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This Week's Citation Classic

Kaplow L S. A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow.
Blood **10**:1023-9, 1955.
[Labs. Mary Fletcher Hosp. and Dept. Pathology, Coll. Med., Univ. Vermont and State Agricultural Coll., Burlington, VT]

A cytochemical azo-dye coupling method is described for demonstrating and semiquantitating leukocyte alkaline phosphatase activity (LAPA) in circulating neutrophils. Cells are rated from 0 to 4+ based on subjective assessment of the amount of intracellular precipitated reaction product. Ratings multiplied by designated factors are summated to provide a 'score' for a given sample. [The *SCI*® indicates that this paper has been cited in over 470 publications since 1961.]

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"In mid-1950, the University of Vermont College of Medicine had an unusual array of established and budding talent in the field of histochemistry. Alex Novikoff was professor of experimental pathology, Bjarne Pierson was chairman, and Roy Korson, now professor, was then assistant professor of pathology. Vittorio Defendi, currently chairman of pathology at New York University, was a young Fulbright fellow recently arrived from Italy. This was prior to my medical training, while I was supervisory technologist at the Laboratories of Mary Fletcher Hospital and simultaneously pursuing a master's degree in pathology.

"Blood cells intrigued me then as now. Their ready availability, representing a biopsy of the circulation, their profound clinical importance, and the aesthetic pleasure in examining a well-stained blood smear explains this not uncommon affection and my interest in leukocyte alkaline phosphatase. During that period of histochemistry, the enzyme had been extensively studied in solid tissues. Paradoxically, blood, so easily obtainable, was greatly neglected. Although, Wachstein[1] in 1946 described a heavy metal method for demonstrating the enzyme in blood cells and reported a marked decrease in LAPA in chronic myelogenous leukemia.

"Stimulated especially by Novikoff and impressed by Dameshek's statement in 1947 that 'morphologic hematology is undoubtedly in for a renaissance...in which it is hoped that appropriate staining technics will point directly to chemical and physiopathologic alterations,'[2] I chose to study alkaline phosphatase in a cat model of leukemia. Despite intensive efforts, neutrophils would not stain. Numerous reports on an azo-dye coupling method suggested that this approach might be superior to the heavy metal method. I determined the optimum fixative and modified the staining method to yield maximum staining of neutrophils on human blood smears and devised a scoring method for assessing overall activity. It worked beautifully and resulted in this *Citation Classic*. Cat cells, however, still would not stain. Only years later did I fully appreciate the bizarre distribution of this enzyme in leukocytes of different species.[3,4]

"There are cogent reasons for the sustained interest in this paper. Methods with clinical applications will always be frequently cited if they are simple, easy to perform, and reliable. The technique described has stood the test of time. The manuscript was written in the early days of hematologic cytochemistry. It sparked an interest in such techniques which has steadily increased in intensity and is presently being actively extended to immunocytochemistry and automated hematology."

1. **Wachstein M.** Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. Clin. Med.* **31**:1-17, 1946.
2. **Dameshek W.** Preface. (Dameshek W, ed.) *Morphologic hematology*. New York: Grune & Stratton, 1947. p. 1-2.
3. **Kaplow L S.** Alkaline phosphatase in peripheral blood lymphocytes. *Arch. Pathol.* **88**:69-72, 1969.
4. ----------. Leukocyte alkaline phosphatase cytochemistry: applications and methods. *Ann. NY Acad. Sci.* **155**:911-47, 1968.