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Research Article

Deterioration of storage phosphor screens with use

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Summary

More than 1000 laboratories worldwide use phosphorimager systems for radioisotopic gel and blot quantification. This method is extraordinary sensitive and has a wide dynamic range, and it is therefore often preferred to conventional film-based analysis. We checked the accuracy of our storage phosphor screen by exposing the same ^{32}P-labelled blot in different positions on the same screen. The first exposure took place at the top of the screen, an area used more often; the second exposure at a relatively little-used area of the screen. As the screens are said to last indefinitely, regardless of how often they are used, we did not expect a difference in the results. In fact, we found a significant impact of the position of the gel on the results. The position that was used more often gave inaccurate results; in contrast, the unused area of the screen gave data that were confirmed by X-ray film autoradiography. We conclude that, contrary to described properties, a deterioration of the phosphor screens takes place with repeated use. To avoid obtaining invalid data, we recommend frequent checks of the screens. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: radioisotopes; storage phosphorimagery; autoradiography; gel electrophoresis

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Introduction

Autoradiography with phosphorimager systems allows quantification of radioactively labelled samples with wide dynamic range and high sensitivity. The phosphorimager technique was developed as a more sensitive alternative to X-ray films.[1] Commercially available imaging plates consist of fine crystals of $BaFBr:Eu^{2+}$ in an organic binder. High-energy radiation (e.g. X-rays, ultraviolet light, gamma rays or beta particles) excites an electron that becomes trapped in the $BaFBr^{-}$ complex, leading to the oxidation of Eu^{2+} to Eu^{3+}. If exposed to light from a helium neon laser (630 nm), the electron is released, resulting in a reduction of Eu^{3+} to Eu^{2+*} and the release of a photon at 390 nm when the Eu^{2+*} returns to ground state. The intensity of luminescence is stored digitally in relation to the position of the scanning laser beam.[1,2] Pixel values of the image are proportional to the incident radiation in the sample, so it can be quantified with the aid of appropriate image analysis software.

The biggest advantage of this storage phosphor technology is that the reactions involved in image formation are completely reversible and therefore the storage phosphor screens are reusable.[1,2] Approximately 80% of the information stored in the screens is erased by scanning, whereas the remaining signal can be extinguished by exposing the screen to visible light for a few minutes.[2] The screens are said not to degrade by repeated exposure to laboratory levels of radioactivity and, with proper care (avoiding compression lines from ball-point pens, acetic acid burns, or contamination with long-lived radioisotopes), they should last indefinitely.[2] We tested the accuracy of the data obtained from a storage phosphor screen following several years of repeated use.

Results and discussion

Relative amounts of β-actin mRNA from 10 rats (2 groups, 5 treated with Cerebrolysin® and 5 treated with a control amino-acid solution) were determined using RT-PCR followed by agarose gel electrophoresis and both phosphorimagery and autoradiography. The same gel was exposed to different areas of the same phosphor screen, a frequently used area near the top (Figure 1A) and a rarely used area at the bottom (Figure 1B). The frequently used area was exposed to samples approximately weekly, on average, whereas the rarely used area was

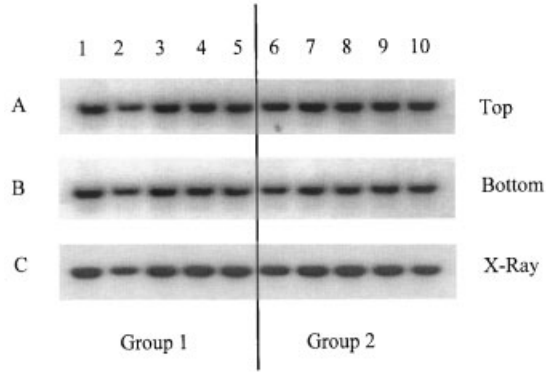

Figure 1. Gel quantitation using storage phorphorimagery and autoradiography reveals inaccurate results from storage phosphor screens. Each band represents the RT-PCR product from a single rat brain. The five bands on the left side belong to group 1 (amino acid-treated rats), and the five bands on the right belong to group 2 (Cerebrolysin®-treated rats). The same gel was used to obtain data in A, B, and C. (A) 30-min exposure from the top of the phosphor screen; (B) 30-min exposure from the bottom of the same screen; (C) exposure to X-ray film

exposed less than once per month. The image from the top of the phosphor screen (Figure 1A) exhibited darker bands on the right side compared to the bands on the left side. The mean pixel value of the right five bands (group 2) was significantly higher than the mean of the left five bands (group 1) ($p < 0.05$) (Figure 2A). The second image (Figure 1B) of the same gel, exposed to the bottom of the same phosphor screen, gave lower mean pixel values for group 2 than in exposure 1A, and there was no significant difference between groups ($p = 0.35$) (Figure 2B).

A third exposure of the same gel was done using X-ray film autoradiography (Figure 1C). This exposure showed no difference between groups 1 and 2 ($p = 0.46$)(Figure 2C) and therefore verifies the results from the bottom portion of the phosphor screen (Figures 1B and 2B).

The results of the exposures to the phosphor screen were independent of the length of the exposure. Insets in Figures 2A and 2B show the pixel values for groups 1 and 2 obtained after 15, 30, 45 and 60 min exposure.

Results were correlated with the amount of usage of the screen rather than with its age, as a second screen of the same age from the same manufacturer, but which was rarely used (e.g. less than once per month), exhibited no such differences across its surface (data not shown).

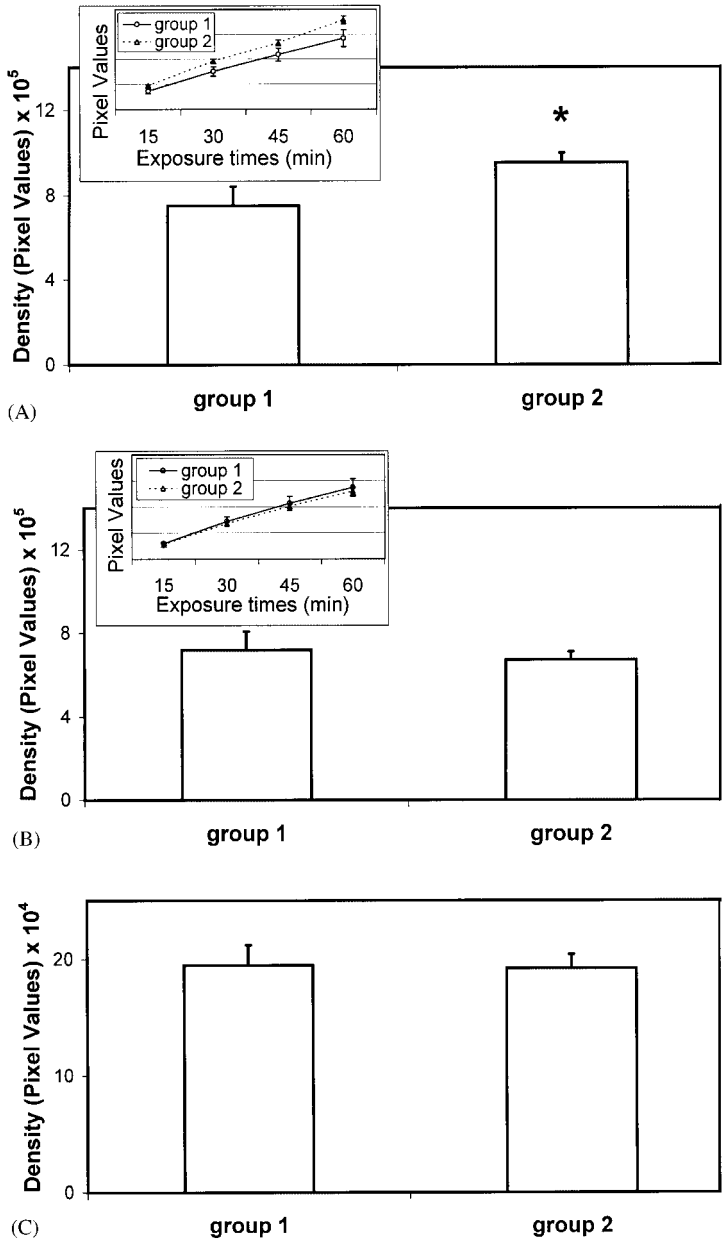

Figure 2. Pixel values of the images in Figure 1. (A), (B) and (C) as in Figure 1 (mean $\pm$SEM). Asterisk represents statistical significance $p < 0.05$. The line graphs in the upper left corner of (A) and (B) show the time-dependence of the densities for both groups (exposure times: 15, 30, 45, and 60 min). Circles and solid line: group 1; triangles and dashed line: group 2

The screen was approximately 5 years old and was used exclusively for ^{32}P quantification from dried gels and blots. The levels of ^{32}P used were similar to those in the experiment described here. The screen was never dropped or exposed to any source of possible damage (acetic acid, long-lived radioisotopes, etc.).[2]

The reactions involved in image formation on the phosphor screen are completely reversible, and the screens are said to 'last indefinitely'.[2] In fact, we demonstrate that areas of the screen subjected to more frequent use give different results than areas of the screen that are less frequently exposed (Figures 1 and 2). One possible explanation for this deterioration is thinning of the plastic coating designed to protect the BaFBr crystals on the screen.

Experimental

Samples and RNA isolation

10 rats (Sprague–Dawley, Charles River, Baie d'Urfé, Quebec) were treated with either an amino acid solution ($n = 5$, group 1) or the nootropic drug Cerebrolysin® (EBEWE, Unterach, Austria) ($n = 5$, group 2) for 7 consecutive days (2.5 ml/kg; ip injections). On day 8, the rats were killed and their brains immediately removed and snap-frozen in liquid nitrogen before storing them at −80°C. Total RNA was isolated as previously described[3] and was DNAse-treated with DNA-free™ (Ambion Inc., Austin, TX, USA) using the manufacturer's protocol.

RT-PCR for β-actin

Total RNA (1 μg) was reverse transcribed into cDNA as previously described,[3] but with oligo d(T) instead of random hexamer primers. Negative controls lacked the MuLV reverse transcriptase. PCR for β-actin was carried out as previously described,[3] but with the addition of 0.5 μCi α-^{32}P-dGTP/reaction (3000 Ci/mmol, Amersham, Arlington Heights, IL, USA) and using AmpliTaq Gold polymerase (Perkin–Elmer, Norwalk, CT, USA) instead of Taq polymerase. The PCR amplification used a "hot start" (12 min denaturation at 94°C) followed by 25 cycles of denaturation at 94°C for 30 s, primer annealing at 64°C for 45 s, extension at 72°C for 1 min and a final extension after 25 cycles at 72°C for 7 min, terminating with a 4°C hold cycle.

Gel electrophoresis and phosphorimage and X-ray film analysis

Each RT-PCR reaction mixture of 10 µl was subjected to gel electrophoresis as described.[3.] The gel was dried and exposed to a 20×25 cm storage phosphor screen (Molecular Dynamics, Sunnyvale, CA, USA) for different lengths of time. Prior to each exposure, the screen was erased by exposing it to a visible light box for 15 min. Following each exposure the screen was immediately scanned in a phosphorimager (Molecular Dynamics, Model 425B) and the stored digital images were analysed using ImageQuant software (Molecular Dynamics) with volume integration and local background subtraction.

The same gel was exposed to X-ray film (X-Omat AR, Kodak, Rochester, NY, USA) to verify the results of the phosphorimager. The exposed film was scanned and analysed using a ScanMaker X6 scanner (MicroTek International, Hillsboro, OR, USA) and Ulead PhotoImpact version 4.2 (Ulead, Torrance, CA, USA).

Statistical analysis

Statistical comparisons between group 1 ($n = 5$) and group 2 ($n = 5$) were based on Kruskall-Wallis ANOVA. The α-error level was fixed at 0.05. All statistics were carried out with a Statistica Version 5 analysis software package (StatSoft Inc., Hamburg, Germany). Data is shown as mean $\pm$ SEM.

Conclusion

Although the literature asserts that the screens do not degrade by repeated exposure,[1,2] we demonstrate here that there is deterioration with repeated use. Storage phosphor autoradiography is a valuable technique, but we strongly recommend frequent checks of screens to avoid obtaining inaccurate data.

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