

# Combined Staining Approach With Myeloperoxidase And Periodic Acid-Schiff's On Routine Bone Marrow Aspiration Smears

S Pattari, N Varma, G Garewal

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## Abstract

Myeloperoxidase (MPO) and Periodic acid schiff's (PAS) are the two basic cytochemical stains, commonly used in diagnosis and typing of acute leukemias. Combined cytochemistry with MPO & PAS can be done in same smear and may have some practical value where material obtained is very scanty.

## BACKGROUND

Myeloperoxidase (MPO) and Periodic acid schiff's (PAS) are the two basic cytochemical stains, commonly used in diagnosis and typing of acute leukemias. MPO positive blasts are quite specific for acute myeloid leukemia (AML). However PAS block positivity is seen in acute lymphoblastic leukemia (ALL), which has significance in absence of MPO positivity. Diffuse or granular PAS positivity has no significance (1).

The combined cytochemistry has been tried to demonstrate two or more cell lines on the same slide and a positive result has been documented in combined esterase stain using alpha-naphthyl acetate esterase and chloroacetate esterase (2) as well as combined staining with chloroacetate esterase and alpha naphthyl butyrate (3). However, combined stain with MPO and PAS has not been tried so far. In the present study, we have succeeded to document this combined approach.

## MATERIALS AND METHODS

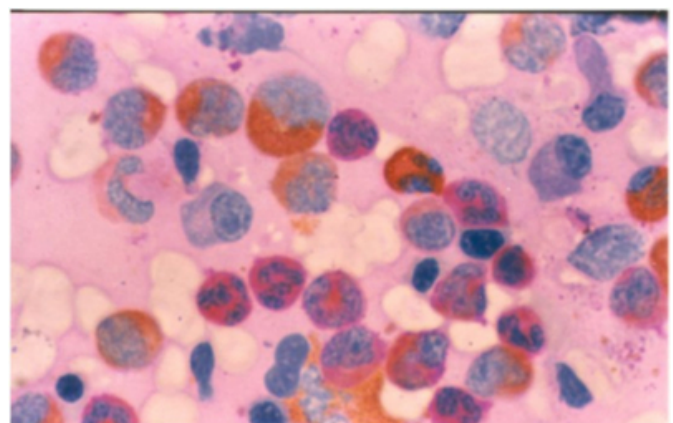
Air dried bone marrow aspiration smears were taken from 40 randomly selected diagnosed cases which includes, normal marrow aspiration (n=7), ALL (n=9), AML (n=5), multiple myeloma (n=2), non-Hodgkin's lymphoma with infiltration (n=1), megaloblastic anemia (n=2) and others (n=14). The staining was performed in multiple batches. First MPO staining was done by the standard method (4). Benzidine was poured over air-dried smears for 1 & 1/2 minutes, followed by hydrogen peroxide for four and half

minutes. Smears were washed with tap water and dried. Alternatively di-amino benzidine can be used as per standardization. After drying, smears were covered with periodic acid for 10 minutes, washed and dried. Then Schiff's reagent was poured over the smears for 20 minutes, washed with running tap water and counterstained with hematoxylin.

## RESULT

Both, MPO and PAS stains were very satisfactory in the same smears (Figure 1).

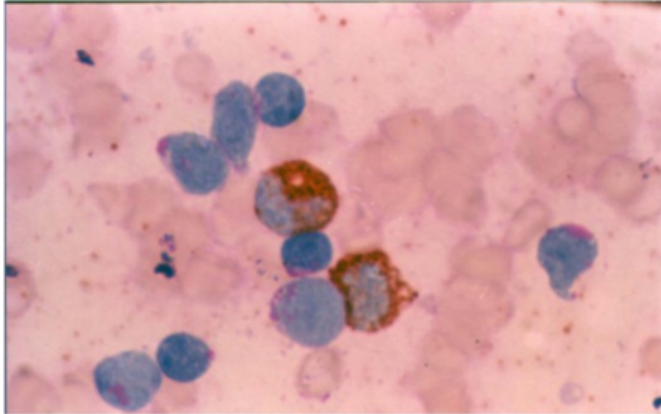
Figure 1



The MPO stain did not hamper the PAS stain. The neutrophils took bright pink color due to positivity for both MPO and PAS. However early myeloid series showed only MPO positivity. Eosinophils were only MPO positive and basophils were both negative. The monocytes were negative

for both MPO and PAS, and appeared as unstained. The block positivity of PAS in ALL was also well highlighted along with MPO positive myeloid series (Figure 2).

**Figure 2**



The staining could be successfully done on unstained smears kept up to 20 days at room temperature.

## **DISCUSSION**

The combined staining with MPO and PAS is a very easy technique and provides good result. Till date the combined method has not been documented. Both the stains can be demonstrated on the same smear. Sometimes, in acute leukemia, material obtained is less and the combined MPO-PAS stain may be helpful. The immunological typing of ALL by flowcytometry/immunocytochemistry has made PAS staining of blasts redundant in advanced countries. However in developing countries like India, cytochemical

stains including PAS are still used. Blasts with L1 or L2 morphology showing block PAS positivity will be classified as lymphoblasts. Therefore, a combined MPO-PAS staining has relevance in the developing countries. Monocytes can be distinguished from other cells by its complete negative staining which may be of help in certain cases like myelodysplastic syndrome. Sometimes eosinophil is difficult to distinguish from neutrophils in routine Romanowsky stain. The combined MPO-PAS stain can clearly distinguish them. Eosinophils shows only MPO positivity which gives it brown color, whether neutrophils shows both MPO and PAS positivity which gives it bright pink color. So combined staining with MPO and PAS may have practical value.

## **CORRESPONDENCE TO**

Dr. Neelam Verma Department of Haematology PGIMER Chandigarh – 160012 India Fax No. 91-172-744401 Email- varmaneelam@yahoo.com

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**Author Information**

**Sanjib Kumar Pattari, MD**

Senior Resident, Department of Pathology, PGIMER

**Neelam Varma, MD**

Associate Professor, Department of Hematology, PGIMER

**Gurjeevan Garewal, MD**

Professor & Head, Department of Hematology, PGIMER