ORIGINAL RESEARCH ARTICLE

In-house Development of Protein Electrophoresis with Hippurate Buffer and Validation with Control Sample

Gopala Krishnan Karuppaiah[1], Sapna Patel[2], Piyush Tailor[3], Hina Baraiya[4]

Received on: 04 February 2025; *Accepted on:* 23 April 2025; *Published on:* 30 August 2025

Abstract

Introduction: Protein electrophoresis with the use of a buffer like Tris-EDTA is commonly used for the detection of multiple myeloma. So an in-house developed and validated electrophoresis method can be cost-effective to implement in a small laboratory.

Aim: To develop and establish in-house hybrid, apparatus for protein electrophoresis with minimum expenditure methodology for preparation of gel and required reagents and to use it in clinical utility.

Materials and methods: In-house prepared hippuric acid buffer and 1% agarose gel. With X-ray film sheet, boundaries on nonsiliconized rough glass slide. About <1 mm thin agarose gel is prepared. The sample applicator is made from a sharp blade, which gave thin band separation. The electrophoretic run is done. The slide is placed for fixation, staining, and destaining in in-house made reagents.

Result: Electrophoresis runs for 5 samples (2 control and 3 patient samples) is done. Bands were perfectly aligned with control of pure albumin as well as with multiple myeloma. The other patient's sample gave albumin, alpha-1, alpha-2, beta, and gamma bands to prove diagnosis of hypoalbuminemia and hypergammaglobulinemia and normal condition too.

Conclusion: Methodology helps to develop hybrid-type electrophoresis apparatus with completely in-house preparation of all the reagents—including buffer, stain, destain, etc.—with minimum expenditure and valid specificity to distinguish abnormal condition from physiological condition.

Keywords: Hypergammaglobulinemia, Hypoalbuminemia, Tris EDTA.

Indian Journal of Medical Biochemistry (2025): 10.5005/jp-journals-10054-0282

Introduction

Electrophoresis is a simple, inexpensive, and valid methodology for the separation of biomolecules based on charge and molecular weight. Protein electrophoresis with different types of buffer is commonly used for detection of multiple myeloma in suspected cases of pathological fracture and multiple osteoporotic lesions in X-rays. Commercially prepared and automated electrophoresis instruments and reagents are not always feasible financially for any small laboratory and for every teaching institute. So the in-house developed electrophoresis method can be cost-effective and later it can be verified with a control sample for its accuracy before using it for clinical samples.[1,2]

Aim

- To develop and establish in-house hybrid apparatus for protein electrophoresis with minimum expenditure.
- To develop and establish methodology for preparation of gel as well as required reagents.
- To validate methodology for use it in clinical utility.

Materials and Methods

Preparation of hippurate buffer of pH 8.8 (Table 1).

Preparation for 1% Agarose Gel

- Take 100 mg of agarose powder in a flask in such a way that it should not touch the side surface of the flask
- Dissolve agarose powder into 10 mL hippurate buffer
- Boil it until the agarose is completely dissolved and the solution becomes clear and homogenous.[3]

[1–4]Department of Biochemistry, Government Medical College, Bhavnagar, Gujarat, India

Corresponding Author: Gopala Krishnan Karuppaiah, Department of Biochemistry, Government Medical College, Bhavnagar, Gujarat, India, Phone: +91 7338799427, e-mail: gope2011@gmail.com

How to cite this article: Karuppaiah GK, Patel S, Tailor P, *et al*. In-house Development of Protein Electrophoresis with Hippurate Buffer and Validation with Control Sample. Indian J Med Biochem 2025;29(3):202–204.

Source of support: Nil

Conflict of interest: None

Table 1: Preparation of hippurate buffer

Reagent	*Quantity*
Sodium hippurate	10 gm
Tris base	7.5 gm
Hippuric acid	2.25 gm

Add above mentioned reagents in to 1000 mL distilled water Check pH. It should be 8.8

Preparation of Agarose Gel on Glass Slide

- X-ray film sheet was used to make boundaries on a nonsiliconized rough glass slide. It is cut into rectangular shape with a 0.5 cm width and placed around a glass slide and stuck on it with wax.
- As it is show in below (Fig. 1).

© The Author(s). 2025 Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

- Prepared 10 mL of liquid 1% agarose gel with hippurate buffer is poured over a nonsiliconized rough glass slide immediately after boiling. So, equilibrium of agarose concentration and thickness should be maintained.
- It was challenging to get thin gel. So later we covered pored agarose gel (which is on nonsiliconized glass slide) with siliconized glass slide carefully without entrapping air bubble. After 10 minutes, remove the siliconized slide.

Immediately apply the sample to prevent excess drying (Fig. 2).

Bromophenol Blue Preparation

- Dissolve 10 mg of BPB dye in 10 mL of buffer solution.

Sample Preparation and Sample Application

- Collect the random blood sample in a red-plain Vacuette. Allow the sample to clot completely and centrifuge at 3,000 rpm for 10 minutes. Take a 200 µL serum sample into an aliquot and add 20 µL of bromophenol blue dye and mix it.
- Firstly, we used a commercially provided sample comb applicator (Fig. 3A) which gave a thick band. Later, we made a comb by X-ray sheet (Fig. 3B), and it gave backward running of band due to left-over sample on the backside of the comb. Finally, we made an applicator by sharp blade (Fig. 3C) which gave a thin band separation.
- Sample was applied over the applicator on a nonsiliconized rough slide, lift up the sample applicator and wipe the back side of applicator for excess sample so that backward running of sample can be prevented.
- Place the applicator over the gel and wait for 2 minutes to allow the samples to get completely absorbed within the gel.
- Remove the applicator.

Electrophoresis Run

Fill the cold buffer in the two compartments. Put the gel slide in the electrophoretic chamber. Connect the gel with buffer through a strip of filter paper wick. We kept filtering paper wick width to cover the whole glass slide width. Covered the chamber with its lid.

- Connected the cathode (–) electrode on the side of sample application and opposite with the anode (+) electrode of the power supply. Set voltage at 300 volts and run for 10 minutes.

Fixation Slide after Run

- Slide is placed for fixation for 10 minutes.

Amido Black Stain Preparation

- Drying of gel for 5 minutes in oven at 50°C. Then staining it for 2 minutes with Amido black (Table 2).

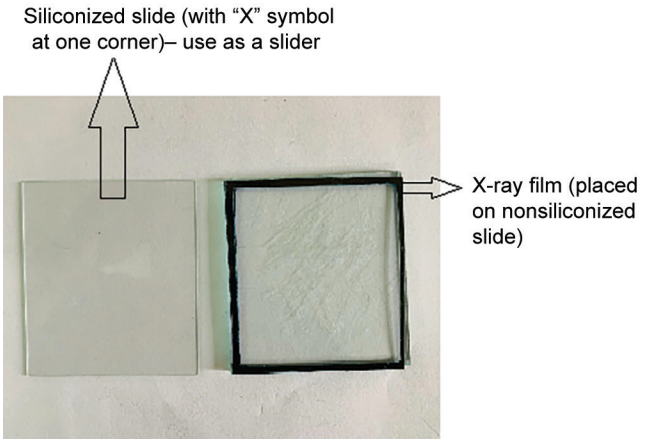


Fig. 1: Glass slide used for preparation

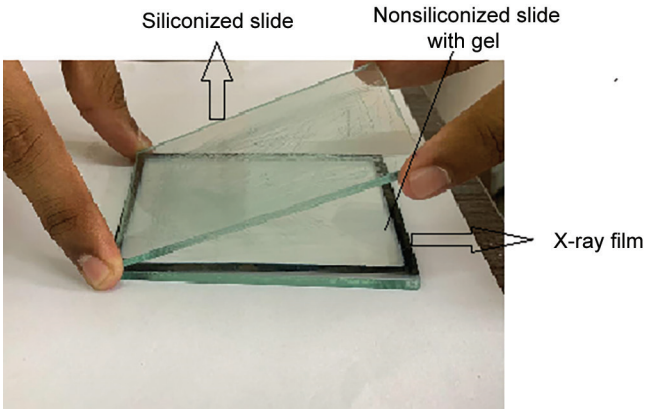
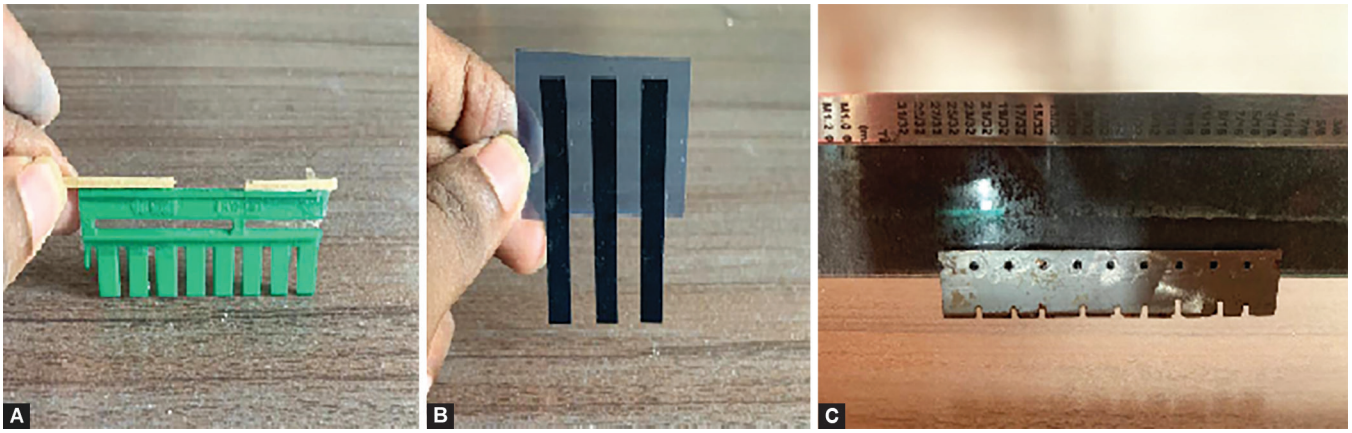


Fig. 2: Nonsiliconized glass slide on base with gel and siliconized glass slide on top



Figs 3A to C: Comb applicator: (A) Sample comb applicator; (B) Comb by X-ray sheet; (C) Comb sharp blade

Table 2: Amido black stain

Reagents	*Quantity*
Amido black dye	2.5 gm
Glacial acetic acid	100 mL
Methanol	450 mL
Distilled water	450 mL

Destain: 5% Glacial Acetic Acid Preparation

- Take 5 mL of concentrated glacial acetic acid and add 95 mL of distilled water. Use it for destaining for 30 min.

Evaluation of Electrophoretic Band

The below picture shows electrophoresis run with control and patient samples (Fig. 4).

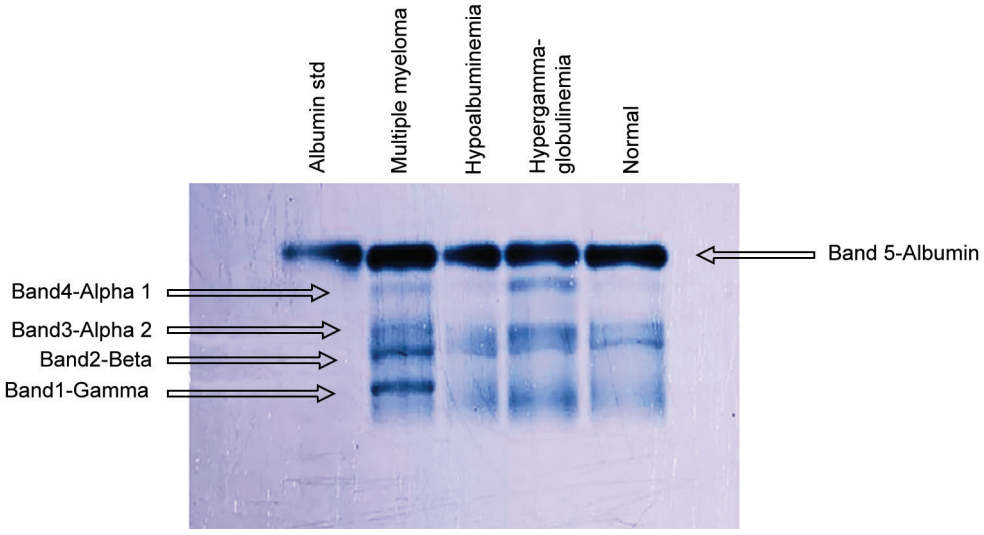


Fig. 4: Electrophoresis run with control and patient samples

The above figure shows electrophoresis run for 2 control and 3 patient samples. As control, in the first two positions from the right side, subsequently, a pure albumin and serum sample of diagnosed patient of multiple myeloma are taken. Then subsequently, two patients' samples are run with the diagnosis of hypoalbuminemia and hypergammaglobulinemia. And in last, extreme left, normal person's sample run.[4–6]

Conclusion

- This methodology helps to develop hybrid-type electrophoresis apparatus with completely in-house preparation of all the reagents, including buffer, stain, destain, etc., with minimum expenditure.
- The result of the electrophoresis reflects that bands appeared at appropriate locations as per the used control of abnormal conditions as well as in physiological conditions.

Orcid

Gopala Krishnan Karuppaiah https://orcid.org/0009-0008-3636-649X
Sapna Patel https://orcid.org/0000-0001-6059-1656
Piyush Tailor https://orcid.org/0009-0001-4922-8909
Hina Baraiya https://orcid.org/0000-0002-7347-8633

References

1. Nwogbo OV, Jin Y, Sliker T, et al. Analysis of multiple bands on serum protein immunofixation electrophoresis: Challenge in interpretation of clonality in a patient with light chain–predominant multiple myeloma. Lab Med 2021;52(5):503–508. DOI: 10.1093/labmed/lmab001.
2. Srinivas PR. Introduction to protein electrophoresis. Protein Electrophoresis: Methods and Protocols 2012;23–28. DOI: 10.1007/978-1-4939-8793-1_2.
3. Ream JA, Lewis LK, Lewis KA. Rapid agarose gel electrophoretic mobility shift assay for quantitating protein: RNA interactions. Anal Biochem 2016;511:36–41. DOI: 10.1016/j.ab.2016.07.027.
4. Dimopoulos M, Terpos E, Comenzo RL, et al. International myeloma working group consensus statement and guidelines regarding the current role of imaging techniques in the diagnosis and monitoring of multiple Myeloma. Leukemia 2009;23(9):1545–1556. DOI: 10.1038/leu.2009.89.
5. Harousseau JL, Dreyling M. Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21:v155–v157. DOI: 10.1093/annonc/mdq178.
6. Quach H, Prince HM. Clinical practice guideline: Multiple myeloma. Medical Scientific Advisory Group (MSAG): Myeloma Australia 2019. pp. 1–53.