting in a false result for the PRM assay.

On the other hand, the dipstick kits which Naruse et al. [1] used to quantify proteinuria are more sensitive to albumin rather than to protein, because the strip tests are based on the principle of the protein error of tetrabromophenol blue, a pH indicator.

The used dye is the same as the one, which has been in use for about 6 decades in the Albustix® strip-based method [4], which is known to be very robust. Over the last 60 years, there are few reports dealing with analytical interferences in test strip analysis: false positive results have been reported in presence of disinfectant (quaternary ammonium compound or chlorhexidine) [4].

When comparing PRM to albuminuria test strips, one must take into account the huge difference in analytical specificity towards albumin. While test strips show a high selectivity towards albumin, the pyrogallol red is not selective at all. Tamm–Horsfall protein (also known as uromodulin, a glycoprotein expressed exclusively by renal tubular cells lining the thick ascending limb of the loop of Henle) shows a marked affinity towards pyrogallol red [5]. The presence of Tamm–Horsfall protein creates a variable "noise level" in assaying proteinuria since it is not a biomarker for glomerular quality. Table 1 summarizes the most important analytical issues for PRM and related methods for assessing proteinuria in a clinical setting.

Considering these features, it is obvious to reveal discordant results between test strips and PRM method-based proteinuria especially when test strip results are negative. In addition, the low specificity of PRM assay for measuring proteinuria precludes the conclusion [1] that all subjects with a urine protein classification of (±) or more should undergo a UPCR-based measurement.

As PRM recipes currently on the market show a broad variation, it is not possible to expand conclusions on a particular PRM assay to a wider universe.

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Table 1 Overview of PRM and related methods

Method	Issues (analytical and other)	Standardisation
Pyrogallol red-molybdate	Variable affinity of various urinary proteins	No
	Drug interferences (positive and negative)	
	Tamm–Horsfall protein background	
Quantitative albuminuria	"Immuno-unreactive albumin", questionable price is higher	NIST SRM 3666
Urine test strip	False positive in presence of disinfectants often expressed as ordinal scale	NIST SRM 3666

Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

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