Failure of Processed Quality-Control Materials to Detect Interference from Labile Form of Hemoglobin A\textsubscript{1c}

To the Editor:

Our laboratory uses the Bio-Rad Variant\textsuperscript{TM} system to measure hemoglobin A\textsubscript{1c}. The procedure begins with an autodiluter aspirating 5 µL of blood and dispensing it with 1 mL of hemolyzing reagent. The hemolyzing reagent also contains chemicals to remove the Schiff base-labile form (pre-A\textsubscript{1c}).

A technologist incorrectly connected wash solution to the autodiluter. Bio-Rad’s hemolysis reagent, wash solution, and buffer solutions all come in rectangular clear plastic bottles of identical shape and size. The bottles are labeled on only one side, and that side of the hemolysis reagent bottle lies next to the autodiluter (to minimize the distance the plastic tubing runs between the front of the autodiluter and the bottle cap, allowing the tubing to reach the bottom of the bottle). Therefore, no other technologists saw that the wrong solution was being used as hemolyzing reagent for 3 weeks. Calibration and quality-control results were acceptable on every run because those stabilized preparations contain no labile form.

After the error was discovered, we retested the 618 available specimens (specimens older than 14 days had been discarded) whose incorrect results (A\textsubscript{1c} plus pre-A\textsubscript{1c}) exceeded our A\textsubscript{1c} reference range. In the statistics that follow, results from specimens older than Bio-Rad’s recommended storage time of 7 days were excluded. The mean difference between the original results (range, 6.6–17.0%) and the corrected results (after exclusion of three outlier results >4.5 SD from mean) was 1.0% (n = 292; range, 0.2–2.1%; median, 0.9%; SD = 0.36%; near-gaussian distribution with a skewness of 0.81 and kurtosis of 0.24). These results contradict Bio-Rad’s claim that “Schiff base seldom exceeds 1.0% of total hemoglobin in nondiabetic and diabetic individuals” (1). The difference (presumed labile form) correlated linearly with the A\textsubscript{1c} percentage (presumed labile form = 0.116[A\textsubscript{1c}] + 0.049; \(r^2 = 0.327; \text{ANOVA } F\text{-value} = 25.7, P < 0.0001; \text{power } 1.000)\).

To assess whether part of the difference between the original incorrect results and the repeated results with the appropriate hemolyzing reagent was attributable to loss of A\textsubscript{1c} with storage, I examined the relationship between the paired differences and the intervals between specimen receipt and the repeat assay. Blood tubes had been stored at 2–8 °C for 1–14 days. There was no significant correlation between storage times and A\textsubscript{1c} differences (n = 616, 3 outliers excluded; linear regression, \(r^2 = 0.03; \text{ANOVA } F\text{-value} = 0.80; P = 0.37; \text{power } 0.139\)). This implies that the differences between the original incorrect results and the repeated A\textsubscript{1c} results were attributable to the removal of hemoglobin labile form and not to degradation of A\textsubscript{1c} over time. Differences in the pH and buffer composition of the hemolysate caused by use of wash solution instead of hemolyzing reagent could have affected the A1c results in ways unrelated to labile form. However, chromatography was not discernibly affected by the inadvertent use of wash solution: Retention times, peak widths, and baselines were unchanged.

Glycohemoglobin differences of 0.5% or more can be clinically significant. Only 6 of the 618 retested specimens had differences <0.5%. We delivered 612 corrected A\textsubscript{1c} reports. We offered free specimen recollection and A1c testing to patients with increased A\textsubscript{1c} results whose specimens had been discarded before discovering the error.

To avoid this problem in the future, we added a patient control at the beginning of each hemoglobin A\textsubscript{1c} run. The patient control is a repeat analysis of a specimen from a previous run. We require the repeat result to be within 0.4% of the original. We asked Bio-Rad to change its A\textsubscript{1c} instruction manual describing the inability of stabilized control materials to assess labile form removal and recommending the additional use of a fresh or frozen patient control to assure that the hemolysis reagent removes the labile form.

References


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Sample Requirements for Plasma
Renin Activity and Immunoreactive Renin

To the Editor:

Renin, an aspartic protease, is rate limiting in the generation of angiotensin (Ang) II, which is a key regulator in the maintenance of salt and water balance by regulating aldosterone secretion and blood pressure (1). Renin is synthesized and secreted into the blood exclusively by the juxtaglomerular cells of the kidney from prorenin, an inactive precursor, which is produced not only by the kidney, but by other tissues, including the adrenal glands, gonads, and the uteroplacental unit (2). Whereas the secretion of renin is tightly controlled, prorenin secretion is not and is ~10-fold higher than renin (3).

Although peripheral conversion of prorenin to renin is limited and does not typically occur in vivo, in vitro conversion of prorenin to renin can occur by cryoactivation, so sample handling is critical (4). Historically, samples for renin activity have been collected on ice, frozen, and assayed at 2–8 °C. Cryoactivation occurs when samples are exposed to refrigerated temperatures (4 °C) for ex-