TECHNICAL NOTE

Toby L. Wolson,[1] *B.S. and Willard C. Stuver,*[1] *M.S.*

Simultaneous Electrophoretic Determination of Phosphoglucomutase Subtypes, Adenosine Deaminase, Erythrocyte Acid Phosphatase, and Adenylate Kinase Enzyme Phenotypes

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ABSTRACT: Many of the conventional agarose phosphoglucomutase (PGM) subtyping systems presently in use fail to provide a good separation between the 1+ and 2− bands as well as the 2+ band and the more anodic moving bands. Use of a 1-mm-thick gel composed of 1% ISO GEL (FMC Corp.) and phosphate-citric acid gel and tank buffers with a pH of 5.3 provided exceptionally good separation between all four of the major subtyping bands. The additional criteria for this procedure is a voltage of 21 V/cm and a run time of 4 h. Utilization of this procedure using case samples of varied ages proved the reliability of the procedure. Also examined were the effects of several reducing agents on the enzyme band patterns and the use of this system for the simultaneous determinations of the adenosine deaminase (ADA), erythrocyte acid phosphatase (EAP), and adenylate kinase (AK) enzyme phenotypes.

KEYWORDS: pathology and biology, serology, electrophoresis, genetic markers, phosphoglucomutase, adenosine deaminase, erythrocyte acid phosphatase, adenylate kinase

Phosphoglucomutase (PGM) is an enzyme which is routinely used in determining the enzyme profile of a sample. By the use of conventional electrophoresis, PGM can be separated into the three major phenotypes with the following population frequencies for whites in Miami, FL: PGM 1:60.1%, PGM 2-1:33.1%, and PGM 2:6.8% [*1*]. It has been demonstrated that each of these PGM phenotypes may be further divided into subgroups by the use of other conventional electrophoresis systems or by isoelectric focusing. PGM subtyping is valuable because it divides the three major PGM phenotypes into ten phenotypes, each of which has a lower population frequency than the three major forms.

At present several problems exist with the conventional methods for determining PGM subtypes. In tests done using a phosphate-citric acid buffer system [*2*] at pH 5.5 and tests of an acetic acid buffer system [*3*] at pH 5.3, there were problems in obtaining readable banding pat-

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[1]Criminalist II and criminalist III, respectively, Metro-Dade Police Department, Miami, FL.

terns. Tests of a phosphate-citric acid buffer system which used a 1% isoelectric focusing agarose gel [4] provided good results but were difficult to reproduce.

Another area of consideration in the analysis of blood and body fluids is the sample quantity. Limited sample size restricts the amount of information which may be determined about it. Electrophoresis systems that provide information on multiple enzyme groups are preferable to systems that provide information on only one enzyme group because large sample quantities are not necessary. Many of the conventional PGM subtyping systems are not multi-enzyme systems.

It has been demonstrated that modification of the phosphate-citric acid buffer system by lowering the pH and using isoelectric focusing agarose provides PGM subtype information that has a good band separation and reproducible results. It has also been demonstrated that the same plate can be developed to determine the adenosine deaminase (ADA), erythrocyte acid phosphatase (EAP), and adenylate kinase (AK) enzyme information.

Methods and Materials

Buffers

Tank Buffer—The tank buffer is a phosphate-citric acid buffer composed of 19.21 g of citric acid, anhydrous (Sigma C 0759) dissolved in 800 mL of distilled water. The buffer is then adjusted to pH 5.3 at 23°C by the addition of approximately 35 g of sodium phosphate, dibasic (Sigma S 0876) and brought up to a final volume of 1 L.

Gel Buffer—The gel buffer is a phosphate-citric acid buffer composed of 0.48 g of citric acid, anhydrous dissolved in 800 mL of distilled water. The buffer is then adjusted to pH 5.3 at 23°C by the addition of approximately 0.75 g of sodium phosphate, dibasic and brought up to a final volume of 1 L.

Gel and Plate Specifications

The gel is composed of 1 g of Isogel (FMC Corp.) dissolved in 100 mL of gel buffer and poured onto a glass plate which is 24 cm long and 25 cm wide. The plate should have borders that are 1 cm wide and 1 mm thick. The gel should be allowed to solidify completely before being placed on a cooling plate.

Samples and Electrophoresis

The blood samples were obtained from regular case evidence which was in the laboratory for routine analysis. The samples were placed in slots cut into the gel 7 cm from the cathode and then wetted with 2 μL of 0.01 *M* Cleland's Reagent (Sigma D 0632).

Electrophoresis is at a constant temperature of 5°C and a voltage of 21 V/cm for 4 h with the samples being run toward the anode.

Development

The reaction buffers and reaction mixtures used for the development of the EAP, ADA, and AK enzymes are the same as those specified in Culliford [5] and Wraxall [6].

The EAP is developed first in the area covering from the origin, 8.5 cm toward the anode. After the EAP overlay is in position the AK and ADA overlays may be poured. The AK overlay is positioned at the origin running 4 cm toward the cathode and the ADA overlay is positioned from the edge of the anodic wick 5 cm back toward the origin. The plate is then placed in an incubator adjusted to 37°C for 30 to 60 min.

After recording the EAP, AK, and ADA results the PGM subtype can be developed. The PGM subtype reaction buffer and reaction mixture is the procedure described by Sutton and

Burgess [7]; the position of the overlay is from the origin, 8.5 cm toward the anode. After pouring the overlay the plate should be placed in a 37°C incubator for 60 to 90 min or until the bands have developed a sufficient density.

Results and Discussion

PGM Subtypes

The pH 5.3 phosphate-citric acid buffer system provides very good resolution and separation of the PGM bands. The separation which occurs between the PGM 2+ band and the more anodic moving bands is excellent but diminishes with each usage of the tank buffer. As a result of this effect the tank buffer should be used a maximum of five times and then discarded.

When determining the PGM subtypes of samples it is recommended that the conventional PGM type be determined in advance. This aids in the reading of the subtype bands and prevents samples from being misread. This is very important in samples that are PGM 1+1+, because these samples may exhibit an additional band which occurs in the PGM 2 region. The band when present is located midway between the positions of the 2+ and 2− bands and can be identified by the use of a 4− band standard and knowledge of the sample's conventional PGM type. The nature of this band is unknown at this time. It may be a degradation product which is evidenced by the failure of the band to occur in fresh blood samples.

EAP

The resolution and separation of the EAP band pattern is extremely good. The band patterns are very sharp with a strong intensity which provides for good visualization under ultraviolet lighting. Because of the intensity of the band patterns the degradation band that occurs anodic to the primary B band is more easily visualized. This problem is minimized when 0.01*M* Cleland's Reagent is applied to the samples at the time the plate is spotted. It is essential to use minimal amounts of the Cleland's Reagent, because it migrates to the position of the AK 1 band and when used in excess will obscure this band.

ADA and AK

The resolution and intensity of the band patterns is comparable to that which occurs when analysis is done using the pH 5.5 phosphate-citric acid buffers and a starch gel [8].

Conclusions

The pH 5.3 phosphate-citric acid buffer system and use of a 1% isoelectric focusing support gel provides information on four enzyme groups simultaneously. This minimizes the quantity of sample expended during analysis, while maximizing the enzyme information obtained.

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Address requests for reprints or additional information to
Toby L. Wolson
Metro-Dade Police Department
Crime Laboratory Bureau/Serology Section
1320 NW 14th St., Rm. 602
Miami, FL 33125