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An *o*-Toluidine Method for Body-Fluid Glucose Determination

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Featured Article: Dubowski KM. An *o*-toluidine method for body fluid glucose determination. Clin Chem 1962; 8:215–35.[2]

This report described a simple, rapid, and versatile method for manual measurement of glucose in whole blood, serum, cerebrospinal fluid (CSF), and other body fluids, with results fully compatible with the then–widely used automated Hoffman procedure for body-fluid glucose: reduction of alkaline ferricyanide to ferrocyanide *(1)*, adapted to the AutoAnalyzer. It also yielded results essentially specific for glucose, encompassed the full physiological range while discriminating readily between closely adjacent values, and was free from interference by most relevant substances.

This work occurred in the context of our establishing a central clinical laboratory, with state-of-the-art clinical chemistry and toxicology services, in the newly opened (1958) Teaching Hospital and Clinics of the University of Florida College of Medicine. Automated clinical chemistry methods were just coming on stream, chiefly with the AutoAnalyzer, invented by Leonard Skeggs in 1957, using continuous segmented flow analysis. The new automated methods increased speed, accuracy, and precision and decreased the cost of manually performed clinical analyses. However, these automated methods also brought a new challenge: compatible manual procedures with results identical to those of the automated methods. We needed just such a manual blood, serum, and CSF glucose procedure for satellite laboratories, individual "stat" tests, and after-hours use and to eliminate the need for different result interpretation criteria depending on the analysis method.

Our solution was to optimize 6% ortho-toluidine in glacial acetic acid as the reagent for glucose in any body fluid, after deproteinization with 3.0% trichloroacetic acid. We heated the reaction mixture (1:3 protein-free filtrate + reagent) at 100 °C for 10 min, rapidly cooled it to room temperature, and measured its absorbance spectrophotometrically at 630 nm. The analysis principle was condensation of glucose with a primary aromatic amine in glacial acetic acid, forming an equilibrium mixture of a glycosylamine and the corresponding Schiff base. An analysis could be performed in 20 min or less. The new method had excellent accuracy and precision and yielded a stable end product. It was readily adaptable to micromethods and body fluid glucose measurement without deproteinization *(2)*, as well as automated analysis *(3)*, and was suitable for human clinical and animal studies. The results were identical to those obtained with the then-recognized reference methods for glucose.

Glucose measurement in blood and other body fluids has long been the most commonly performed clinical chemistry procedure. The *o*-toluidine method introduced in the 1962 *Clinical Chemistry* article was soon adopted by US Armed Forces clinical laboratories and has since undergone scores of minor modifications. Modified versions of the *o*-toluidine reagent have been patented, although not by this author *(4)*. Surveys by the College of American Pathologists documented that the *o*-toluidine method for glucose determination was the single most "popular" method for that analyte during 1969–79, by a wide margin (Gilbert RK, et al. National programs guarantee quality assurance. Lab World 1979;30:81–7.) It thus could be considered as the single most often used analysis method in all of clinical chemistry during that decade. It remained dominant for some years thereafter, until largely replaced by newer automated enzymatic procedures, but clinical studies using the original 1962 method were published as recently as 2007 *(5)*. Its frequent citation in the literature is probably partly due to the many modifications it has undergone in other hands and its widespread use in animal and clinical studies worldwide.

In 1974, the US Food and Drug Administration proposed a new system of standards for "In Vitro Diagnostic Products for Human Use," the initial one being a "Product Class Standard for Detection or Measurement of Glucose" *(6)*. The FDA-adopted reference method for glucose measurement was that reported in *Clinical Chemistry* in 1962, described as "simple and

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[2] This article has been cited more than 568 times since publication.

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straight-forward." An Internet search with the Google search engine in April 2008 produced 2780 results for "blood glucose analysis with *o*-toluidine" in 0.24 s. Time marches on.

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