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# The Eyes as a Postmortem Interval Estimation Method

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**Abstract**

After death, the eyes change physically and chemically in ways that can be measured over time. The postmortem analysis of the changes in the eyes has the potential to become a major contributor to estimating a postmortem interval (PMI). This review will focus on three changes in the eye used to estimate PMI. These include the physical features, the temperature, and the vitreous fluid of the eye. These changes have specific correlations with PMI. This allows for the development of mathematical formulas and standards for use in criminal investigations. However, the validity of the method and an investigation's evidence can be affected by outside influences on postmortem changes. Three examples of these influences are ambient temperature, application time, and diseases. These are not completely understood by pathologists. Understanding these influences on the eyes and other PMI indicators is necessary for producing reliable results in the future.

**Keywords:** postmortem interval, eyes, vitreous fluid, cornea, pupil

## Introduction

The Postmortem Interval (PMI) is the time period after an organism has died. After an unexplained death occurs, various methods provide a PMI estimation based on changes within or around the body. One part of the body that undergoes measurable changes after death is the eyes. The eyes may provide a PMI method that is comparatively less influenced by environmental or pathological factors [1, 2]. These factors have hindered the creation of an accurate method that can be applied to all unexplained deaths. Unexplained deaths are often encountered in criminal investigations and reliable PMI estimation methods are needed to provide evidence for the criminal justice field.

Additionally, the eyes as a PMI indicator increase the potential range of application time because they have both thermal and biochemical features. Within the first 24 hours of death, the current methods use temperature to assess how much the body has cooled with time [3]. Beyond a 24 hour period, current methods use the biochemistry of the body's tissue and fluids [1, 4], rigor mortis [3], and the surrounding entomological and bacterial communities [4, 5] to estimate PMI. This review will focus on the use of the physical features, temperature measurements, and vitreous fluid from the eyes to determine a PMI estimate.

## Physical Features of the Eye

The postmortem physical changes in eyes provide a pattern that can lead to a new way of estimating PMI. The opacity in the eye is one pattern that generally increases as the time period since death increases [6]. When images of the human eyes, taken every twenty minutes

over a 15 hour time period, were analyzed using a computer program, the corneal regions decreased in brightness by 7% and non-corneal regions decreased by 20% as time progressed [6]. However, this pattern varied when the ambient temperature and moisture content of the air was considered [7]. Corneal transparency generally decreased faster in warm weather than cold and faster in moist weather than dry weather [7]. This effect on transparency patterns proposes a factor that must be considered when applying this method.

Lenses are another part of the eye that change in transparency over time. Instead of using a computer program to track this change, one study used a spectrophotometer to measure the absorbance of a rabbit lens [8]. The absorbance of rabbit lenses decreased with time because the lens' protein fibers degraded [8]. The effects of the liquid environment on fiber integrity need to be considered since it could potentially change postmortem as well. This liquid is known as the aqueous humor. Aqueous humor changes the membrane potential and pH of lenses when there is variance in its potassium, ammonium, or chloride levels [9]. Resting membrane potentials are important for transport of molecules across the membrane, so any change could result in adverse effects to the structure of the lens. The integrity of the lens could also be affected by the pH change because all biological processes are sensitive to pH. The effects of membrane potential and pH ultimately affect the application of lens opacity as a PMI estimation method.

The use of pilocarpine eye drops may provide a more convenient way of estimating PMI by using the size of the pupil. The pupils do not independently change as time progresses after death [6], but pilocarpine drops can be used to constrict the pupil. The diameters of the pupils before and after the application of the drops had a negative correlation with the increase of PMI [10]. The method's reliance on a chemical change introduces the possibility of the biochemistry of the eye affecting the accuracy of the results. Nevertheless, pilocarpine eye drops have the potential for onsite crime scene application. Unlike the other two methods, laboratory equipment

or computer software would not be needed for this method. Before being applied, this relationship between pupil diameter and PMI must first be further confirmed with future studies.

**Table 1.** Comparison of PMI Estimation Methods that use the Eye

| Method | Measured Variable | Location of Variation | Application Time | Limitations | References |
|---|---|---|---|---|---|
| Matlab Computer Software Image Analysis | Transparency | Cornea | <36 hours | AT, air exposure, and moisture content | 6-7 |
| Spectrophotometry | Transparency | Rabbit Lens | 24-96 hours | AT, air exposure, and aqueous humor biochemistry | 8-9 |
| Pilocarpine Eye Drops (MA) | Size | Pupil | <15 hours | Long reaction onset period and difficult measuring procedure | 10 |
| Algor Mortis Equation (MA) | Temperature | Eye and Rectum | <24 hours | AT and hair | 2, 4, 11-12 |
| Capillary Electrophoresis | Hypoxanthine | Vitreous Fluid | >24 hours | AT and VF pretreatment | 1, 14-15 |
| Flame Photometry or Ion-Selective Electrode Method | Potassium | Vitreous Fluid | >24 hours | AT, VF pretreatment, and cold chambers | 1, 13-14, 16 |
| Paper-Based Colorimetric Analysis (MA) | Iron (II) Ion | Vitreous Fluid | <24 hours | AT, concentrations and volume of reagents used and sample volumes | 17 |
| Gas Diffusion of Ammonium after NaOH addition (MA) | Ammonium | Vitreous Fluid | 10-250 hours | AT, concentrations and volume of reagents used and sample volumes | 18 |

**AT = Ambient Temperature**
**(MA) = Mobile Application**
**VF = Vitreous fluid**

In order to achieve standardization, the limitations of each of these methods will need to be addressed [Table 1]. If transparency is to be used for ever day application, then research must determine whether spectrophotometry or computer programs are more accurate. Pilocarpine eye drops introduces a technique with no laboratory equipment, but the accuracy of the measurement tools affects the accuracy of the PMI estimation. The proper use of these methods also relies upon their valid application times [Table 1]. The research so far has only considered either too narrow of a time period or has not fully explored a set time period in which the method would be most accurate.

**Temperature**

PMI estimations based on temperature typically come from a core temperature measured from the rectum that are used in cooling calculation equations. The following external influences can affect the accuracy of the PMI estimated from temperature: clothing, body weight, ambient temperature, and the location of thermometer application [4]. These influences affect the eye's change in temperature less than the rectum's because the eyes are enclosed in the eye socket [1]. In order to examine this in the context of PMI, one study measured both the rectal and eyeball temperature from thirty human cadavers at various intervals within the first three hours of death [11]. The predicted PMI was compared to the known PMI, and the PMI calculations using only rectal temperature were less accurate than calculations using both rectal and eye temperature [11]. This is because core temperature measurements from the rectum are susceptible to the temperature plateau that occurs directly after death, thus increasing the error in the PMI estimation [2]. A temperature plateau is seen when the temperatures stay consistently the same over an extended period of time. Temperatures measured from the head tissue have little to no signs of a temperature plateau in early PMI that would affect the accuracy of the predicted time of death [2].

While there is not a lot of data collected on this method, it has the potential to replace current methods with a more accurate system. Not only is it more accurate, it only required equipment is a thermometer. A thermometer is a mobile tool that can be taken to a crime scene and used to estimate PMI quickly. It should be noted that the eye cooling pattern must be standardized before there is everyday implementation of this method. Additionally, there are limitations that could affect the estimations made using temperature. One example is the presence or absence of hair. Two cases where the cadavers had bald or shaved heads showed that the difference in predicted PMI and observed PMI was higher than the differences in cases

where hair was present [12]. This introduces an influence that can limit the universal application of this method.

**Vitreous Fluid**

The postmortem changes in concentration of biochemicals in vitreous fluid provides a useful tool for estimating PMI. Vitreous fluid is a clear gel-like fluid that fills the eyeball. When estimating PMI, vitreous fluid is used over other body fluids for biochemical analysis [1, 13]. There are various chemicals measured to analyze how they change with increased PMI. A few of them are potassium, urea, sodium, and glucose [1]. When potassium is compared to six other analytes in vitreous fluid, it has the highest correlation with increased PMI [13]. A strong positive correlation is also seen in hypoxanthine [14]. These correlations are only improved when ambient temperature is taken into consideration [14]. Another factor to consider when applying this method is the pretreatment of vitreous fluid so that it is not so viscous [1]. For example, the concentration of hypoxanthine varies when the sample of vitreous fluid is pretreated with varying enzyme, heat, sonification, or centrifuge treatments [15]. This introduces the need for a standardized treatment to assure accurate and reliable results.

Even though a method that measures potassium and hypoxanthine levels has potential, it would require laboratory equipment and the analysis could not be performed immediately in the field. Bodies are removed from their death location and placed in a cold chamber for storage until an autopsy can be performed. By the time the autopsy is performed, the cold chambers have been shown to affect the rate of change in potassium levels [16]. In order to avoid this effect, a few studies have considered creating an onsite analysis method. One technique used a paper-based device that colorimetrically measured the amount of the iron (II) ion in vitreous fluid

and compared that concentration to that measured by mass spectrometry [17]. These two measurements were not statistically different as shown by a t-test [17]. Another study built upon this work using the ammonium produced by the vitreous fluid after sodium hydroxide was added [18]. This creates a color change that could be measured by a smart phone camera [18]. The measurements were compared to those produced by capillary electrophoresis and the strong correlation was showed by a $R^2$ value of 0.9367 [18]. If a standard correlation can be created for the iron (II) ion and ammonium as PMI increases, then these methods can be used at crime scenes.

## Conclusion

This review addressed the methods used to analyze eyes as a way of estimating PMI. Physical changes of the eyes after death are one measurable change to consider, which include the opacity of the eye and the reaction of the pupil to pilocarpine drops postmortem [6, 8, 10]. Temperatures measured from the eye demonstrates a method that increased the accuracy of a PMI calculation when used alongside the rectal temperature measurement [11]. Finally, methods analyzing the biochemical makeup of vitreous fluid [1] are useful beyond a 24 hour postmortem period and onsite at crime scenes [4, 17, 18]. Currently, the most potential lies within methods that do not require laboratory equipment. Techniques such as pilocarpine eye drops, eye temperature, and chemical paper for vitreous fluid have introduced mobility and faster results to PMI methodologies.

Because of the overlap between the PMI and the criminal justice field, it is critical to create standard methods that produce reliable time of death estimations. Research of the eyes as a PMI estimator need to use larger population sizes, more trials, and address various

environmental or pathological factors that influence the postmortem changes. Valid application times and standardized patterns also need to be reproduced. Table 1 summarizes the limitations and application times of each method. It is possible that one technique's limitations may overlap with another even if the effects have not been directly studied. In the future, it is possible that these methods need to be combined when analyzing a death based off of specific crime scene evidence or medical history. However, it is only from research of each method's limitations that a dependable method or combination of methods can be determined.

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