CC/NUMBER 12
MARCH 23, 1981

This Week's Citation Classic

Zlatkis A, Zak B & Boyle A J. A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.* **41**:486-92, 1953. [Depts. Chem., Pathol., and Med., Wayne Univ., and City of Detroit, Receiving Hosp., Detroit, MI]

The authors present a new, sensitive, and stable color reaction for the simple determination of serum cholesterol by direct treatment of the serum with a reagent composed of ferric chloride dissolved in a glacial acetic acid-sulfuric acid mixture. [The *SCI*® indicates that this paper has been cited over 1,110 times since 1961.]

Bennie Zak
Department of Pathology
Wayne State University School of Medicine
Detroit, MI 48201
and
Albert Zlatkis
Department of Chemistry
Houston University
Houston. TX 77004

January 27, 1981

"This procedure developed out of a need for both simplicity in handling micro samples as well as sensitivity of signal achieved in a project in which many serum samples drawn from treated and control rabbits had to be evaluated for their cholesterol concentrations for the purpose of a special investigation into the formation and reversal of atherosclerotic plaques.[1] The idea of generating these plaques in a susceptible animal such as the rabbit was not a new one; however, the attempt to cause the reversal of those atherosclerotic lesions by some potentially appropriate chemical treatment with a chelating agent was a newer one. In a sense, the then somewhat unusual direct approach was made possible by the use of a specially purified glacial acetic acid which was glyoxal-free and thereby did not result in an interfering Hopkins-Cole reaction of the matrix reagent with either the tryptophane of the still intact proteins or the free tryptophane of the serums. In the ensuing years many modifications appeared, a few from ourselves, many from others. Then, with the advent of continuous flow mechanization and its adaptation to the automated handling of many serum samples, it became an often used reaction in the modified cholesterol procedures of many routine chemistry laboratories. Commercial organizations were quick to cash in on its virtues for easy salability, and modified procedures ensured profits for those enterprising enough to package them in kit forms for both manual and automated systems.[2,3] In more recent years the Bureau of Standards felt the reaction itself was important enough to study the elucidation of the mechanism.[4]

"Since the introduction of enzymes as reagents into cholesterol methodologies, a new simplicity of technology without the need for a viscous strong acid matrix of reaction has begun to replace the latter and a waning popularity is the result even though comparisons have shown a similarity in results between the new and the old methodologies. Any future for strong acid reactions now could only reside in the comparative economics of such a simple re-agent over that of the more complex sequenced enzyme reagent systems that have begun their displacement process, for the old procedures may only serve as cheaper screening devices in the future for this important and popular determination.

"It is always difficult to understand why a simple procedure is referred to enough to make it a *Citation Classic*. One could take the optimistic view that it filled an immediate need for investigators who could use a simple, sensitive, and stable color reaction as compared to a less sensitive, unstable, and more complicated reaction system of the past. The method was, in spite of its simplicity, easily amenable to alteration, so between application and modification it appeared in the literature many times. We are still studying its reaction characteristics and how they are affected by matrix modification. Hopefully, a few more references are still to appear."[5]

1. **Uhl H S, Brown H H, Zlatkis A, Zak B, Myers G B & Boyle A J.** Effect of ethylenediamine tetraacetic acid on cholesterol metabolism in rabbits. *Amer. J. Clin. Pathol.* **23**:1226-33, 1953.
2. **Wybenga D R, Pileggl V J. Dirstine P H & DiGiorgio J.** Direct manual determination of serum total cholesterol with a single stable reagent. *Clin. Chem.* **16**:980-4, 1970.
3. **Levine J B & Zak B.** Automated determination of serum total cholesterol. *Clin. Chim. Acta* **10**:381-4, 1964.
4. **Burke R W, Diamondstone B I, Velapoldi R A & Menis O.** Mechanisms of the Lieberrnann-Burchard and Zak color reactions for cholesterol. *Clin. Chem.* **20**:794-801, 1974.
5. **Zak B.** Cholesterol methodologies: a review. *Clin. Chem.* **23**:1201-14. 1977.