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Introducing a Rapid and Safe Method for Myeloperoxidase Staining

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Abstract

Background: Myeloperoxidase staining is used to differentiate leukemias since several decades. Despite implementation of flow cytometric, cytogenetic and molecular techniques for identification of leukemic blasts, histochemical stains such as myeloperoxidase stain are persistently used for better classification of leukemias. The myeloperoxidase staining is a time consuming and hazardous procedure. The present report describes a sensitive, rapid and easy method for assessment of peroxidase activity. Materials and Methods: Bone marrow aspiration slides were stained with Dako product: Code number: K3467 containing DAB chromogen (3,3-diaminobenzidine in chromogen solution) and substrate buffer (Imidasole-HCL buffer, PH 7.5 containing hydrogen peroxide and an anti microbial agent) in a rapid procedure taking only ten minutes time. The staining needs no material preparation steps. Neutrophils in the slide are taken as positive control or another normal smear was co-stained to be used as control. All cases were followed up with flow cytometry and cytogenetic studies. Result: The reaction product of this stain is brown and granular. Promyelocytes and myelocytes are the most strongly staining cells with positive (primary) granules. Lymphoblasts are negative. The result of classification of leukemias with this technique was in concordance with flow cytometric immunophenotyping. Discussion: Many practical techniques have been described using benzidine as an indicator for myeloperoxidase staining. Benzidine is a carcinogenic material and its usage is severely restricted in laboratory. Formerly we

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prepared requisite materials for myeloperoxidase staining by hazardous ways (boiling), but we decided to apply ready to use 3,3-diaminobenzidine (DAB), which is used in final step of immunohistochemistry stains. Conclusion: Use of 3,3-diaminobenzidine (DAB) is highly recommended for myeloperoxidase staining, while the result is extraordinary and fully compatible with flow cytometry and the method is safe and rapid.

Keywords

Myeloperoxidase Staining, DAB 3,3-Diaminobenzidine, Safe and Rapid Method

1. Introduction

Histochemical stains are still valuable in identification of leukemias. Several stains are used in practice such as myeloperoxidase, Periodic Acid Schiff (PAS) and nonspecific esterases.

The main aim of performing these stains on bone marrow aspiration slides or blood smears is to differentiate myeloblasts from lymphoblasts. Myeloperoxidase stain is mainly used to identify myeloblasts while it is negative in lymphoblasts. In contrast PAS stain is positive as large blocks in lymphoblasts.

The demonstration of leukocyte myeloperoxidase has been important for hematologists from the beginning of this century [1]-[8].

Myeloperoxidase (MPO) is located in some cells like neutrophils (in primary and secondary granules), eosinophils and monocytes. MPO splits hydrogen peroxidase (H_2O_2) and in the presence of a chromogenic electron donor forms an insoluble reaction product [5].

Many practical techniques have been described using benzidine as an indicator, of which 3,3-diaminobenzidine (DAB) is the preferred chromogen [5]. This compound when oxidized, precipitates as brown or blue insoluble crystals or granules [6].

There are some problems in staining for peroxidase activity in older procedures; like enzyme inhibition, cellular distortion, taking a long time and furthermore, benzidine base is carcinogenic so its use is limited [6].

Some methods substitute benzidine dihyrocholoride as the indicator. This compound is also carcinogenic but more granular and less powdery than the base and thus less dangerous to handle but the procedure needs preparation of a mixture from different substances which is time consuming [6].

The present report describes a highly sensitive and rapid method for assessment of peroxidase activity on blood smears and bone marrow aspiration slides, using a ready to use kit and the staining is performed in only 10 minutes.

To the best of our knowledge this method is not yet introduced so we found it interesting to report.

2. Methods

Bone marrow aspiration slides were prepared and stained with DAB chromogen. This substrate-chromogen system is a highly sensitive DAB system suitable for use in peroxidase-based immonohistochemical (IHC) and in situ hybridization (ISH) staining methods [5]. Upon oxidation, DAB forms a brown end-product at the site of the target antigen or nucleic acid.

The staining procedure is discussed below in detail:

Reagents: Dako product: Code number: K3467 [7].

1. DAB chromogen (3,3-diaminobenzidine in chromogen solution);

2. Substrate buffer (Imidasole-HCL buffer, PH 7.5 containing hydrogen peroxide and an anti microbial agent).

Practical methods:

1. Use fresh smears of blood or bone marrow in EDTA or heparin;

2. Fix air-dried smears for 60 seconds at room temperature in 10 percent formal-ethanol;

3. Rinse thoroughly in gently running tap water for 30 seconds;

4. Add the mixture of reagents (1 ml of substrate buffer + 1 drop of DAB chromogen) on the slides completely covering the sample;

5. Incubate for 10 minutes;
6. Wash for 30 seconds with gently running tap water;
7. Counter stain with Hematoxylin for 1 - 5 minutes, rinse in running tap water and air dry.

3. Results

Myeloperoxidase stain and Periodic Acid Schiff (PAS) stain are used for classification of leukemias in our center. Myeloblasts often show positivity with myeloperoxidase and lymphoblasts show negative staining. In contrast, PAS staining is positive in lymphoblasts and is seen as red pink large blocks [5].

The reaction product by myeloperoxidase staining is brown and granular. Promyelocytes and myelocytes are the most strongly staining cells with positive (primary) granules. Metamyelocytes and neutrophils have fewer (secondary) granules. Eosinophil granules stain strongly with this stain. The most primitive myeloblasts are negative. Lymphocytes are negative. Monoblasts and monocytes may be negative or positive and when positive the granules are smaller than in neutrophils and are diffusely scattered throughout cytoplasm. Red cells show diffuse brown cytoplasmic staining [2] [5].

We have been staining with this method for more than a year in our center and we used neutrophils in the slide as positive control or co stain another normal smear to be used as control. All cases were followed up with flow cytometry and cytogenetic studies. The staining result was extraordinary and fully compatible with flow cytometry. So we recommend this simple method in centers who work with hematology oncology patients.

4. Discussion

Histochemical stainings such as myeloperoxidase are used in classification of acute leukemias in conjunction with flow cytometry and cytogenetic and molecular studies.

Morphologic subtyping by cytochemical stainings is backed to 1950 and 1960's [8] and although by the advent of flow cytometry their use was largely abandoned, however, during coming years, the flaws of flow cytometry has made them as important as before in diagnostic workup of leukemias.

Most staining methods for myeloperoxidase need preparation of reagents from different substances and need heating which result in production of dangerous fumes so the preparation of stain solution must be done under the hood. Besides benzidine powder is carcinogenic and its handling is difficult. The stability of the stain solution is limited and fresh stain should be prepared after a while.

Due to all these shortcomings, myeloperoxidase stain was rather abandoned in our center until we encountered a proposal by one of our technicians to use Liquid DAB product. We tried the stain and found that the procedure is very simple and rapid, so we decided to represent it to other colleagues who work for oncology patients. Besides the stain is very stable and you can use it even after expiration date of kit.

It is noteworthy that myeloperoxidase stain can also be performed on paraffin embedded sections of bone marrow biopsy but this method requires several steps for antigen retrieval and the results are affected by putting tissue in acid prior to processing. In this method, peroxidase is bond to antibody but in our method no antibody is used and peroxidase activity is endogenous. We do not need any antigen retrievals steps either.

The only shortcoming is that liquid DAB is considered a carcinogen and should be handled with care and the waste should be discarded in sink with large amount of running water.

5. Conclusion

We recommend use of 3,3-diaminobenzidine (DAB) for myeloperoxidase staining, while the results are extraordinary and fully compatible with flow cytometry.

Conflict of Interest Disclosure

We also declare that this article has not been published elsewhere. It is not being considered for publication elsewhere. It has been submitted with the full knowledge and approval of all authors. The authors had no potential conflicts of interest during study.

Authors' Contributions

Concepts, design, definition of intellectual content were done by Dr. Mahjoub and Sharifian, Dr. Jahanzad and

Vaziri have done literature search manuscript review, data analysis, clinical studies and manuscript editing, Firouzjaie Karder was responsible for literature search, manuscript preparation and experimental studies.

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