



ASOCIACIÓN NACIONAL
DE
MÉDICOS FORENSES

Spanish Journal of Legal Medicine

Revista Española de Medicina Legal

www.elsevier.es/mlegal



REVIEW

Review of cadaveric dating methods and new perspectives from the necrobiome[☆]

Ángel M. Aragonés, Silvana Teresa Tapia-Paniagua^{*}

Departamento de Microbiología, Campus de Teatinos, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

Received 24 February 2021; accepted 30 May 2021

KEYWORDS

Necrobiome;
Thanatomicrobiome;
Forensic
microbiology;
Post-mortem interval

Abstract Nowadays there are numerous scientific strategies that helping to clarify forensic cases, including time since death. The absence of reliable quantitative methods to estimate the *post-mortem* interval explains the increase in promising new lines of research for this purpose. After the appearance of the new techniques of massive sequencing and bioinformatics, also arises the study of the necrobiome through a new and little studied area within the forensic sciences, Forensic Microbiology. In this review, a tour of the existing techniques and procedures of cadaveric dating is made, which includes new cutting-edge techniques in different areas of knowledge and also mentions the utilities of Forensic Microbiology, where the thanatomicrobiome, present from the moment of death, according to recent studies, points to be a promising method for estimating the *post-mortem* interval in the future.

© 2021 Asociación Nacional de Médicos Forenses. Published by Elsevier España, S.L.U. All rights reserved.

PALABRAS CLAVE

Necrobioma;
Tanatomicrobioma;
Microbiología
forense;
Intervalo *post mortem*

Revisión sobre las nuevas perspectivas de datación cadavérica desde el necrobioma

Resumen Hoy en día existen numerosas estrategias desde un punto de vista científico que ayudan a esclarecer los casos forenses, entre ellas la datación cadavérica. La ausencia de métodos fiables cuantitativos para estimar el intervalo *post mortem* explica el incremento de nuevas líneas de investigación prometedoras con dicha finalidad. Tras la aparición de las nuevas técnicas de secuenciación masiva y bioinformáticas, surge también el estudio del necrobioma

DOI of original article: <https://doi.org/10.1016/j.reml.2021.05.001>

[☆] Please cite this article as: Aragonés ÁM, Tapia-Paniagua ST. Revisión sobre las nuevas perspectivas de datación cadavérica desde el necrobioma. Rev Esp Med Legal. 2022;48:30–35.

^{*} Corresponding author.

E-mail address: stapia@uma.es (S.T. Tapia-Paniagua).

como un área novedosa y poco estudiada dentro de las ciencias forenses, que se ha llegado a denominar como “microbiología forense”. En esta revisión se realiza un breve recorrido por las técnicas y procedimientos existentes de datación cadavérica, centrándose en la utilidad del tanatomicrobioma, o conjunto de microorganismos presentes en el momento de la muerte, que podría ser un método prometedor para la estimación del intervalo *post mortem* en el futuro.
© 2021 Asociación Nacional de Médicos Forenses. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

It is still a challenge to establish the *post mortem* interval (PMI) precisely. Several methods have been used to elucidate the PMI for several decades, many of which are imprecise and require a certain degree of subjective interpretation¹. In spite of this, they are still used and show no significant improvements in their reliability². The PMI can only be determined within certain limits of probability. To do so reliably it is only possible to empirically determine this on the basis of the statistical analysis of errors using the data obtained in field studies, and the reliability of the value obtained is then shown together with its degree of uncertainty³. The methods used to estimate the PMI are currently of limited applicability and precision. To improve the methods used a study timeframe will have to be selected in which to study the PMI. Nevertheless, it is possible that restricting this timeframe to a set period of time may not make sense. This is because the usefulness of cadaveric dating techniques is lined to the decomposition process, and this process varies depending on the influence of biotic factors. These factors include the intrinsic *ante mortem* properties of the body, internal and external bacteria, fungi, protozoa, necrophagic insects, vegetation and carrion-eating scavengers. Abiotic factors are also important, such as the presence or absence of a covering, radiation on remains, temperature, humidity and/or pH, among others⁴.

The use of any single method to estimate the PMI will not be very reliable, including Henssge's known nomogram method⁵, which is based on a single measurement of rectal temperature. This method is designed for use in the first 24 h, and the cooling curves it employs do not consider all of the variables. Some authors therefore believe it to be of limited applicability, and they recommend that it should be combined with other cadaveric dating techniques⁶. Other researchers seek to establish universal formulae⁷, although these have been said to be unreliable⁸, as it would be hard for a universal formula to give an exact estimation of the PMI because of the large number of variables involved in the decomposition process. In spite of the work that has gone into searching for a reliable quantitative technique for the estimation of the PMI, a suitable analyte or ideal sampling site have yet to be identified. The estimation of the PMI in human remains using bone analysis with radionuclides such as ²¹⁰Pb, ²¹⁰Po or ¹⁴C stands out at the present

time, as do measurements based on tissue citrate or nitrogen content^{9,10}. In less advanced stages of decomposition, evaluation of the changes undergone by molecules such as proteins¹¹, DNA and/or RNA after death are promising strategies. The relative quantity of a specific range of proteins has been shown to fall with increasing *post mortem* time^{2,12}.

With regard to the tissues analysed and suggested as possible indicators, vitreous humour has been shown to be less susceptible to suffer swift autolysis, making it very useful for sampling during the first hours *post mortem*¹³. The facial orifices are known to be the first sites of oviposition used by necrophagous insects¹⁴, and this process commences a few seconds or minutes after death¹⁵. Knowledge of the life-cycles of these necrophagous insects may offer useful information for the estimation of the PMI, although forensic entomology is restricted to corpses in an advanced stage of decomposition, as they have to be colonised beforehand (oviposition). This fact depends on environmental conditions, seasonality, light¹⁶, temperature, accessibility (whether the remains are located indoors or outside, or wrapped in plastic, etc.). Additionally, it has to be said that the type of insects will vary depending on the geographical location where the body was found¹⁷, thereby complicating the process of analysis and dating.

On the other hand, the commensal microbiota and external bacteria are now being studied for possible use in analysis for cadaveric dating. The commensal microbiota consists of a set of microorganisms which coexist with the host until the moment of death. The immune system ceases to function at that moment, permitting bacterial population to change without any restrictions or host barriers¹⁸. This factor can be analysed, as it remains isolated and protected from environmental factors and animal decomposition, and it is present from the moment of death. However, study of this phenomenon and its possible use for cadaveric dating commenced recently and it is still hardly understood¹⁹. Notwithstanding this, the arrival of new-omic techniques and analytical methodologies may revolutionise microbiological knowledge in estimation of the PMI.

The aim of the following chapters is to use a non-systematic review to describe the latest studies of the necrobiome and methodology used in cadaveric dating. It also covers the variables which most influence *post mortem* bacterial communities and which therefore must be taken into account when determining the PMI.

Necrobiome and cadaveric dating

The set of species which actively participate in the process of decomposing a cadaver is known as the "necrobiome"²⁰. These microorganisms (bacteria, fungi and protozoa) play a very important role within the necrobiome that has hardly been studied, offering information that is highly useful in forensic microbiology (FM). To date, the chief medical-legal reasons for requesting a microbiological analysis in a forensic autopsy are usually unexpected death with the suspicion of an infectious cause, sudden death in children, investigations into alleged medical malpractice or cardiac death in which a viral myocarditis is suspected²¹. Nevertheless, it has been seen that FM is able to offer vital information in medical-legal investigations regarding the time, place, cause and manner of death, as well as supplying valuable data for the identification of suspects²². When FM is used to determine the time of death, some studies found no variations in the decomposition process in association with sex, weight or the cause of death²³. These methods for estimating the PMI are based on the fact that the set of microorganisms that drive mammalian decomposition are similar and reproducible in specific hosts and environments²⁴. Within the necrobiome, the set of *post mortem* microbiome used to estimate the PMI may be classified into two groups: epinecrotic communities and the thanatomiobiome.

Epinecrotic communities are the set of microorganisms which live and/or move on the surfaces of decomposing remains, including surface epithelial tissues, facial mucosa and distal orifices of the digestive tract²⁵. A wide range of sampling sites have been used, including the skin and oral, nasal and auditory cavities²⁶, and soil associated with a cadaver has also been analysed²⁷ when they were buried or remained on the surface²⁸. Nevertheless, these locations are more influenced by abiotic factors because they are exposed to the environment, and this may lead to more errors when estimating the PMI. For example, the microbiological profile of the soil associated with cadavers has been found to vary significantly between two different seasons (summer and winter)²⁹.

On the other hand, after death the commensal microbiota of individuals undergoes a dysbiosis or alteration which several authors have termed the thanatomiobiome. This is defined as the set of bacteria which are found in the organs and internal cavities after death²⁵. In this case, samples are taken from gastrointestinal locations which contain abundant bacteria, although other authors have studied its progression through the internal organs during decomposition³⁰. Due to the wide range of sampling sites in a cadaver, which in some cases use procedures that are too invasive, there is still no agreement or normalisation of any specific place for taking samples²⁴.

Different studies indicate that the thanatomiobiome should be used to estimate the PMI, as it is the most promising source of information³¹, given that unlike the epinecrotic communities, it remains more isolated and stable³². However, to achieve a better estimation of the PMI and to guarantee the design of a single model for general use in analysis^{18,24} the different variables which may affect the thanatomiobiome must be taken into account. Many dif-

ferent variables which influence the speed of decomposition and therefore the composition of the thanatomiobiome have been described²⁴. Temperature is the most influential variable, as many microorganisms have an optimum temperature for growth and activity, with a notable affect on decomposition¹⁸. A study in rats showed a higher quantity of *proteobacteria*, followed by *Firmicutes* in spring, and this filum was the most abundant in summer³³. Another study in pigs showed that some bacteria are season-specific, as *Carnobacterium*, *Marinomonas*, *Aeromonas* and *Bacteroidales* were only present in autumn, and *Polaribacter* and *Bacteroidales* were only present in winter³⁴. The type of environment has also been found to have a strong affect, so that it is important to use comparative models in different contexts. Several studies indicate that the predominant microbial communities during mammalian decomposition in terrestrial environments differ from those in aquatic media²⁴. Other studies have analysed the changes that occur in case of freezing. Hyde et al.⁴ studied the cadaveric thanatomiobiome in bodies that had been frozen for months, detecting *Firmicutes*, *Actinobacteria* and *Proteobacteria*; the second filum fell and the third increased with advancing time.

Other variables should also be assessed to discover whether they play an important role in the estimation of the PMI based on the thanatomiobiome. For example, the presence of necrophagous insects and carrion-eating animals will act together with the microorganisms, competing for the decomposition of remains¹⁸. Microorganism activity may also be affected by pH, the availability of oxygen and the degree of humidity¹⁸. On the other hand, other intrinsic *ante mortem* variables of an individual will also have to be taken into consideration, such as their stage of development. This is because larger amounts of *Actinobacteria*, *Fusobacteria* and *Gammaproteobacteria* have been found in infantile cadavers, with smaller amounts of *Firmicutes* than is the case in adults³⁵. The influence of sex on the estimation of the PMI should also be taken into account, as one study found differences in the coronary thanatomiobiome, where in men the *Firmicutes* filum was abundant, together with *Bacilli* and *Streptococcaceae*, while *Proteobacterias* predominated in women, together with *Pseudomonadales* and *Gammaproteobacterias*³⁶. Other variables may be the presence of infections *ante mortem*, drug use, alcohol abuse, antibiotic use and the type of diet etc¹⁸. Recent studies by Wójcik et al.³⁴ underlined the importance of knowing the medical history of the dead individual, as previous diseases altered the microbiota. The said factors alter the composition of the commensal microbiome during life, and in turn this is reflected in the thanatomiobiome¹⁸.

It is now no longer too much of a challenge to obtain a microbiological registry of a specific sample, thanks to the existence of mass sequencing techniques and taxonomically informative universal genes. These genes supply useful information for the identification of organisms (e.g., rRNA 16S, rRNA 18S, ITS genetic region), making it possible to obtain an instantaneous picture of the microbial taxa in a sample at a specific time²⁴. The majority of thanatomiobiome studies are based on the amplification and sequencing of the rRNA 16S gene¹⁸. Thanks to techniques of this type, studies during the different phases of

decomposition have determined that the diversity and richness of the organisms within the thanatomicrobiome fall over time, although the relative abundance of the groups which remain increases^{18,24,37}. This varies according to the stages of decomposition, with the opening up of the cadaver to the environment and the availability of oxygen, as is the case with the availability of nutrients^{31,37}. The human gastrointestinal tract has been shown to contain a high microbial burden, and the most important changes over time consist of a fall in the *Bacteroides* and *Parabacteroides* groups in favour of other communities, such as *Clostridium*, *Anaerospaera*, *Ignatzschineria* and *Wohlfahrtiimonas*³⁸. A study in 12 human cadavers found that the relative abundance of *Bacteroides* and *Lactobacillus* in the large intestine of these individuals fell exponentially over time, suggesting that these groups may be indicators quantitative *post mortem* indicators²³. On the other hand, a study in animal models undertaken in rats also showed a general tendency: it found that the relative abundance of the *Firmicutes* and *Proteobacteria* fila fell over the first 10 days after death, and that as a whole, diversity of the gut thanatomicrobiota tended to fall³⁷. This study determined that the *Prevotella*, *Enterococcus*, *Proteus* and *Sporosarcina* genii were the main microbial communities to appear after death, and their relative abundance changed together with the decomposition process in the cadavers, suggesting a possible correlation with the PMI³⁷. Nevertheless, other studies observed that at higher taxonomic levels, the variability found is explained. Analyses at family and genus level explained 21% of the correlation models that were drawn up, while when they switched to species level, they explained 65% of the same. This suggests that it is necessary to study and identify microorganisms at species level, so that they can be evaluated as possible biomarkers³⁹.

The most exact estimations of the PMI have used automatic learning methods that employ changes in the relative abundance of all the microorganisms in the thanatomicrobiome²⁴. Automatic learning is a part of computing, and it is a branch of artificial intelligence that automates computers so that they can learn data, identify patterns and make predictions without being expressly programmed to do so¹⁹. It has been widely used in biomedical research, from cancer diagnosis to study of the human microbiome¹⁹. Automatic learning is a powerful tool for the discovery of patterns in complex data sets, such as the thanatomicrobiome, which has large numbers of sequences representing different types of microorganisms in it. The regression methods obtained using automatic learning with the thanatomicrobiome have excellent potential to predict the PMI in many mammalian species, in controlled environments as well as in the open air¹⁹. To construct and calibrate a robust model for the estimation of the PMI a series of samples must be taken at known intervals, taking into account the above-mentioned variables which affect the succession and composition of the thanatomicrobiome. Additionally, it is probable that the exactitude of the technique may be improved if the experiments include daily or hourly sampling over a period of time¹⁹. Researchers have met this challenge by vertebrate decomposition experiments using human bodies that had been donated to science, or animal models, rodents or pigs, which are useful as they make it possible to introduce a large number of variables and replicas. The

sole environmental characteristic which has been included to date in a microbial decomposition regression model is the temperature²⁴. As new experiments are published with larger data sets, it will be possible to know whether their results can be extrapolated.

Several different techniques exist within the field of automatic learning, and they have yet to be systematically compared²⁴. Some authors prefer “Random Forest”, as it is said to be robust and more precise in comparative evaluative tests of sets of microbiome data¹⁹. Nevertheless, the study by Liu et al.³¹ showed a higher level of precision when using “artificial neuronal networks”, another algorithm technique used in automatic learning. Using operative taxonomic units (OTU) of blind samples the authors obtained a model fit of 1.5 ± 0.8 h in the first 24 h of decomposition, and of 14.5 ± 4.4 h up to the first 15 days of decomposition. It is important to point out that, although it has proven appropriate to date, some authors now consider the bioinformatics approach used to process all of the OTU and make functional predictions to be obsolete²⁹. Amplicon sequence variants (ASV) may offer a richer taxonomic image. While OTU grouping methods try to unify similar sequences in a consensus sequence, thereby minimising the influence of any sequencing error within the group of readings, the ASV method tries to move in the opposite direction. Future studies have yet to show whether ASV will improve predictive models of the PMI based on the thanatomicrobiome.

Final considerations

This paper described microbial succession after death and its potential within the forensic sciences. In a not-too-distant future the FM may offer useful information for establishing the time of death by studying and analysing the thanatomicrobiome. It is still not known whether this methodology for estimating the PMI will be sufficiently exact in real cases, or whether it will have to be combined with other existing techniques to improve its reliability. The progression of the thanatomicrobiome has been described in numerous biological replicas using animal models. The best models created using automatic learning techniques are those which have been obtained from identical individuals in controlled environments. However, there are actually many variables, such as those mentioned in this review, which affect decomposition and the thanatomicrobiome. It will be necessary to see which of these variables add the most weight to fit models to improve their precision, and which should be rejected because they only have a minimum influence on microbial progression. This has been observed, for example, in terms of cadaveric mass or the type of soil, both of which influence the thanatomicrobiome to a lesser degree²⁴.

Many projects are currently underway in the attempt to shed some light on all of these questions, such as the Human Post-mortem Microbiome Project [HPMP]³⁹. This centres on collecting and analysing data that is representative and is an indication of the abundance and variety of the microorganisms involved in the decomposition of human cadavers. It has the aim of supplying and collecting data corresponding to the thanatomicrobiome as well

as the necrobiome and the microbiota in the surrounding soil. This has the purpose of improved our comprehension of decomposition, and it attempts to resolve aspects such as the cause and manner of death, together with PMI estimations. It will also try to bring together work to validate and standardise protocols to establish a widespread and normalised usage of the thanatomicrobiome in forensic investigations.

All of the above considerations show the potential of the thanatomicrobiome within the field of forensic science. Moreover, new emerging analytical techniques now complement all of the existing ones. They are increasingly more accurate thanks to the constant improvement in techniques and sequencing processes, as well as mass data analysis. Nevertheless, there are still difficulties to overcome in data interpretation and regression, due to the high number of variables which are involved.

References

- Chandra J, Sabharwal K. Determination of time since death from a study of various postmortem changes. *J Indian Med Assoc.* 1968;7:336–41.
- Brooks JW. Postmortem changes in animal carcasses and estimation of the postmortem interval. *Vet Pathol.* 2016;53:929–40.
- Madea B. Methods for determining time of death. *Forensic Sci Med Pathol.* 2016;12:451–85.
- Hyde ER, Haarmann DP, Lynne AM, Bucheli SR, Petrosino JF. The living dead: bacterial community structure of a cadaver at the onset and end of the bloat stage of decomposition. *PLoS One.* 2013;8:e77733.
- Althaus L, Henssge C. Rectal temperature time of death nomogram: sudden change of ambient temperature. *Forensic Sci Int.* 1999;99:171–8.
- Bañón González RM, Hernández del Rincón JP. Determinación de la data en el periodo precoz de la muerte. *Métodos instrumentales.* *Rev Esp Med Legal.* 2010;36:83–6.
- Vass AA. The elusive universal post-mortem interval formula. *Forensic Sci Int.* 2011;204:34–40.
- Cockle DL, Bell LS. Human decomposition and the reliability of a “Universal” model for post mortem interval estimations. *Forensic Sci Int.* 2015;253:136.e1–9.
- Schwarcz HP, Agur K, Jantz LM. A new method for determination of postmortem interval: citrate content of bone. *J Forensic Sci.* 2010;55:1516–22.
- Ubelaker DH. Radiocarbon analysis of human remains: a review of forensic applications. *J Forensic Sci.* 2014;59:1466–72.
- Díaz Martín RD, Camacho-Martínez Z, Ambrosio Hernández JR, Valencia-Caballero L. La proteómica como una nueva herramienta en las ciencias forenses. *Rev Esp Med Legal.* 2018;45:114–22.
- Mathur A, Agrawal YK. An overview of methods used for estimation of time since death. *Aust J Forensic Sci.* 2011;43:275–85.
- Jashnani KD, Kale SA, Rupani AB. Vitreous humor: biochemical constituents in estimation of postmortem interval. *J Forensic Sci.* 2010;55:1523–7.
- Iancu L, Carter DO, Junkins EN, Purcarea C. Using bacterial and necrophagous insect dynamics for post-mortem interval estimation during cold season: novel case study in Romania. *Forensic Sci Int.* 2015;254:106–17.
- Perper J. Time of death and changes after death. Part-1: Anatomical considerations. In: Spitz WU, editor. *Spitz and fisher’s medicolegal investigation of death.* 4th edition Charles C Thomas publishers; 2004. p. 87–127.
- Archer MS, Elgar MA. Yearly activity patterns in southern Victoria (Australia) of seasonally active carrion insects. *Forensic Sci Int.* 2003;132:173–6.
- Tomberlin JK, Sanford MR. Forensic entomology and wildlife. In: *Wildlife forensics*; 2012. p. 81–107.
- Dash HR, Das S. Thanatomicrobiome and epinecrotic community signatures for estimation of post-mortem time interval in human cadaver. *Appl Microbiol Biotechnol.* 2020;104:9497–512.
- Metcalfe JL, Xu ZZ, Bouslimani A, Dorrestein P, Carter DO, Knight R. Microbiome Tools for Forensic Science. *Trends Biotechnol.* 2017;35:814–23.
- Benbow ME, Lewis AJ, Tomberlin JK, Pechal JL. Seasonal necrophagous insect community assembly during vertebrate carrion decomposition. *J Med Entomol.* 2013;50:440–50.
- Fernández-Rodríguez A, Alberola J, Cohen MC. Análisis microbiológico post mórtem. *Enferm Infecc Microbiol Clin.* 2013;31:685–91.
- Oliveira M, Amorim A. Microbial forensics: new breakthroughs and future prospects. *Appl Microbiol Biotechnol.* 2018;102:10377–91.
- Hauther KA, Cabaugh KL, Jantz LM, Sparer TE, DeBruyn JM. Estimating time since death from postmortem human gut microbial communities. *J Forensic Sci.* 2015;60:1234–40.
- Metcalfe JL. Estimating the postmortem interval using microbes: knowledge gaps and a path to technology adoption. *Forensic Sci Int Genetics.* 2019;38:211–8.
- Javan GT, Finley SJ, Abidin Z, Mülle JG. The thanatomicrobiome: a missing piece of the microbial puzzle of death. *Front Microbiol.* 2016;7:225.
- Johnson HR, Trinidad DD, Guzman S, Khan Z, Parziale JV, DeBruyn JM, et al. A machine learning approach for using the postmortem skin microbiome to estimate the postmortem interval. *PLoS One.* 2016;11:1–23.
- Adserias-Garriga J, Hernández M, Quijada NM, Rodríguez Lázaro D, Steadman D, García-Gil J. Daily thanatomicrobiome changes in soil as an approach of postmortem interval estimation: an ecological perspective. *Forensic Sci Int.* 2017;278:388–95.
- Finley SJ, Pechal JL, Benbow ME, Robertson BK, Javan GT. Microbial signatures of cadaver gravesoil during decomposition. *Microb Ecol.* 2016;71:524–9.
- Robinson JM, Pasternak Z, Mason CE, Elhaik E. Forensic applications of microbiomics: a review. *Front Microbiol.* 2021;11(January):608101.
- Javan GT, Finley SJ, Can I, Wilkinson JE, Hanson JD, Tarone AM. Human thanatomicrobiome succession and time since death. *Sci Rep.* 2016;6:1–9.
- Liu R, Gu Y, Shen M, Li H, Zhang K, Wang Q, et al. Predicting postmortem interval based on microbial community sequences and machine learning algorithms. *Environ Microbiol.* 2020;22:2273–91.
- Pechal JL, Crippen TL, Tarone AM, Lewis AJ, Tomberlin JK, Benbow ME. Microbial community functional change during vertebrate carrion decomposition. *PLoS One.* 2013;8:e79035.
- Benbow ME, Pechal JL, Lang JM, Erb E, Wallace JR. The potential of high-throughput metagenomic sequencing of aquatic bacterial communities to estimate the postmortem submersion interval. *J Forensic Sci.* 2015;60:1500–10.
- Wójcik J, Tomsia M, Drzewiecki A, Skowron R. Thanatomicrobiome – States of the art and future directions. *Postepy Mikrobiologii.* 2021;60:21–30.
- Pechal JL, Schmidt CJ, Jordan HR, Benbow ME. Frozen: thawing and its effect on the postmortem microbiome in two pediatric cases. *J Forensic Sci.* 2017;62:1399–405.

36. Bell CR, Wilkinson JE, Robertson BK, Javan GT. Sex related differences in the thanatomiobiome in postmortem heart samples using bacterial gene regions v1–2 and v4. *Lett Appl Microbiol.* 2018;67:144–53.
37. Li H, Yang E, Zhang S, Zhang J, Yuan L, Liu R, et al. Molecular characterization of gut microbial shift in SD rats after death for 30 days. *Arch Microbiol.* 2020;202:1763–73.
38. DeBruyn JM, Hauther KA. Postmortem succession of gut microbial communities in deceased human subjects. *PeerJ.* 2017;5:e3437.
39. Javan GT, Finley SJ. What is the “Thanatomiobiome” and what is its relevance to forensic investigations? *Forensic Ecogenomics.* 2018:133–43.