Comparison of Green Fluorescent Protein (GFP) fluorescence intensity and tissue quality using two fixation methods by immersion

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1 INTRODUCTION

Green Fluorescent Protein (GFP) is a naturally fluorescent gene product of the jellyfish *Aequorea Victoria.* As new drugs in development are geared towards gene therapy, GFP is being used as a reporter gene to ensure that the future therapeutic gene will be expressed in targeted cells. Therefore, monitoring GFP expression accurately is an important issue. The fastest and most cost-effective way to monitor GFP expression is by direct fluorescence microscopy to detect its autofluorescence. Other methods to detect GFP expression on histology slides are immunohistochemistry or immunofluorescence but they are more time-consuming and expensive. Autofluorescence intensity can be quenched by different parameters including the type of fixative used prior to the freezing procedure. A period of fixation is necessary prior to freezing in order to retain the GFP, which is soluble, within cells; otherwise, the protein leaks out of cells that have lost membrane integrity. The fixation method of choice for GFP detection by direct fluorescence microscopy (i.e. to ensure good tissue quality and good fluorescence intensity) is by perfusion with 4% paraformaldehyde which is not always available in terms of equipment and cost. This poster compares two fixation methods by immersion and their results in terms of tissue quality *vs* GFP fluorescence intensity in rats.

2 MATERIALS AND METHODS

GFP transgenic Sprague Dawley rats (SD-Tg(GFP)2BalRrrc) were obtained from the Rat Resource and Research Center, University of Missouri, Columbia, MO. Rats were necropsied and the following tissues retained: eyes, brain, heart, kidney, liver, pancreas, lung, spleen, testis and skeletal muscle. For each tissue, samples were fixed by immersion in either 4% paraformaldehyde or 4% formalin in PBS with 7% picric acid for either 2, 14 or 22 hours. After fixation, tissue samples were put in 15% sucrose-PBS for 2 hours , transferred in 30% sucrose-PBS for 2 to 3 hours as a cryoprotective agent and then frozen in Tissue-Tec OCT (Fisher; Pittsburgh, PA). 8µm sections were cut and mounted on glass slides with an anti-fading mounting media (VECTASHIELD HardSet Antifade Mounting Medium with DAPI – Vector Laboratories). Slides were stored at 4˚C. During the whole processing procedure, tissues, blocks then slides were kept away from light as much as possible to avoid quenching of the fluorescence.

3 RESULTS AND DISCUSSION

Results are summarized in table 1.
GFP fluorescence was detected in all tissues with either fixative. Fixation time did not influence fluorescence intensity as the signal was similar for a given fixative.
Comparing both fixatives, GFP fluorescence was more intense in tissues fixed in paraformaldehyde. The down side of this fixative was a mediocre tissue quality: cells were shrunken and there were gaps within the tissue (Fig. 1a). This appearance was variable depending on each tissue's fragility (Fig. 2, 3a), pancreas and liver structures being better preserved than other tissues. Tissue quality was well preserved using formalin/picric acid fixation and GFP fluorescence was detected even though less intense than with paraformaldehyde fixation (Fig. 1b). Blocks were stored at -20˚C for 6 months and new sections were cut and mounted on glass slides to evaluate GFP direct fluorescence. It appeared that block quality was altered after long term storage. Cryomicrotome sectioning was difficult and resulted in mediocre tissue quality. Quality was still markedly better with formalin/picric acid fixation compared to paraformaldehyde. GFP fluorescence was detected in paraformaldehyde fixed tissues but the signal was much weaker compared to past sections (Fig. 3). No fluorescence could be detected in formalin/picric acid fixed tissues after a 6-month storage. After approximately 1 year of storage, several blocks were sectioned and immunohistochemistry with an anti-GFP antibody could be performed with success (Fig.4).

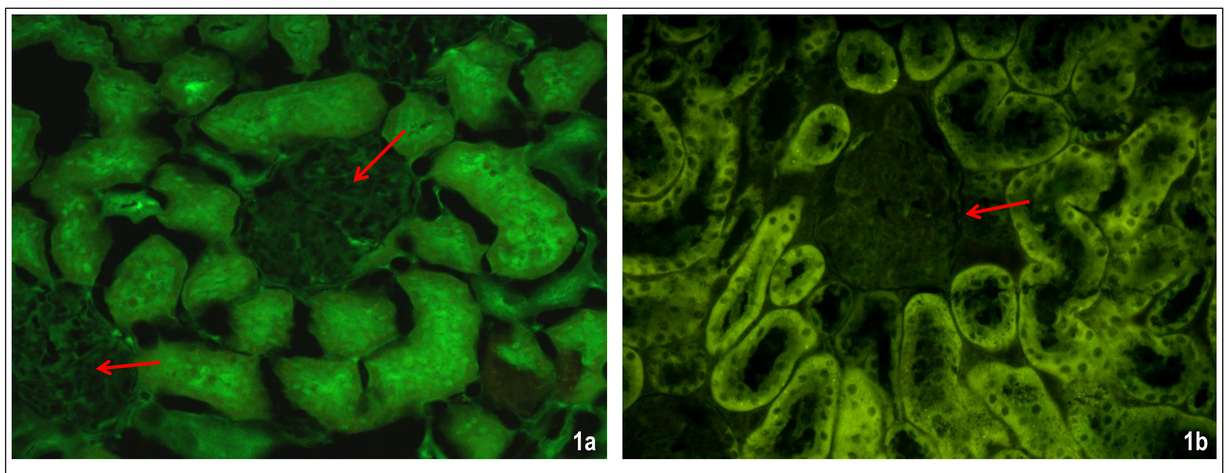

Figure 1 – Kidney, frozen section. In both sections, glomeruli (arrows) present a lower level of GFP expression compared to surrounding tubules; **(1a)** Fixed by immersion in 4% paraformaldehyde for 2 hours. Tubules are shrunken with gaps between them but GFP fluorescence intensity is strong. **(1b)** Fixed by immersion in 4% formalin/7% picric acid for 2 hours. Tubular architecture is well preserved but signal intensity is weaker compared to 1a.

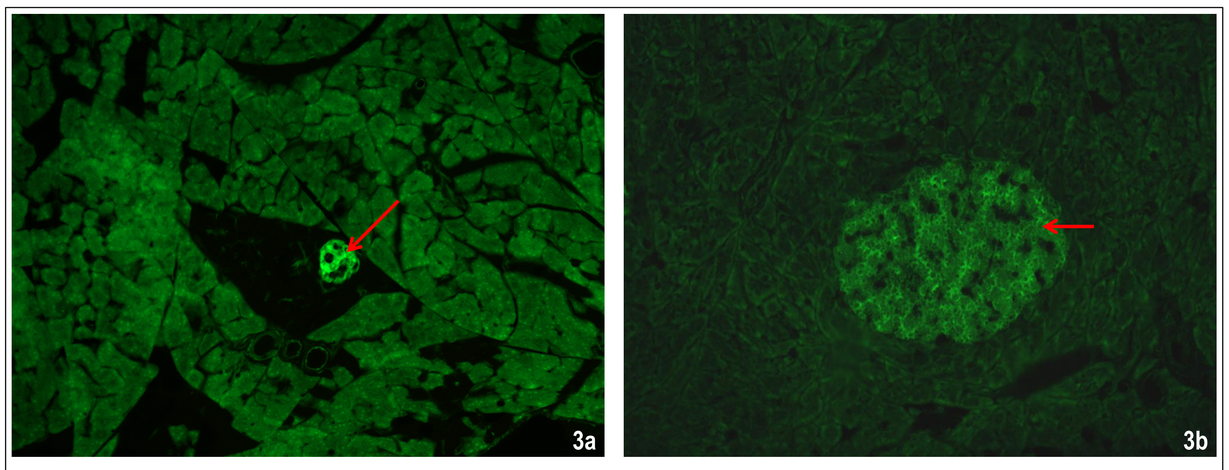

Figure 3 – Pancreas, frozen section, fixed by immersion in 4% paraformaldehyde for 2 hours. Even though fixation was in paraformaldehyde, structure was better preserved than kidney (Fig. 1a). **(3a)** Fresh block. GFP fluorescence is strong, especially in islet cells (arrow). **(3b)** After a 6-month storage, GFP fluorescence is much weaker but still stronger in islet cells (arrow).

Table 1 - Comparison of GFP fluorescence and tissue quality using immersion in 4% paraformaldehyde or 4%formalin/7% picric acid as fixation methods.

	GFP fluorescence intensity		Tissue quality	
Fixative	Fresh blocks	Stored blocks (6 months)	Fresh blocks	Stored blocks (6 months)
4% paraformaldehyde (2, 14 or 22 h)	+++	+	+	+
4% formalin/7% picric acid (2, 14 or 22 h)	++	-	+++	++

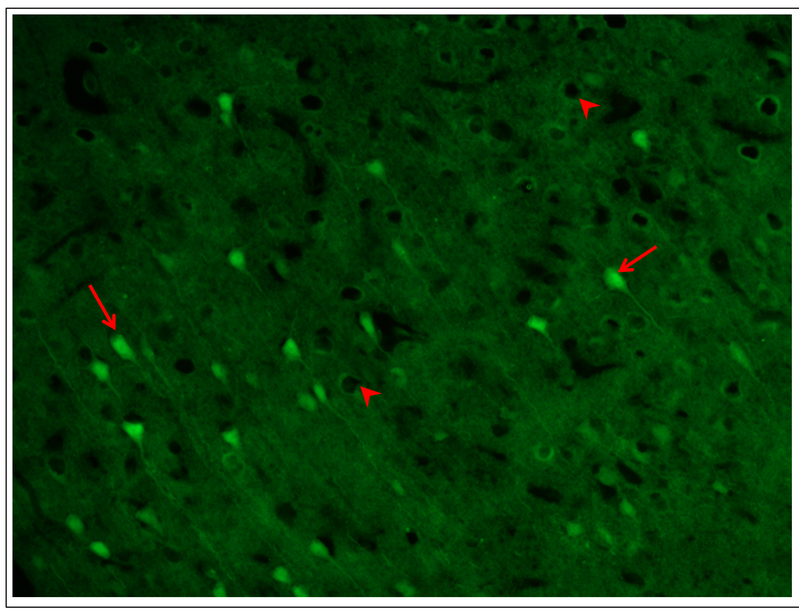

Figure 2 – Brain, frozen section, fixed by immersion in 4% paraformaldehyde for 14 hours. There are artefactual vacuoles (arrowheads) but GFP fluorescence intensity is very good and stronger in neurons (plain arrows).

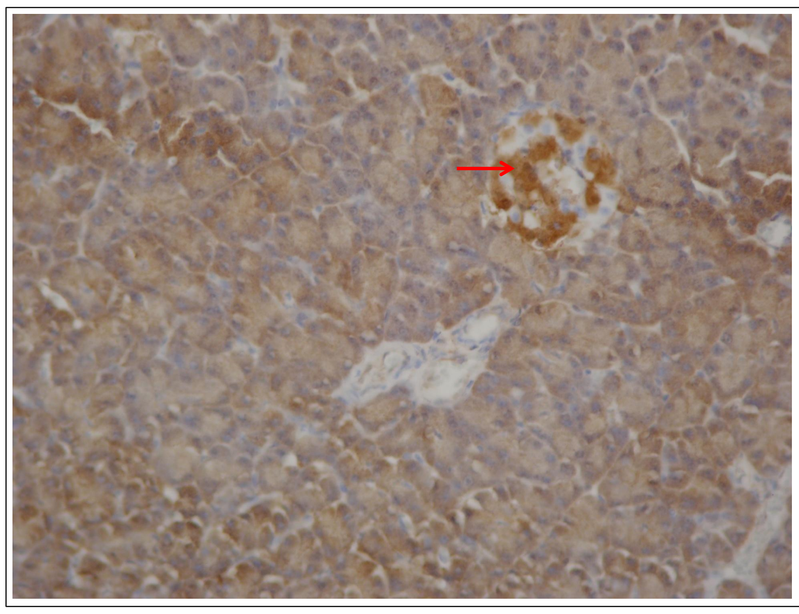

Figure 4 – Pancreas, frozen section. Immunohistochemistry with anti-GFP antibody performed after storage of blocks for approximately one year was of good quality. Again GFP expression is stronger in islet cells (arrow).

4 CONCLUSION

Direct detection of GFP expression under fluorescence microscopy could be achieved by immersion fixation of tissues for up to 22 hours followed by freezing. Even though formalin/picric acid is not a routine fixative, it should be considered when GFP expression is to be evaluated as it allows detection of GFP by direct fluorescence microscopy with very good tissue quality and therefore, can be a more simple and economic alternative to fixation by perfusion. Immersion in paraformaldehyde remains a good option in terms of fluorescence intensity but tissue quality is highly variable. Furthermore, both fixation methods still allow immunohistochemistry to be performed efficiently on frozen sections, even after long term storage.

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