Diffusional Interference with Determination of Salicylate by the Trinder, Abbott TDX, and Du Pont aca Methods

To the Editor:

Diffusional (2',4'-difluoro-4-hydroxy-3-hydroxy-carboxylic acid; Dolobid®, Merck Sharp & Dohme, West Point, PA 19486) is a nonsteroidal, anti-inflammatory, non-narcotic drug with antipyretic properties. Structurally, it is a difluorophenyl derivative of salicylic acid:

\[
\text{Diflunisal} \quad \text{COOH} \quad \text{OH} \\
\text{F} \\
\text{COOH} \\
\text{OH} \\
\text{Salicylic acid}
\]

The strong similarity between this compound and salicylate suggested possible interference by difflunisal in salicylate assays. We prepared three serum pools to contain 100, 200, and 400 mg of diffusional per liter. Salicylate concentrations were determined in each pool by the manual Trinder (1), Abbott TDX (2), and Du Pont aca methods (3).

The interference by diffusional with determination of salicylate is greatest with the TDX method, and to a lesser extent with the Trinder and aca methods. The results for the 100, 200, and 400 mg/L pools were 311, 492, and >800 mg/L; 61, 103, and 195 mg/L; and 78, 122, and 224 mg/L by these methods, respectively.

Because the TDX involves measurement of fluorescence polarization, it was necessary to determine whether diffusional's native fluorescence could be responsible for the elevated values. To assess this possibility, we assayed the three serum pools for another drug, phenobarbital, using the TDX instrument (4). No reaction was noted, thus eliminating the possibility of drug fluorescence as the source of the interference.

Salicylate has a plasma half-life of 2.5 h, diffusional 8 to 12 h (5). Thus, diffusional interference could cause problems for physicians if salicylate concentrations determined by these methods for therapeutic monitoring are used.

We conclude that serum specimens submitted for salicylate determination may result in falsely increased results if diffusional is also present in the patient's serum. Significant problems may also arise in emergency toxicology screening if diffusional is erroneously identified as salicylate.

We acknowledge the assistance of Judith Gull in providing data from the Du Pont aca.

References
2. Abbott Laboratories, Diagnostic Division, Irving, TX 75061, TDX Salicylate Assay Supplement no. 6107c.
3. Du Pont Co., Clinical Instrument Systems Division, Wilmington, DE 19886; SAL, Salicylate Test Methodology.
5. Dolobid® (Diflunisal, MSD) Package Insert, Merck Sharp & Dohme, West Point, PA 19486.

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Comment on NCCLS Guidelines for Skin Puncture

To the Editor:

The National Committee for Clinical Laboratory Standards (NCCLS) should be commended for the approved guidelines H14A and H4A that define devices helpful to skin puncture as well as to blood collection (1, 2). A consensus has developed, however, that perpetuates misconceptions about skin puncture, the test tubes used, and the preference of serum to plasma:

- The approved use of lancets for skin puncture no deeper than 2.4 mm is praiseworthy, because it provides a “ballpark” size to guide manufacturers. But this size is much too long. The number—2.4 mm—was the distance observed by Blumenfeld and coworkers between the skin surface and heelbone periosteum of one premature infant at autopsy (3, 4). This became the basis for stipulating the limit of the lancet’s puncturing tip. Recommendations were also made on the site of heel puncture, so that areas with greater distances between skin and heelbone would be used.

The difficulty in promoting 2.4 mm is that there is marked compression of the skin during skin puncture, so that the real distance between skin and bone must be much less. Try pressing your thumbnail against your forefinger to make this fact self-evident. Other unmeasured factors that could affect the distance include the fluidity of the skin, and the patient’s nutritional and hydration status, but we know, in any case, will be that the depth of puncture should be less than 2.4 mm, and probably much less, so long as the capillary blood vessels are reached.

- Unproved virtues must not be promoted for test tubes used to receive blood from skin puncture. Figure 6 of the NCCLS H14A reference fails to show the ordinary lithium-heparinized polyethylene microtube (with cap) that has been successfully used for many years (5). Once the first drop establishes the channel for blood-flow, the collection is rapid. In our experience this has been much preferred over other test-tube devices designed to promote flow, such as the addition of a capillary or a “scoop” device in the cap, that add unnecessary cost and inconvenience.

For “service” clinical chemistry, plasma is superior to serum, if the dry anticoagulant used does not harm the test desired. Lithium heparin has been most useful. Clotting causes several undesirable changes that have been documented but need further verification. These include hemolysis, shift in K⁺ and Cl⁻, loss of glucose via glycolysis, phosphate change, and others. Samples centrifuged after coagulation is completed may display peaky “clots” of fibrin in the serum layer.

Finally, I should note our observations that plasma obtained from the skin-puncture blood of infants older than two weeks displays hemolysis only as a result of poor blood-collecting technique; and that the usual “squeezing” at the puncture site does not liberate so-called tissue juices, provided there is no edema present (6).

We must encourage the continued healthy progress of the NCCLS, but always bear in mind that timeworn definition about consensus views: a camel is really a horse designed by a committee.

References
2. H4 A; Approved standard procedures for the collection of diagnostic blood specimens by skin puncture. NCCLS 2, 132–149 (1982).