Published in final edited form as:

J Glaucoma. 2013 ; 22(8): . doi:10.1097/IJG.0b013e3182a4a012.

Letter to the Editor: Aqueous Humor Oxygen Measurements

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To the Editor

We read with interest and concern a recent article in the Journal of Glaucoma by Sharifipour *et al.* (2012).¹ These authors estimated the oxygen tension of aqueous humor in samples withdrawn from the anterior chamber and measured using a blood-gas analyzer. They concluded that patients with glaucoma had lower pO₂ in the anterior chamber than patients without glaucoma. Two aspects of this paper were of concern to us; one technical and one ethical. We demonstrate below that the method used by Sharifipour *et al.* to measure pO₂ greatly overestimates the pO₂ in the sample being measured, a problem that had been brought to their attention previously. In addition, they excluded mention of research from our group that contradicted their results, even though they were well aware of our work, having cited it in a paper submitted previously. These issues are described below.

In addition to blood gas analyzers, a variety of other instruments have been used to assess the oxygen partial pressure (pO₂) of solutions. Other studies examining aqueous humor have used polarographic oxygen electrodes (Clark electrodes) and fiber optical sensors (optodes) to measure oxygen within the anterior chamber *in vivo*.²⁻⁴ Sharifipour and colleagues noted significant variability across studies regarding the reported pO₂ values in aqueous humor. While the reasons for this variability may not be clear, we think it is valuable to seek explanations. Complicating factors have been described for measuring oxygen tension using blood gas analyzers. These include problems with air contamination and metabolic consumption of oxygen.⁵⁻⁸

Of particular relevance to air contamination is the type of material that comprises the syringes used for transporting samples. Plastic materials serve as a poor barrier to oxygen. This has been corroborated by a variety of studies examining pO₂ levels in plastic syringes containing human blood samples.⁵⁻⁸ Delay submitting the syringes for analysis resulted in higher recorded pO₂ levels, presumably due to air diffusing into the syringe as well as transfer of the samples into the blood gas analyzer. It is for this reason that standard protocols advise the use of glass rather than plastic syringes for arterial blood samples that

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Conflicts of Interest: Dr. Siegfried receives funding from the National Eye Institute supporting work on oxygen metabolism in the eye. Dr. Beebe currently serves as Editor-In-Chief of the IOVS journal.

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may require greater than 30 minutes of elapsed time prior to being analyzed by a blood gas analyzer.⁹ From a microstructural point of view, the larger pore size and increased density of pores in plastic materials renders them more permeable to oxygen than glass.¹⁰ However, not all studies comparing plastic and glass syringes have consistent results. This may be attributable to leukocyte metabolism in human blood and differences in the methodology of handling samples (e.g., whether or not samples were placed on ice).^{5, 6}

To address this potential source of error, we designed a small experiment to test the effect of time after collection on the pO₂ values in solutions kept inside a syringe and assayed using a blood gas analyzer. We first de-oxygenated a 750 mL glass bottle of balanced salt solution (BSS) by bubbling it with 100% N₂ for 20 minutes. This can be expected to bring the pO₂ of the solution to approximately 0 mm Hg. This was verified with the use of fiberoptic sensor (Oxylab pO₂ optode; Oxford Optronix, Oxford, UK), which read the pO₂ in solution as 1.5 mm Hg. A 30-gauge needle was then used to slowly aspirate the de-oxygenated BSS from the bottle into plastic tuberculin syringes. The dead space of the needle was filled with de-oxygenated BSS to avoid introducing extraneous oxygen into the sample. Samples were read by a blood gas analyzer (Stat Profile pHox Plus C, Nova Biomedical) successive times after aspiration. Samples from group t₁ were analyzed as quickly as possible, within one minute after aspiration. Subsequent samples were analyzed 5, 10, 15, and 20 minutes after aspiration (t₅, t₁₀, t₁₅, t₂₀). In the time between aspiration and analysis, samples were kept on ice, as described by Sharifipour et al.¹ Samples with visible air bubbles were discarded.

Five samples were measured at t₁ and four samples at each of the successive time points. The pO₂ values of each group were averaged and the 95% confidence intervals are displayed in Figure 1. A one-way ANOVA detected a significant difference between the groups (F = 10.09; p < 0.001). As shown in Fig. 1, analysis using the methods described in the paper by Sharifipour and colleagues resulted in grossly elevated oxygen values within one minute after aspiration. These values increased when samples were kept on ice.

Removing aqueous humor from the eye to measure oxygen destroys the gradients of oxygen that are present in the anterior chamber.¹¹ As shown by the data in Fig. 1, it also provides inaccurate results, especially when the samples are kept on ice. These technical defects show that the results reported by Dr. Sharifipour and colleagues must be inaccurate.

We were also disappointed that the paper by Sharifipour and colleagues avoided mention of our previous work, which reported much lower levels of oxygen measured *in situ* in the anterior chamber.¹¹ Two of us were reviewers of papers sent by Dr. Sharifipour and colleagues to other journals. In the first case, they authors cited our published paper. This submission was rejected due to the unrealistically high oxygen values obtained and the presumed unreliability of using a blood gas analyzer (now confirmed in our test). In a subsequent submission, the authors did not mention our previous work. This omission was pointed out as an error by one of us and this manuscript was also rejected. In their paper in the Journal of Glaucoma, Dr. Sharifipour and colleagues again avoided mention of our work studying human aqueous humor measurements *in vivo*. Therefore, although they were well aware of our paper, they chose not to mention it, presumably because our results conflicted so starkly with their data.

We consider the inaccuracy of the blood gas analyzer to be a serious scientific defect, especially since the potential for error was pointed out to Dr. Sharifipour previously and because the authors never validated their method using an oxygen standard. It is our opinion that the omission of any mention of our published work is a significant ethical transgression, since it seems intended to deceive the reader. Because it has been demonstrated to be inaccurate and is ethically flawed, we feel that the appropriate action by the Editor would be

to consider withdrawal of the paper by Sharifipour and colleagues from the Journal of Glaucoma.

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Figure 1. pO₂ values recorded by blood gas analyzer for samples from a de-oxygenized bottle of balanced salt solution. After samples were aspirated by a 30-gauge needle into a plastic tuberculin syringe, they were submitted for analysis at varying times. Error bars represent standard deviation. The red marker indicates the measurement obtained from the OxyLab fiber-optic sensor (optode).