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Research Article

A Staining Protocol of Proteins on Agarose Gel with Amido Black

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Abstract

Amido black is known as a dye for protein staining for a long time. However, the detailed description of the staining time is not described in on agarose gels. In this context we have incubated the protein on agarose gel with Amido black for different time periods. We have observed that in Agarose thick gel 5 minutes incubation with dye is sufficient to analyse the protein. Whereas in agarose slide gel minimum 15 minutes incubation is necessary for qualitative analysis. Our results are exciting because CBB the most popular stain requires minimum overnight staining for visualisation of proteins.

Keywords: Amido Black; Protein Staining; Electrophoresis; Agarose Gel

Abbreviations

BPB: Bromophenol Blue; CBB: Coomassie Brilliant Blue

Introduction

Staining of proteins on the gel is a routine procedure in any biological laboratory. Much discussion has been done on this issue and matter has been reviewed in detail [1-3]. However, to the best of our knowledge for agarose gels stain preparation and staining time is not documented in an elaborated manner as far as amido black staining is concerned. Some publications document experience regarding staining of proteins on agarose gels with amido black [4]. Such publications discuss the results of the experiment but do not document the procedure. On agarose gel protein staining amido black preparation is described earlier. The procedure requires warming up the stain up to 60°C [5]. On thin agarose gels, 0.2% amido black stain is prepared in acetic acid, and that has stained the proteins in 15 minutes [6]. This is not the case for staining on nitrocellulose membranes. Moreover, it is described that on nitrocellulose membrane the staining time is 1 minute and if more time is provided for staining the intensity of the bands does not increase [7]. We have recently observed that on agarose such observation is not valid. Amido black staining is an age-old procedure [5,8]. On agarose gel other than our report detail description of the staining process is not done recently. Our observations set a standard protocol for the process, and we feel that such information should be communicated quickly to the research community.

Material and Methods

Agarose, Amido Black 10B, Tris Free base, Bromophenol Blue, Coomassie Brilliant Blue R-250 were purchased from HiMedia, Glycine, Glycerol from Merck, Methanol from Qualigens 3446Q, Acetic acid glacial from Qualigens 1100Q, Ethyl alcohol (99.9%) from Changshu Hongsheng Fine Chemical Co., Ltd. and all other reagents were of analytical grade. The electrophoresis system was from Genei, Bengaluru, India. ELICO pH meter was used throughout the study.

For agarose gel electrophoresis

Preparation of the reagents

1. **Tris-Glycine buffer (pH~8.3):** 3g of Tris (0.025M) and 14.4g of Glycine (0.192M) was dissolved in 1 litre of distilled water.
2. **Sample buffer:** 2.5 mL of Tris-Glycine buffer, 2 mL of Glycerol, 5.5 mL of distilled water to make 10 mL of sample buffer.

3. **1% Agarose Gel:** 1g of agarose was dissolved in 100mL of Tris-Glycine buffer (pH-8.3 to 8.5). The mixture was heated until the solution becomes clear.

4. **5**% Bromophenol blue (BPB) in Phosphate buffer, 0.1M (pH 7.38).

5. **Amido Black stain:** 1g of Amido black was dissolved in 90 ml of ethanol followed by addition of 10 ml glacial acetic acid.

6. **Amido Back destaining solution:** 7 ml of glacial acetic acid was mixed with 50 ml of methanol, and distilled water was then added to make the final volume of solution 100 ml.

7. **Coomassie Brilliant Blue (CBB) staining solution:** 0.25g of CBB dissolved in 125 ml of methanol followed by addition of 25 ml acetic acid glacial followed by addition of 100ml distilled water.

8. **CBB destaining solution:** 10 ml of acetic acid glacial was mixed with 10ml of methanol followed by addition of 80 ml of distilled water.

Methodology details

For agarose (thick) gel electrophoresis

Sample: 20 µL of diluted plasma (1:1 with sample buffer + 2 µL of the tracking dye). The left oversample of healthy individuals is taken.

Tracking dye: 5% Bromophenol Blue (196 µL sample buffer + 4 µL of the dye).

Electrophoretic conditions: 50 Volts till the tracking dye reached the end of the gel. The electrophoresis apparatus was placed on ice while running.

Staining of the gel after the run: The gels were placed in a container containing Amido Black stain for 5 minutes, 15 minutes and overnight staining at room temperature. Also, the gels are also placed in the CBB staining solution for overnight at room temperature.

Destaining of the gel: After staining the gels were then placed in destaining solution (amido black and CBB) for overnight at room temperature.

Densitometry: Using freely available image J analyser software (https://imagej.nih.gov/ij/download.html) following the instruction of the manufacturer.

For slide gel electrophoresis

The 1% agarose gel is poured with the help of pipette over the clean glass slide and is allowed to get solidified.

Sample loading: 20 µL of plasma (leftover of healthy individuals) and 5 µL of BPB has been mixed with the help of pipette over a clean glass slide. Then the edge of the coverslip is charged with the sample and has been loaded onto the slide gel by puncturing the gel.

Electrophoretic conditions: 50 Volts till the tracking dye reached the end of the gel. The electrophoresis apparatus was placed on ice while running.

Staining of the gel after the run: The gels were placed in a container containing Amido Black stain for 5 minutes, 15 minutes and overnight staining at room temperature. Also, the gels are also placed in the CBB staining solution for overnight at room temperature.

Destaining of the gel: After staining the gels were then placed in destaining solution (amido black and CBB) for overnight at room temperature.

Densitometry: Using freely available image J analyser software (https://imagej.nih.gov/ij/download.html) following the instruction of the manufacturer.

Statistical analysis: The values were represented as mean ± SD (n = 6) and unpaired t-test was used to analyse the data. A p-value < 0.05 was considered as significant.

Results

In agarose (thick) gel

It was observed that in both 5 minutes and 15 minutes stained gels the proteins bands were visible. However, in overnight stained gel, no bands were visualised due to overstaining (Figure 1). The background staining in 5 minutes stained gel is less in comparison to 15 minutes stained gel. The band intensity of globulin in both 5 minutes stained gel and 15 minutes stained gel is represented as mean ± SD.

In slide gel electrophoresis

It was observed that in 5 minutes stained gel the some of the protein bands are not visible in comparison to 15 minutes and overnight stained gel (Figure 2). The background staining is less

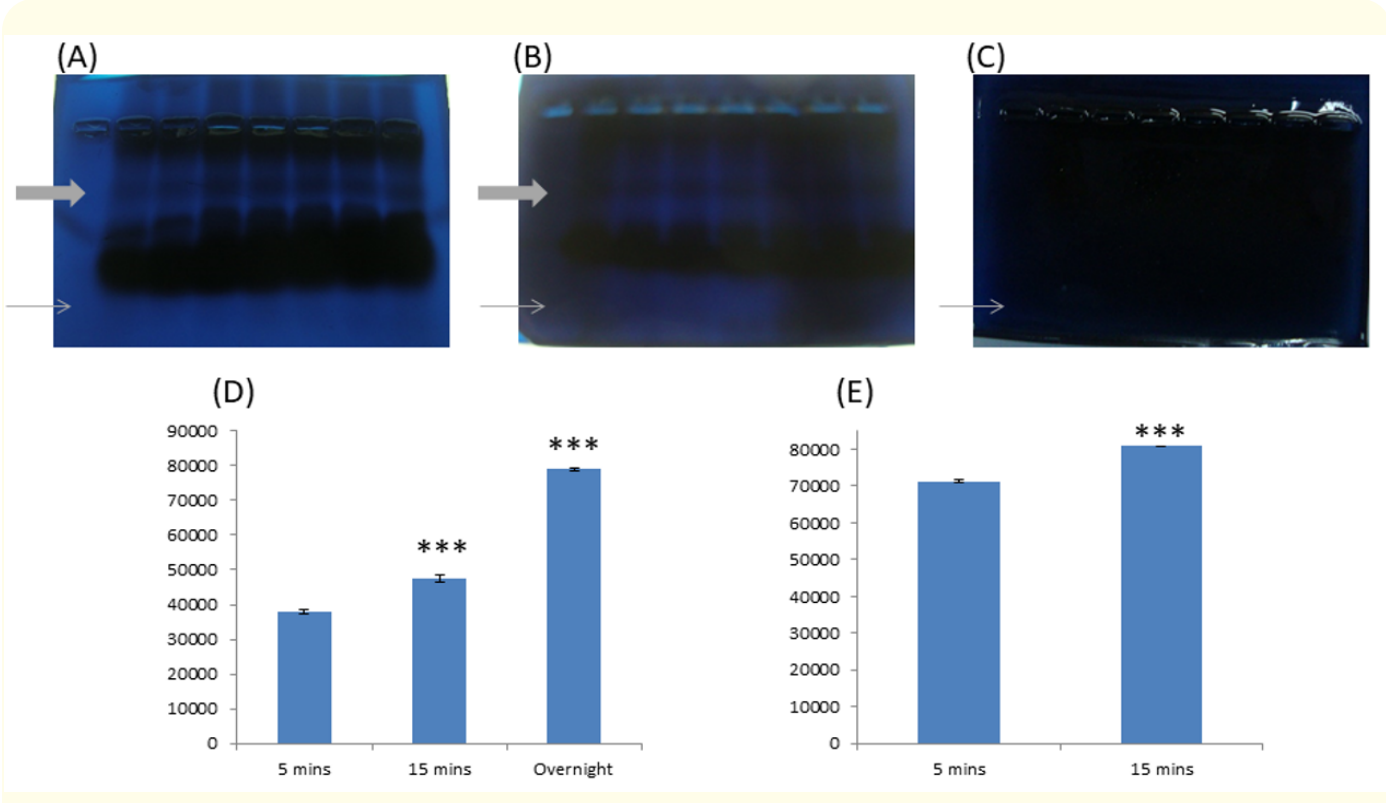

Figure 1: Representative picture of agarose (thick) gel electrophoresis of serum proteins followed by Amido black staining and overnight destaining.

The gel incubated in Amido black stain for (A) 5 minutes, (B) 15 minutes and (C) Overnight.

The densitometry analysis of the background of the gel (→).

Mean ± SD (n = 6) of the background density is shown in (D) for (A), (B) and (C) respectively (Y-axis in square pixel where 96 pixel = 1 inch). *** p-value less than 0.0001.

The densitometry analysis of the globulin band in the gel (➡).

Mean ± SD (n = 6) of the globulin band density is shown in (E) for (A) and (B) respectively (Y-axis in the square pixel where 96 pixel = 1 inches). *** p-value less than 0.0001.

in 5 minutes stained gel in comparison to the 15 minutes and overnight stained gel. The band intensity of globulin in both 15 minutes stained gel and the overnight stained gel is represented as mean ± SD.

Discussion

Amido black staining is popular to detect proteins on gel [9]. However, on the agarose gel, the staining procedure is yet not standardised. Therefore, in this context our observed results are important. It is observed that on overnight staining on the thick agarose gel destaining is not possible in 24 (overnight) hours. That is not the case with slide gel where clear band pattern is visible. So, it is clear from our observation that on thick agarose gel overnight staining is not a preferred procedure. However, in Coomassie Brilliant Blue (CBB) both slide gel and thick gel can be stained for overnight followed by overnight destaining for successful visuali-

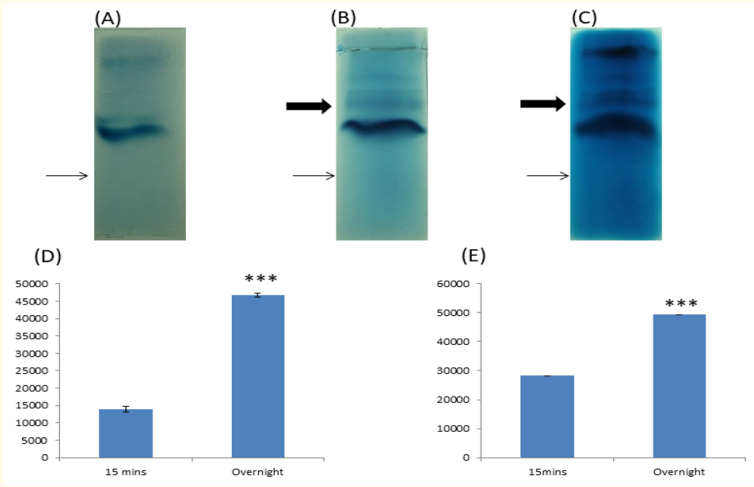

Figure 2: Representative picture of agarose slide gel electrophoresis of serum proteins followed by Amido black staining and overnight destaining.

The slide gel incubated in Amido black stain for (A) 5 minutes, (B) 15 minutes and (C) Overnight.

The densitometry analysis of the background of the gel (→).

Mean ± SD (n = 6) of the background density is shown in (D) for (B) and (C) respectively (Y-axis in square pixel where 96 pixel = 1 inch). *** p-value less than 0.0001.

The densitometry analysis of the globulin band in the gel (⟹).

Mean ± SD (n = 6) of the globulin band density is shown in (E) for (B) and (C) respectively (Y-axis in the square pixel where 96 pixel = 1 inches). *** p-value less than 0.0001.

sation of the bands (Figure 3). It proves that the staining protocol is different in thick gels with amido black and CBB stains. It is known that CBB is more sensitive to detect the proteins on the gel. However, we have observed that in slide gel for 15 minutes of staining Amido Black is producing appreciable bands while CBB does not provide such results. Our observations are confirmative with the previous findings of 15 minutes results is concerned on slide gel [6,10]. So, in slide gel amido black requires less staining time. This advantage of amido black is never highlighted before to the best of our knowledge. But, we emphasise that 5 minutes of staining has made the albumin band visible, and globulin bands are not appreciable on slide gel.

For thick gel, the background staining of 5 minutes staining is less than 15 minutes as expected and the bands are also visible in both. So for thick gel, we recommend 5 minutes staining. Our stain preparation requires no warming up or heating and for 5 minutes staining following the addition of the destaining solution the band appears on gel approximately after 2 hours. This is not the case with CBB where overnight destaining following overnight staining has to be done. This proves that for detection of proteins on thick agarose gels amido black is quicker staining method. Keeping in mind the importance of protein staining on gel we recommend amido black for routine protein staining on agarose gel where the protein has to be stained quickly.

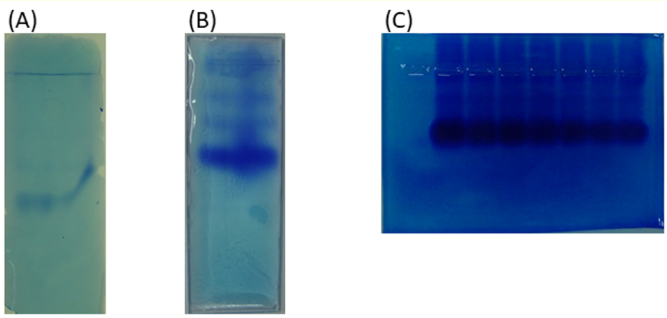

Figure 3: Representative picture of agarose gel electrophoresis of serum proteins followed by CBB staining and overnight destaining.

Agarose slide gel in incubated in CBB stain for (A) 15 minutes (B) Overnight.

Agarose (thick) gel incubated in CBB stain for (C) Overnight.

Conclusion

Amido black staining for 5 minutes in thick agarose gel is sufficient to visualise protein band after overnight destaining whereas in slide gel 15 minutes staining is necessary to visualise the protein bands. It is quicker than CBB to get the proteins visualised on the agarose gel.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

JKK, BB, RK are undergraduate students who have carried out this work at PGIMER, Chandigarh as a part of their training under the guidance of DK. DB overall supervises it.

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