Microtiter-Latex Test for C-Reactive Protein, for Screening Large Numbers of Samples, *Julio M. Coll*

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The slide-latex test for C-reactive protein (CRP) takes a few minutes per sample, but the critical timing involved makes it unsuited for use with large numbers of samples. In addition, almost 40% of CRP-positive samples give negative results by either nephelometry or CRP enzyme-linked immunoassay (1). By using microwells instead of slides, increasing the time of the reaction from 3 to 60 min, and increasing the serum dilution without introducing new steps, we have facilitated the processing of samples and increased the test's specificity.

A local hospital classified serum samples as CRP-positive or -negative by doing an anti-CRP slide-latex test (Lab. Diag., Morganville, NJ).

Anti-CRP antiserum from rabbits was obtained from Llorente, Madrid, Spain. Polyvinyl wells (MRC-96) were from Limbro, Handen, CT. Wash about 600 μL of 0.8-μL particles of latex ("Estapor K109", 10 g/L latex suspension; Rhone-Poulenc, Coubevoie, France) with 10 mL of glycine buffer (50 mmol/L, pH 8.6) containing 30 mmol of NaCl per liter. Resuspend in 20 mL of the same buffer and add 150 μL of CRP antiserum. Incubate at 37 °C for 3 h, with agitation, then wash the latex suspension in the same buffer and resuspend it in 5 mL of dilution buffer (per liter: 200 mmol of borate, 75 mmol of NaCl, 2 mmol of $CaCl_2$, 10 g of bovine serum albumin, 0.1 g of thimerosal, and 0.5 mL of Tween 20, pH 8) and store at 0 °C. Before use, dilute this suspension fourfold in dilution buffer. Pipette 5 μL of serum into each well, add 50 μL of anti-CRP-bound latex, and gently agitate all samples by moving the plates horizontally. Let the samples stand at room temperature for 60 min. Homogeneously dispersed latex corresponds to negative CRP samples; coarse pellets appear in the positive CRP samples.

Different anti-CRP latexes must be titrated to ascertain the correct amount of sample and latex, to adjust sensitivity. We use a cutoff for positive CRP of 6 mg/L. We tested several types of wells in different shapes and materials; polyvinyl V-shaped wells over black backgrounds gave the greatest contrast, the differences between positive and negative wells being easily visible. The percentage of false positives was decreased from 38.8% in the slide-latex method (1) to 6.2% in the microtiter-latex test. The percentage of true values increased from 61.2% to 76.3% (n = 80). The increased specificity can be due to avoidance of the artefacts caused by reading-time differences from sample to sample when a large number of samples are involved. Evaporation artefacts are also avoided. Interference from rheumatoid factor and highly lipemic sera is minimized by diluting the sample 10-fold (done simultaneously with the addition of latex) compared to the 1:1 dilution factor in the slide-latex test. Prozone phenomena were also decreased because of this higher latex/serum ratio.

By using automatic pipettes we could dispense 100 samples and latex suspensions in 11.4 s per sample. This time could be shortened if multichannel pipettes are used. Working time per sample is decreased mainly because no stick mixing of sample and latex is required. To check for prozone effect, one can re-assay the negative samples after 1 h by adding another 50 μL of latex per well to the negative samples, agitating the wells, and waiting another hour before reading the results.

This method is useful for screening large number of samples requiring only qualitative (yes/no) answers.

Reference
1. Martinez J, Coll J. Clinical preliminary studies on C-reactive protein quantification by ELISA. In preparation.

A Modified Method for Quantifying Urinary Vanillylmandelic Acid, *Penelope J. Parks and Stephen N. Thibodeau*

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A commonly used method for measuring VMA is the "VMA-Skreen" colorimetric procedure (Biochemical Diagnostics, Inc., Farmingdale, NY). In this method, VMA is separated from other urinary metabolites by several organic extractions and reacted with diazotized p-nitroaniline to form a colored product. Although the VMA standards used range from 5 to 25 mg/L, the patient's results are usually expressed with reference to the urinary creatine concentration (μg of VMA per mg of creatinine).

With our particular patient population, we noticed that the urinary concentrations of VMA (mg/L) in >50% of our patients' specimens (Figure 1) were less than our lowest standard, an area where the absorbance change is marginal ($\Delta A_{570} \simeq 0.04$ at 5 mg/L). More importantly, results for 26% of this group were abnormally increased when normalized to the creatinine concentration. Because a substantial number of our abnormal patients' results were being generated from values obtained within the least sensitive and least precise portion of our standard curve (<5 mg/L), we decided to explore various assay modifications that might help alleviate the problem.

First, the sample volume was increased from 3.0 to 6.0 mL, and secondly, an additional standard (2.5 mg/L) was incorporated into the assay. All of the remaining variables remained unaltered. Although the extraction efficiency decreased slightly, we were still able to nearly double the adsorbance readings for each of the standards. The only adverse effect of this change was a decrease in the upper limit of detection, which changed from 25 to 20 mg/L. The interassay variability (CV) for the old and the modified assay was 6.3% ($\bar{x}$ = 17.5 mg/L, n = 41) and 7.9% ($\bar{x}$ = 14.5 mg/L, n = 37), respectively. Additionally, a comparison of patients' results (n = 19) determined with the old assay (x) and with the modified assay (y) showed good correlation ($y = 0.89x + 0.08$, $r = 0.994$).
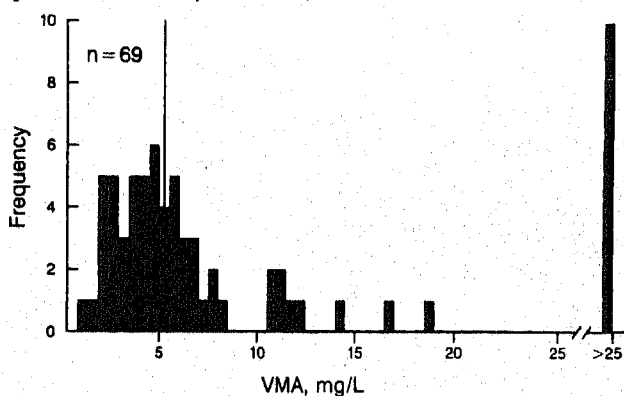

Fig. 1. Distribution of patients' concentrations of urinary VMA over a six-month period before modifying the VMA procedure

A *vertical line* at 5 mg/L indicates the lowest-concentration standard used in the unmodified method