Review of Postmortem Interval Estimation Using Vitreous Humor: Past, Present, and Future

Brandi C. McCleskey, Daniel W. Dye, Gregory G. Davis

ABSTRACT

For decades, forensic scientists have sought a means of estimating the postmortem interval using laboratory analyses. The best known of these attempts uses a linear regression formula based on the increasing concentration of potassium ions in vitreous humor following death. Like all laboratory analyses, the determination of a potassium concentration is subject to pre-analytical, analytical, and post-analytical errors. Any error is magnified when entered into a regression formula that itself is subject to statistical variation, typically with a 95% confidence interval. Estimating the postmortem interval based solely on the concentration of potassium in vitreous humor proved too simplistic for accurate modeling of the myriad factors that influence postmortem changes. Research continues, using more complicated algorithms involving multivariate ion and chemical analyses and genomic sequencing of the postmortem biome. However refined estimates of the postmortem interval based on laboratory analysis become, sound medical practice will still require the integration of scene findings and information concerning the last time that a given decedent was known to be alive with the results of postmortem examination and laboratory analyses into a medical opinion concerning the postmortem interval. *Acad Forensic Pathol. 2016 6(1): 12-18*

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INTRODUCTION

In cases of unwitnessed death, forensic investigators and family members often want to know the time between death and discovery of a body. Family members may ask whether some intervening action could have prevented death, and if so, whether something or someone is to blame for this death. Forensic investigators could use an accurate postmortem interval to corroborate a witness's statements, prove or disprove alibis, ease tension between family members, and ideally as evidence in a court of law (1-10). Despite decades of research, no forensic scientist has found a metric to determine the postmortem interval with sufficient accuracy to be adopted universally. This article reviews past and present attempts to estimate the postmortem interval by analyzing samples collected during postmortem examination.

BACKGROUND

In the immediate postmortem period, determination of the postmortem interval by examination of the body relies on subjective methods such as assessment of livor and rigor mortis and objective measurements of body cooling, rectal temperature, and changes in the vitreous chemistries (1, 7, 8, 11-15). Also described, mostly in European literature, analysis of the supravitality of tissues with skeletal muscle stimulation or pupillary response to chemical stimuli has been applied in the immediate postmortem period (usually less than 20 hours postmortem) (1, 16, 17). Most published work has focused on postmortem analysis of vitreous humor. Vitreous humor is a unique source of physical evidence in forensic pathology owing to its location, relative protection from bacterial contamination, ease of sampling, and general availability during postmortem examination (5, 6, 9, 15, 18-26). The vitreous sample is typically collected via a scleral puncture and aspiration of gelatinous contents of the posterior chamber of the eye, with care to avoid blood or retinal cell contamination, which can yield a variety of false results (3, 12, 23, 27-31). Common practice during postmortem examination is to collect vitreous humor samples at one time point from both eyes and combine them into one sample (3, 11, 32-36). Few authors have demonstrated variability between the eyes, assuming that the eyes appear to be in a similar state of preservation; however, recent work has shown that the variability is minimal during repetitive sampling from a single corpse over the course of many hours (5, 15, 30, 37, 38).

DISCUSSION

The loss of active ATP-mediated ion transport, loss of barrier integrity between cells, membranes, and fluids, as well as general cellular degradation and autolysis during decomposition have allowed researchers to examine a plethora of ion kinetics in the postmortem period using vitreous humor with varying success (1, 6, 7, 9, 15, 36, 39-46). With the knowledge that the baseline potassium concentration in the vitreous humor is within a physiological range of 3.5 to 5.0 mmol/L and that potassium leaks into the vitreous humor from neighboring cells via diffusion, many researchers proposed that the concentration of potassium in the vitreous humor changed at a predictable rate following death (32, 33, 35, 47-56). These various researchers plotted data and obtained a linear relationship between the postmortem interval and the rise in vitreous potassium concentration in the postmortem period spanning a few hours to a few days. They then used linear regression to determine a formula that they proposed would allow an individual to determine the postmortem interval in a new case by measuring the concentration of potassium in the vitreous humor and entering this number as the independent variable into their linear regression formula. A more recent publication by Zilg et al. demonstrated a nonlinear correlation that approached asymptotic after five days (1). Few studies have evaluated the use of vitreous potassium in the late postmortem period, as correlation weakens beyond a few days postmortem, likely due to decomposition and volume changes (1, 48). Some researchers are evaluating inorganic content of bone in their attempt to determine the postmortem interval when a body has entered the stage of putrefactive decay (57, 58).

Estimating the postmortem interval from the concentration of potassium in vitreous humor sounds like an elegantly simple method of estimating the time of death, so why does it not work? Analysis of a sample of vitreous humor obtained at postmortem examination is subject to all the sources of error that affect clinical laboratory analyses – pre-analytical error, analytical error, and post-analytical error. Tight though the control of the potassium concentration in the body is, it still varies from 3.5-5.0 mmol/L, meaning that a normal concentration of 5.0 mmol/L exceeds a normal concentration of 3.5 mmol/L by 40%. On that basis alone, one could argue that an estimated postmortem interval of three hours could vary by up to roughly 75 minutes in either direction, providing an estimated time of death ranging between 1.75-4.25 hours prior to collection of the vitreous humor. This range may be longer than family members and investigators would find useful and is little better than making an educated guess based on reliable evidence that shows the last time that the decedent was known to be alive.

Another source of variability are factors that affect the degree and speed at which potassium leaks from the inner cell mass of the eye into the vitreous humor. Health conditions such as renal failure and diabetes mellitus can affect the overall homeostasis of electrolytes throughout the body, and thereby alter baseline values, leading to erroneous estimations of time of death (1, 59). More recent publications have looked at the effect on potassium diffusion of ambient temperature and time within that environment, including the controlled temperature environments of the morgue, room temperature, and elevated environmental temperatures (1, 34, 46, 60-64). It appears that the relative increase in potassium is affected by higher temperatures and the duration that a body is at those temperatures. Obviously, a corpse left at room temperature or higher for a considerable amount of time will also progress through the stages of decomposition quickly and further complicate the ability to utilize vitreous humor for testing. Interestingly, some investigators report that a decedent's age affects the concentration of vitreous potassium, although this is not universally agreed upon (1, 3, 7, 65, 66). The effect of age is hypothesized to be due to globe size, vitreous composition, and cell mass or volume (1). It may be assumed that the agonal period and numerous neurotransmitters released during this period may cause shifts in cell ion concentrations; however, few have demonstrated that this affects concentration of potassium seen in the vitreous including samples collected from victims of hanging, burns, trauma, or poisoning (1, 49, 59).

Additional errors in accuracy and precision have been shown due to sources of pre-analytic variability including sample preparation and pretreatment as well as analytic variation regarding instrumentation available and technique utilized (15, 17-19, 29, 34, 36, 37, 67-73). It is currently recommended to centrifuge the sample in order to assist with liquefying the vitreous sample for testing and for each institution to develop standardized values since there are no normalized ranges for postmortem vitreous chemistries established at this time (29, 74, 75). More important still is that different researchers used different techniques to analyze the potassium concentration in vitreous humor. Because the concentration of potassium depends in part upon the technique used for analysis, a given regression formula determined by a given researcher is only truly valid if one uses the same analytical process as was used in the original research. Thus, entering a potassium concentration into a regression formula determined by a different technique creates post-analytical error. Moreover, any regression formula should be published with a confidence interval, typically a 95% confidence interval, and this confidence interval must also be allowed for when using a formula to estimate the postmortem interval, further decreasing the accuracy of the estimate.

Determining the postmortem interval from the concentration of potassium in vitreous humor was very attractive when it was proposed because the determination is easily made and the formula easily calculated, but the formula proved too simple to accommodate variability from decedent to decedent and from scene to scene, rendering controversial conclusions (6, 17, 33, 59, 75, 76). That does not mean that the work already done is not useful. Science advances in this sort of way – simple theories are proposed, rejected because their simplicity is inadequate to accurately model circumstances, and eventually replaced by more complicated models that do accurately model the behavior of the system being studied. A simple test that would accurately estimate the postmortem interval would be useful, but it is simplistic to think that one test will answer a medical question. A diagnosis of myocardial infarction based solely on an elevated troponin concentration is less convincing than a diagnosis based on a history of chest pain with tingling in the left arm, ST segment elevation on an electrocardiogram, and the appropriate elevation of troponin. In the same way, vitreous humor analysis alone cannot be used for estimating the postmortem interval with accuracy. Forensic pathologists can expect that the most accurate estimation of the postmortem interval will incorporate information from the combination of data retrieved during investigation, postmortem examination, and ancillary testing (77).

CONCLUSION

In order for postmortem laboratory analysis to provide a useful estimation of the postmortem interval, the analysis must be valid, reproducible, accurate, accessible, and quick. To achieve this, researchers must continue to provide relevant data details, utilize varied populations with adequate power in each group, utilize validated testing parameters, provide confidence intervals, and rigorously test the mathematical model in a blinded and controlled fashion (1, 15, 29, 75). Researchers can begin this testing themselves by using whatever formula they develop for estimating the postmortem interval in a blinded fashion on a new group of cases for which one individual knows the postmortem interval by means of alternate information (e.g., a witnessed death). If a person blinded to the correct answer can use the formula or algorithm to provide an accurate estimate of the time between examination and death, then the researchers can be confident that they are reporting a useful model. If the proposed method for estimating the postmortem interval does not predict the time of death accurately for a control group where the time of death is known, then the researchers will know that they must continue their work and temper their enthusiasm for the usefulness of their current model when they describe it in presentations and publications.

The Future

Multivariate analysis has supplanted a simple linear regression formula for estimating the postmortem interval (58, 78), but even this advanced analysis is prone to error of up to 5.5 hours. This may help suggest whether death occurred on one day or another, but the time frames that are useful for homicide investigations often depend on smaller intervals than 5.5 hours, and so this approach has limited usefulness. Analysis of the concentrations of multiple chemicals requires sophisticated computer algorithms, and the future of postmortem interval determination is likely to proceed along this path, accompanying the rest of medical practice into a world of sophisticated technology and testing platforms including molecular tools and next generation sequencing. One area with great potential is genetic sequencing of the microbial community from grave soil and the human gut microbiome (8, 79-84). It is well-understood that a corpse left in the environment will be inhabited by regular successions of organisms and microbes. Research emerging over the last few years has investigated the use of microbial diversity and the "postmortem microbial clock" to determine the postmortem interval (2, 85). Recent work by Metcalf, Carter, Hauther and many others, well summarized by Finley, has shown similar findings among the microbial environments in both a controlled environment using non-human species as well as an outdoor environment using donated human corpses (2, 8, 79, 85). Although the microbial community demonstrates variation among geographical areas, soil conditions, and seasons, it appears that the relative population of certain microbes can be used to provide a wealth of information and perhaps assist in determining the estimated time of death. Given the increased availability and cost-effectiveness of sequencing and identification methods, this area will likely provide many avenues for research toward determining an algorithm that will bring us closer to a laboratory method useful for determining the postmortem interval in each case.

REFERENCES

1) Zilg B, Bernard S, Alkass K, et al. A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature. *Forensic Sci Int*. 2015 Sep; 254:158-66. PMID: 26232848. http://dx.doi.org/10.1016/j.forsciint.2015.07.020.
2) Metcalf JL, Wegener Parfrey L, Gonzalez A, et al. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. *Elife*. 2013 Oct 15; 2:e01104. PMID: 24137541. PMCID: PMC3796315. http://dx.doi.org/10.7554/elife.01104.
3) Coe JI. Vitreous potassium as a measure of the postmortem interval: an historical review and critical evaluation. *Forensic Sci Int*. 1989 Aug; 42(3):201-13. PMID: 2676789. http://dx.doi.org/10.1016/0379-0738(89)90087-x.
4) Sturner W, Gantner GE Jr. The postmortem interval: a study of potassium in the vitreous humor. *Am J Clin Pathol*. 1964 Aug; 42: 137-44. PMID: 14202146.
5) Chandrakanth HV, Kanchan T, Balaraj BM, et al. Postmortem vitreous chemistry--an evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 h after death). *J Forensic Leg Med*. 2013 May; 20(4):211-6. PMID: 23622461. http://dx.doi.org/10.1016/j.jflm.2012.09.001.
6) Swain R, Kumar A, Sahoo J, et al. Estimation of post-mortem interval: A comparison between cerebrospinal fluid and vitreous humour chemistry. *J Forensic Leg Med*. 2015 Nov; 36:144-8. PMID: 26454503. http://dx.doi.org/10.1016/j.jflm.2015.09.017.
7) Jashnani KD, Kale SA, Rupani AB. Vitreous humor: biochemical constituents in estimation of postmortem interval. *J Forensic Sci*. 2010 Nov; 55(6):1523-7. PMID: 20666922. http://dx.doi.org/10.1111/j.1556-4029.2010.01501.x.
8) Hauther KA, Cobaugh KL, Jantz LM, et al. Estimating time since death from postmortem human gut microbial communities. *J Forensic Sci*. 2015 Sep; 60(5):1234-40. http://dx.doi.org/10.1111/1556-4029.12828.
9) Kalra JBN. Vitreous humor: a short review on post-mortem applications. *J Clin Exp Pathol*. 2015; 5(1). http://dx.doi.org/10.4172/2161-0681.1000199.
10) Reddy NKS. The essentials of forensic medicine and toxicology. 24th ed: New Delhi: K. Suguna Devi Publication; 2005. 569 p.
11) Sturner WQ. The vitreous humour: postmortem potassium changes. *Lancet*. 1963 Apr 13; 1(7285):807-8. PMID: 13978991. http://dx.doi.org/10.1016/s0140-6736(63)91509-5.
12) Muñoz JI, Suárez-Peñaranda JM, Otero XL, et al. A new perspective in the estimation of postmortem interval (PMI) based on vitreous. *J Forensic Sci*. 2001 Mar; 46(2):209-14. PMID: 11305419.
13) Madea B, Henssge C. Forensic medicine: clinical and pathological aspects. London: Cambridge University Press; c2003. Chapter 9, Timing of death; p. 91-114.
14) Muggenthaler H, Sinicina I, Hubig M, Mall G. Database of post-mortem rectal cooling cases under strictly controlled conditions: a useful tool in death time estimation. *Int J Legal Med*. 2012 Jan; 126(1):79-87. PMID: 21538225. http://dx.doi.org/10.1007/s00414-011-0573-6.
15) Mihailovic Z, Atanasijevic T, Popovic V, et al. Estimation of the postmortem interval by analyzing potassium in the vitreous humor: could repetitive sampling enhance accuracy? *Am J Forensic Med Pathol*. 2012 Dec; 33(4):400-3. PMID: 22922550. http://dx.doi.org/10.1097/paf.0b013e31826627d0.
16) Dettmeyer R, Verhoff MA, Schütz HF. Forensic medicine: fundamentals and perspectives. Heidelberg: Springer Science & Business Media; 2014. 584 p.
17) Doberantz E, Döppes D, Henssge C, et al. Estimation of the time since death. 3rd ed. Boca Raton: CRC Press; 2015. 282 p.
18) Coe JI. Postmortem chemistry update emphasis on forensic application. *Am J Forensic Med Pathol*. 1993 Jun; 14(2):91-117. PMID: 8328447. http://dx.doi.org/10.1097/00000433-199306000-00001.
19) Madea B. Is there recent progress in the estimation of the postmortem interval by means of thanatochemistry? *Forensic Sci Int*. 2005 Jul 16; 151(2-3):139-49. PMID: 15939145. http://dx.doi.org/10.1016/j.forsciint.2005.01.013.
20) Honey D, Caylor C, Luthi R, Kerrigan S. Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. *J Anal Toxicol*. 2005 Jul-Aug; 29(5):365-9. PMID: 16105262. http://dx.doi.org/10.1093/jat/29.5.365.
21) Harper DR. A comparative study of the microbiological contamination of postmortem blood and vitreous humour samples taken for ethanol determination. *Forensic Sci Int*. 1989 Sep; 43(1):37-44. PMID: 2591842. http://dx.doi.org/10.1016/0379-0738(89)90120-5.
22) Zilg B, Alkass K, Berg S, Druid H. Postmortem identification of hyperglycemia. *Forensic Sci Int*. 2009 Mar 10; 185(1-3):89-95. PMID: 19167848. http://dx.doi.org/10.1016/j.forsciint.2008.12.017.
23) Madea B, Musshoff F. Postmortem biochemistry. *Forensic Sci Int*. 2007 Jan 17; 165(2-3):165-71. PMID: 16781101. http://dx.doi.org/10.1016/j.forsciint.2006.05.023.
24) Coe JI. Postmortem chemistry: practical considerations and a review of the literature. *J Forensic Sci*. 1974 Jan; 19(1):13-32. PMID: 4853713.
25) Egger SF, Buxbaum A, Georgopoulos M, et al. Bacterial growth in human vitreous humor. *Exp Eye Res*. 1997 Dec; 65(6):791-5. PMID: 9441702. http://dx.doi.org/10.1006/exer.1997.0385.
26) Garg V, Oberoi S, Gorea R, Kaur K. Changes in the levels of vitreous potassium with increasing time since death. *J Indian Acad Forensic Sci*. 2004 Oct; 26(4):136-9.
27) Muñoz Barús JI, Suárez-Peñaranda J, Otero XL, et al. Improved estimation of postmortem interval based on differential behaviour of vitreous potassium and hypoxantine in death by hanging. *Forensic Sci Int*. 2002 Jan 24; 125(1):67-74. PMID: 11852204. http://dx.doi.org/10.1016/s0379-0738(01)00616-8.
28) Madea B, Käferstein H, Hermann N, Sticht G. Hypoxanthine in vitreous humor and cerebrospinal fluid—a marker of postmortem interval and prolonged (vital) hypoxia? Remarks also on hypoxanthine in SIDS. *Forensic Sci Int*. 1994 Mar; 65(1):19-31. PMID: 8206451. http://dx.doi.org/10.1016/0379-0738(94)90296-8.
29) Blana S, Musshoff F, Hoeller T, et al. Variations in vitreous humor chemical values as a result of pre-analytical treatment. *Forensic Sci Int*. 2011 Jul 15; 210(1-3):263-70. PMID: 21511417. http://dx.doi.org/10.1016/j.forsciint.2011.03.023.
30) Mulla A, Massey KL, Kalra J. Vitreous humor biochemical constituents: evaluation of between-eye differences. *Am J Forensic Med Pathol*. 2005 Jun; 26(2):146-9. PMID: 15894848.
31) Bito LZ. The physiology and pathophysiology of intraocular fluids. *Exp Eye Res*. 1977; 25 Suppl:273-89. PMID: 338321. http://dx.doi.org/10.1016/s0014-4835(77)80024-9.
32) Jaffe F. Chemical postmortem changes in the intraocular fluid. *J Forensic Sci*. 1962; 7:231-7.
33) Adjutantis G, Coutselinis A. Estimation of the time of death by potassium levels in the vitreous humour. *Forensic Sci*. 1972 Apr; 1(1):55-60. PMID: 5054823. http://dx.doi.org/10.1016/0300-9432(72)90147-1.
34) Rognum TO, Hauge S, Øyasaeter S, Saugstad OD. A new biochemical method for estimation of postmortem time. *Forensic Sci Int*. 1991 Oct; 51(1):139-46. PMID: 1752591. http://dx.doi.org/10.1016/0379-0738(91)90214-4.

35) Coe JI. Postmortem chemistries on human vitreous humor. *Am J Clin Pathol*. 1969 Jun; 51(6):741-50. PMID: 5770672.

36) Henssge C, Madea B. Eye changes after death. In: Henssge C, Knight B, Krompecher T, et al, editors. The estimation of the time since death in the early postmortem period. London: Edward Arnold; 1995. p. 106-37.

37) Pounder DJ, Carson DO, Johnston K, Orihara Y. Electrolyte concentration differences between left and right vitreous humor samples. *J Forensic Sci*. 1998 May; 43(3):604-7. PMID: 9608696.

38) Balasooriya B, St Hill C, Williams A. The biochemistry of vitreous humour. A comparative study of the potassium, sodium and urate concentrations in the eyes at identical time intervals after death. *Forensic Sci Int*. 1984 Oct; 26(2):85-91. PMID: 6489885. http://dx.doi.org/10.1016/0379-0738(84)90064-1.

39) Ingham AI, Byard RW. The potential significance of elevated vitreous sodium levels at autopsy. *J Forensic Leg Med*. 2009 Nov; 16(8):437-40. PMID: 19782311. http://dx.doi.org/10.1016/j.jflm.2009.07.013.

40) Mihailovic Z, Atanasijevic T, Popovic V, Milosevic MB. Could lactates in vitreous humour be used to estimate the time since death? *Med Sci Law*. 2011 Jul; 51(3):156-60. PMID: 21905571. http://dx.doi.org/10.1258/msl.2011.010124.

41) Palmiere C, Mangin P. Postmortem chemistry update part I. *Int J Legal Med*. 2012 Mar; 126(2):187-98. PMID: 21947676. http://dx.doi.org/10.1007/s00414-011-0625-y.

42) Palmiere C, Mangin P. Postmortem chemistry update part II. *Int J Legal Med*. 2012 Mar; 126(2):199-215. PMID: 21984165. http://dx.doi.org/10.1007/s00414-011-0614-1.

43) Bévalot F, Cartiser N, Bottinelli C, et al. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: a review. *Forensic Toxicol*. 2016; 34(1):12-40. PMID: 26793276. PMCID: PMC4705140. http://dx.doi.org/10.1007/s11419-015-0294-5.

44) Sebag J. The vitreous: structure, function, and pathobiology. New York: Springer-Verlag; 1989. 173 p.

45) Farmer JG, Benomran F, Watson AA, Harland WA. Magnesium, potassium, sodium and calcium in post-mortem vitreous humour from humans. *Forensic Sci Int*. 1985 Jan; 27(1):1-13. PMID: 3988191. http://dx.doi.org/10.1016/0379-0738(85)90099-4.

46) Siddamsetty AK, Verma SK, Kohli et al. Estimation of time since death from electrolyte, glucose and calcium analysis of postmortem vitreous humour in semi-arid climate. *Med Sci Law*. 2014 Jul; 54(3): 158-66. PMID: 24166687. http://dx.doi.org/10.1177/0025802413506424.

47) Madea B, Rödig A. Time of death dependent criteria in vitreous humor: accuracy of estimating the time since death. *Forensic Sci Int*. 2006 Dec 20; 164(2-3):87-92. PMID: 16439082. http://dx.doi.org/10.1016/j.forsciint.2005.12.002.

48) Hansson L, Uotila U, Lindfors R, Laiho K. Potassium content of the vitreous body as an aid in determining the time of death. *J Forensic Sci*. 1966 Jul; 11(3):390-4. PMID: 5967742.

49) Adelson L, Sunshine I, Rushforth NB, Mankoff M. Vitreous potassium concentration as an indicator of the postmortem interval. *J Forensic Sci*. 1963 Oct; 8(4):503-14.

50) Hughes WM. Levels of potassium in the vitreous humour after death. *Med Sci Law*. 1965 Jul; 5(3):150-6. PMID: 5828803.

51) Lie J. Changes of potassium concentration in the vitreous humor after death. *Am J Med Sci*. 1967 Aug; 254(2):136-43. PMID: 4951791. http://dx.doi.org/10.1097/00000441-196708000-00002.

52) Naumann HN. Postmortem chemistry of the vitreous body in man. *Arch Ophthalmol*. 1959 Sep; 62:356-63. PMID: 14426195. http://dx.doi.org/10.1001/archopht.1959.04220030012003.

53) Dominiczak MH, Woodward M. Flesh and bones of metabolism. New York: Elsevier Health Sciences; 2007. 156 p.

54) Hall JE, Guyton AC. Guyton and Hall textbook of medical physiology. London: Saunders; 2011. 1264 p.

55) James RA, Hoadley PA, Sampson BG. Determination of postmortem interval by sampling vitreous humour. *Am J Forensic Med Pathol*. 1997 Jun; 18(2):158-62. PMID: 9185933. http://dx.doi.org/10.1097/00000433-199706000-00010.

56) Madea B, Herrmann N, Henssge C. Precision of estimating the time since death by vitreous potassium—comparison of two different equations. *Forensic Sci Int*. 1990 Jul; 46(3):277-84. PMID: 2376367. http://dx.doi.org/10.1016/0379-0738(90)90313-n.

57) Schwarcz HP, Agur K, Jantz LM. A new method for determination of postmortem interval: citrate content of bone. *J Forensic Sci*. 2010 Nov; 55(6):1516-22. PMID: 20681964. http://dx.doi.org/10.1111/j.1556-4029.2010.01511.x.

58) Vass AA, Barshick SA, Sega G, et al. Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. *J Forensic Sci*. 2002 May; 47(3):542-53. PMID: 12051334.

59) Madea B, Henssge C, Hönig W, Gerbracht A. References for determining the time of death by potassium in vitreous humor. *Forensic Sci Int*. 1989 Mar; 40(3):231-43. PMID: 2731842. http://dx.doi.org/10.1016/0379-0738(89)90181-3.

60) Komura S, Oshiro S. Potassium levels in the aqueous and vitreous humor after death. *Tohoku J Exp Med*. 1977 May; 122(1):65-8. PMID: 918956. http://dx.doi.org/10.1620/tjem.122.65.

61) Bray M. The effect of chilling, freezing, and rewarming on the postmortem chemistry of vitreous humor. *J Forensic Sci*. 1984 Apr; 29(2):404-11. PMID: 6427391.

62) Schoning P, Strafuss AC. Determining time of death of a dog by analyzing blood, cerebrospinal fluid, and vitreous humor collected at postmortem. *Am J Vet Res*. 1980 Jun;41(6):955-7. PMID: 7436089.

63) McLaughlin PS, McLaughlin BG. Chemical analysis of bovine and porcine vitreous humors: correlation of normal values with serum chemical values and changes with time and temperature. *Am J Vet Res*. 1987 Mar; 48(3):467-73. PMID: 3565904.

64) Henssge C. Rectal temperature time of death nomogram: dependence of corrective factors on the body weight under stronger thermic insulation conditions. *Forensic Sci Int*. 1992 Apr; 54(1):51-66. PMID: 1618454. http://dx.doi.org/10.1016/0379-0738(92)90080-g.

65) Blumenfeld TA, Mantell CH, Catherman RL, Blanc WA. Postmortem vitreous humor chemistry in sudden infant death syndrome and in other causes of death in childhood. *Am J Clin Pathol*. 1979 Feb; 71(2):219-23. PMID: 425937.

66) Mason JK, Harkness RA, Elton RA, Bartholomew S. Cot deaths in Edinburgh: infant feeding and socioeconomic factors. *J Epidemiol Community Health*. 1980 Mar; 34(1):35-41. PMID: 7189206. PMCID: PMC1052037. http://dx.doi.org/10.1136/jech.34.1.35.

67) Thierauf A, Musshoff F, Madea B. Post-mortem biochemical investigations of vitreous humor. *Forensic Sci Int*. 2009 Nov 20; 192(1-3): 78-82. PMID: 19729257. http://dx.doi.org/10.1016/j.forsciint.2009.08.001.

68) Coe JI, Apple FS. Variations in vitreous humor chemical values as a result of instrumentation. *J Forensic Sci*. 1985 Jul; 30(3):828-35. PMID: 4031810.

69) Bocaz-Beneventi G, Tagliaro F, Bortolotti F, et al. Capillary zone electrophoresis and artificial neural networks for estimation of the post-mortem interval (PMI) using electrolytes measurements in human vitreous humour. *Int J Legal Med*. 2002 Feb; 116(1):5-11. PMID: 11924710. http://dx.doi.org/10.1007/s004140100239.

70) McNeil AR, Gardner A, Stables S. Simple method for improving the precision of electrolyte measurements in vitreous humor. *Clin Chem*. 1999 Jan; 45(1):135-6. PMID: 9895352.

71) Tagliaro F, Manetto G, Cittadini F, et al. Capillary zone electrophoresis of potassium in human vitreous humour: validation of a new method. *J Chromatogr B Biomed Sci Appl*. 1999 Oct 15; 733(1-2): 273-9. PMID: 10572986. http://dx.doi.org/10.1016/s0378-4347(99)00338-2.

72) Zhou B, Zhang L, Zhang G, et al. The determination of potassium concentration in vitreous humor by low pressure ion chromatography and its application in the estimation of postmortem interval. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007 Jun 1;852(1-2): 278-81. PMID: 17347058. http://dx.doi.org/10.1016/j.jchromb.2007.01.027.

73) Gagajewski A, Murakami MM, Kloss J, et al. Measurement of chemical analytes in vitreous humor: stability and precision studies. *J Forensic Sci*. 2004 Mar; 49(2):371-4. PMID: 15027563. http://dx.doi.org/10.1520/jfs2003152.

74) Lendoiro E, Cordeiro C, Rodriguez-Calvo MS, et al. Applications of Tandem Mass Spectrometry (LC-MSMS) in estimating the post-mortem interval using the biochemistry of the vitreous humour. *Forensic Sci Int*. 2012 Nov 30; 223(1-3):160-4. PMID: 22981211. http://dx.doi.org/10.1016/j.forsciint.2012.08.022.

75) Mitchell R, Charlwood C, Thomas SD, et al. An audit of the contribution to post-mortem examination diagnosis of individual analyte results obtained from biochemical analysis of the vitreous. *Forensic Sci Med Pathol*. 2013 Dec; 9(4):515-20. PMID: 23852932. http://dx.doi.org/10.1007/s12024-013-9469-8.

76) Stephens RJ, Richards RG. Vitreous humor chemistry: The use of potassium concentration for the prediction of the postmortem interval. *J Forensic Sci*. 1987 Mar; 32(2):503-9. PMID: 3572343.

77) Vass AA. The elusive universal post-mortem interval formula. *Forensic Sci Int*. 2011 Jan 30; 204(1-3):34-40. PMID: 20554133. http://dx.doi.org/10.1016/j.forsciint.2010.04.052.

78) Bortolotti F, Pascali JP, Davis GG, et al. Study of vitreous potassium correlation with time since death in the postmortem range from 2 to 110 hours using capillary ion analysis. *Med Sci Law*. 2011; 51 Suppl 1:S20-3. PMID: 22021629. http://dx.doi.org/10.1258/msl.2010.010063.

79) Carter DO, Metcalf JL, Bibat A, Knight R. Seasonal variation of postmortem microbial communities. *Forensic Sci Med Pathol*. 2015 Jun; 11(2):202-7. PMID: 25737335. http://dx.doi.org/10.1007/s12024-015-9667-7.

80) Tuomisto S, Karhunen PJ, Vuento R, et al. Evaluation of postmortem bacterial migration using culturing and real-time quantitative PCR. *J Forensic Sci*. 2013 Jul; 58(4):910-6. PMID: 23550887. http://dx.doi.org/10.1111/1556-4029.12124.

81) Matuszewski S, Bajerlein D, Konwerski S, Szpila K. Insect succession and carrion decomposition in selected forests of Central Europe. Part 1: Pattern and rate of decomposition. *Forensic Sci Int*. 2010 Jan 30; 194(1-3):85-93. PMID: 19914786. http://dx.doi.org/10.1016/j.forsciint.2009.10.016.

82) Centeno N, Maldonado M, Oliva A. Seasonal patterns of arthropods occurring on sheltered and unsheltered pig carcasses in Buenos Aires Province (Argentina). *Forensic Sci Int*. 2002 Mar 28; 126(1):63-70. PMID: 11955835. http://dx.doi.org/10.1016/s0379-0738(02)00037-3.

83) Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature*. 2007 Oct 18; 449(7164):804-10. PMID: 17943116 . PMCID: PMC3709439. http://dx.doi.org/10.1038/nature06244.

84) Payne JA. A summer carrion study of the baby pig Sus scrofa Linnaeus. *Ecology*. 1965 Sep; 46(5):592-602. http://dx.doi.org/10.2307/1934999.

85) Finley SJ, Benbow ME, Javan GT. Microbial communities associated with human decomposition and their potential use as postmortem clocks. *Int J Legal Med*. 2015 May; 129(3):623-32. PMID: 25129823. http://dx.doi.org/10.1007/s00414-014-1059-0.