

## AGAROSE GEL ELECTROPHORESIS TECHNIQUE FOR THE DETERMINATION OF AMYLASE ISOENZYMES

The broad term "amylase" refers to enzymes that catalyze the hydrolysis of starch and glycogen. Amylase is distributed throughout the human body and can be detected in numerous body fluids, such as blood serum, urine, peritoneal fluid, and saliva. Amylase levels in fluids can be determined by assays in which a colored dye is released and expressed upon amylase-catalyzed hydrolysis of a starch.

Amylase found in the body consists not of a single enzyme but rather includes several enzymes having different structures but which perform substantially the same function and are referred to as isoenzymes of amylase or isoamylases. Broadly, there are two types of isoamylases, salivary(S) and pancreatic(P); however, each of these two classes include several isoenzymes which differ in certain properties. The different isoamylases can be distinguished according to their electrophoretic mobilities.

A variety of pathologic conditions give rise to elevated amylase levels which can be detected in blood serum, urine and other body fluids. Because elevated amylase is strongly indicative of a pathological condition, an assay revealing elevated total amylase is generally followed up by more comprehensive investigation in an attempt to diagnose the cause of the elevated amylase, and substantial medical costs are incurred investigating the causes of elevated amylase levels.

Because various pathologic conditions which give rise to elevated total amylase levels do not affect the levels of all isoenzymes equally, knowledge of the respective levels of the various isoamylases contribute significantly to defining the cause of an elevated amylase level. While a number of investigators have demonstrated widely divergent amounts of the isoamylases in patients with various conditions, the results of such investigations have not been generally applied in clinical testing. Previously described electrophoretic techniques have not been adapted for clinical testing and have not sufficiently separated all significant isoamylase bands desirable for unambiguous interpretation of results. An example of a previously reported electrophoresis protocol is in P. Leclerc et al., *Clin. Chem.* 28, n.1 37-40, 1982; however, identification of certain isoamylase bands, i.e., the P<sub>1b</sub> and S<sub>5</sub> bands, is not reported.

A clinical test for isoamylase is commercially sold under the tradename Phadebas® Isoamylases Test by Pharmacia Diagnostics. This test only provides levels of salivary isoamylase and pancreatic isoamylase and is performed by assaying for total isoamylase (salivary plus pancreatic) while performing a simultaneous assay for amylase in the presence of an inhibitor that suppresses activity of the salivary isoamylases but has little inhibitory effect on the pancreatic isoamylases.

It would be desirable to have an electrophoresis protocol which would clearly separate substantially all of the isoamylases for which diagnostic significance is attributed to their presence or absence and/or to their respective levels. It would be further desirable to provide such an electrophoresis protocol which is easily and reliably performed in a clinical laboratory and which may be adapted to be supplied as a prepackaged test kit.

## SUMMARY OF THE INVENTION

The present invention provides an agarose gel electrophoresis system which is performable on relatively small test plates and which provides clear, distinct separation of the P<sub>1</sub>, P<sub>1b</sub>, P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>5</sub> bands. The support medium is agarose, and the buffer is tris-sodium barbital-barbital at a barbital anion concentration of between about 0.04 and about 0.08 M and a pH of between about 8.4 and about 9.2. The concentration of tris is between about 0.03 M and about 0.07 M and the concentration of sodium is between about 0.03 M and about 0.07 M. The electrophoresis plates are backed by a cooling block that is maintained at a temperature of between about 2° C. and about 25° C. as the gels are subjected to between about 50 volts and about 400 volts, causing differential migration of the isoamylases.

## IN THE FIGURE

The FIGURE is a photograph of an actual electrophoresis performed in accordance with the present invention, the various isoamylase bands being indicated.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the invention, a protocol is provided in which biological samples, such as blood sera, are electrophoresed on agarose gel using a tris-sodium barbital-barbital buffer at basic pH's. The electrophoresis plate is then exposed to a chemical system including a starch or similar substance that hydrolyzes in the presence of amylase, developing a dye which stains the gel in the region of the isoamylase bands. The electrophoresis separates the P<sub>1</sub>, P<sub>1b</sub>, P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>5</sub> bands. Separation of the bands is effected within a relatively short running distance, permitting short length electrophoresis plates to be used. At running voltages of between about 50 and 400, separation is complete within about one half to about six hours. The small size of the plates and the stable nature of the agarose gel support provides that the electrophoresis plates might be prepared in advance and sold as components of prepackaged clinical test kits. The relatively short running time of the electrophoresis, i.e., within the working day of a clinical technician, enhances its applicability to performance in the clinical laboratory.

A significant factor in achieving the excellent separation of isoamylase bands is the selection of a buffer system. The tris-sodium barbital-barbital buffer system is not in itself unique, and related buffer systems, including sodium barbital buffers, have been used previously to electrophoretically separate isoamylase. However, this is the first use of a tris-sodium barbital-barbital buffer system for isoamylase electrophoresis and, surprisingly and unexpectedly, it is found that a tris-sodium barbital-barbital buffer gives a substantially improved separation relative to a sodium barbital buffer, such as that reported by Leclerc et al., supra. This improved separation results in an electrophoresis pattern in which substantially all clinically significant bands, including P<sub>1b</sub> and S<sub>5</sub>, are each clearly distinguished.

Specifically, the tris-sodium barbital-barbital buffer in accordance with the present invention comprises barbital anion at a concentration of between about 0.04 and about 0.08 M. The concentration of the tris (2 amino-2-hydroxymethyl-1,3-propanediol) cation is between about 0.03 and about 0.07 M; the concentration of the