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Narrative review

Post-mortem microbiology in sudden death: sampling protocols proposed in different clinical settings

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ABSTRACT

Background: Autopsies, including minimally invasive autopsies, are a powerful tool for determination of the cause of death. When a patient dies from an infection, microbiology is crucial to identify the causative organism. Post-mortem microbiology (PMM) aims to detect unexpected infections causing sudden deaths; confirm clinically suspected but unproven infection; evaluate the efficacy of antimicrobial therapy; identify emergent pathogens; and recognize medical errors. Additionally, the analysis of the thanatomicrobiome may help to estimate the post-mortem interval.
Aims: The aim was to provide advice in the collection of PMM samples and to propose sampling guidelines for microbiologists advising autopsy pathologists facing different sudden death scenarios.
Sources: A multidisciplinary team with experts in various fields of microbiology and autopsies on behalf of the ESGFOR (ESCMID – European Society of Clinical Microbiology and Infectious Diseases – study group of forensic and post-mortem microbiology and in collaboration with the European Society of Pathology) developed this narrative review based on a literature search using MedLine and Scopus electronic databases supplemented with their own expertise.
Content: These guidelines address measures to prevent sample contamination in autopsy microbiology; general PMM sampling technique; protocols for PMM sampling in different scenarios and using minimally invasive autopsy; and potential use of the evolving post-mortem microbiome to estimate the post-mortem interval.
Implications: Adequate sampling is paramount to identify the causative organism. Meaningful interpretation of PMM results requires careful evaluation in the context of clinical history, macroscopic and

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histological findings. Networking and closer collaboration among microbiologists and autopsy pathologists is vital to maximize the yield of PMM. **A. Fernández-Rodríguez, Clin Microbiol Infect 2019;25:570**

Introduction

This review is part of the objectives launched by the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) Study Group for Forensic and Post-mortem Microbiology (ESGFOR), aiming to standardize post-mortem microbiology (PMM) protocols, including those related to sampling techniques. It is the result of a collaboration between microbiologists and autopsy pathologists who are members of the ESGFOR and of the European Society of Pathology (ESP). It includes a comprehensive overview of the literature relating to PMM sampling and the authors' own recommendations based on their collaborative practical experience. With the ultimate aim of improving the yield of post-mortem sampling to detect infections, the proposed protocols address the most frequently encountered clinical scenarios. In the setting of PMM, microbiologists and autopsy pathologists share the same interest: identifying or ruling out an infectious cause of death (COD). This applies to clinical and forensic autopsies. Any death that is sudden, suspicious, or clearly criminal should be investigated by the judicial authority and a full autopsy should be performed. Especially in young people, the autopsy can provide very useful information, as sudden deaths are often caused by genetic cardiac diseases, and the results can inform further genetic investigations in family members. Alternatively, with a few exceptions, if an infectious disease is found, such as viral myocarditis, a genetic cardiomyopathy can be ruled out. In deaths of uncertain or suspicious origin, the confirmation or exclusion of an infection as the cause or contributory factor to death helps to direct the judicial authority's investigation.

Updated European guidelines for autopsy practice and mortuary accreditations are lacking. However, these have already been introduced in individual countries, such as the UK. The Royal College of Pathologists has developed specific guidelines and regulations for autopsies in the different scenarios [1]. Mortuaries are part of the Laboratory accreditation programme (ISO/IEC 17025/2017). Accreditation and surveillance visits are conducted by UKAS, the national accreditation body and operates under a Memorandum of Understanding with the Government [2].

Estimation of the post-mortem interval (PMI) can be important in the investigation in some unexpected deaths. Several studies have shown that forensic microbiology may provide crucial information about the PMI. Sampling techniques for estimation of the PMI using the thanatomicrobiome are also discussed.

Forensic microbiology is a term with a wider meaning than PMM, as it also covers a larger area of clinical and public health-related issues such as bioterrorism, food-borne pathogens, rape cases, and other investigations with application in forensic pathology and criminalistics, such as the PMI.

The literature search was conducted using the MedLine electronic database National Library of Medicine, Bethesda, MD (via Ovid) and Scopus database (2008–2017) with searches based on the MeSH terms “post-mortem microbiology”, “forensic microbiology” and others specific for the different post-mortem scenarios considered.

PMM has proven useful when aseptic measures are taken at autopsy [3,4], and the interpretation of the results is jointly done by experienced microbiologists and autopsy pathologists in light of histopathological findings [4–7]. In 1969, Roberts [8] concluded that PMM correlated well with ante-mortem results if multiple sites were sampled for culture and antibiotic therapy had not been administered. According to Pryce et al. [6], post-mortem sampling is as valuable as ante-mortem sampling in the context of an infectious COD.

A recent survey demonstrated that most pathologists routinely or occasionally use PMM as part of their examinations, and, of these, the majority consider it a useful resource [7]. Although there are national recommendations for PMM [9], these mostly refer to cases of sudden unexpected death in infancy (SUDI). In particular countries, such as the UK [4,10] or South Africa [11], there is wide variation in the number and nature of the samples collected, and guidelines for the different scenarios of PMM are frequently lacking [7,12].

Few guidelines, such as the Kennedy Protocol used in the UK, describe what samples can be procured in the emergency department [7]. Pryce et al. [13] showed that the value of post-mortem samples did not differ if they were taken immediately following death or during the autopsy.

Measures to prevent PMM sample contamination and maintain operator safety

Careful efforts to prevent contamination of microbiological samples collected during autopsies are required. Sample collection requires scrupulous planning before entering the post-mortem room. Usually there will be only one opportunity to obtain samples that have not become contaminated. Contamination can be linked to either external or corpse-related factors.

To reduce the external contamination, the autopsy room and instruments should allow easy cleaning and disinfection. Controlled air circulation, closed doors and restricted access, well-trained autopsy personnel, and use of personal protective equipment are also required [6,14].

Contact with overtly infected cadavers with tuberculosis or haemorrhagic fevers is far more infectious than contact with living infected persons [15], thus posing a risk for the autopsy staff. Additionally, modern medicine creates a new era of immunocompromised patients dying from infections with far less virulent micro-organisms such as *Aspergillus fumigatus* complex or *Pneumocystis jirovecii*. This highlights the growing importance and acknowledgment of preventing possible environmental contamination of microbiological samples collected at autopsy.

Sample contamination can be assigned to the corpse itself since translocation of micro-organisms occurs as soon as natural organ barriers disintegrate [15]. Therefore, microbiological sampling should preferably be done within the first 24 hours post-mortem and before heavy manipulation or opening cavities.

Puncture sites are disinfected with alcohol-based solutions containing chlorhexidine or iodine. Depending on the requirements for toxicological testing, it may be necessary to switch to an aqueous chlorhexidine-containing solution [16].

The subclavian vein is the most appropriate sampling site for peripheral blood, rather than the jugular or femoral veins [17]. Cardiac blood should only be collected when peripheral blood is not available [3].

Internal organs should be sampled while they are *in situ*, immediately after opening the body, and after appropriate antiseptic preparation or searing of the external surface of the organ [4]. Separate sets of sterile instruments (i.e. scalpel and forceps) should be used to collect each sample, to avoid cross-contamination [5]. Bowel content samples, if collected, should be obtained last during evisceration, to prevent contamination of nearby organs by the gut microbiome.

Safety measures are required to limit the risk of infection among staff working in the post mortem room. Ventilation should be adapted in autopsy rooms, personnel should be trained properly in biosafety risks and personal protective equipment such as N-95 respirators, gowns, aprons, and face shields should be available. Immunization against hepatitis B virus, tuberculosis screening, and procedures to be followed in case of exposure to blood-borne pathogens are common practice. Special concern should be given in case of a possibility of Creutzfeld Jakob's disease since this requires specific cleaning and disinfection measures for instruments [18,19]. In cases where a highly infective agent is suspected (such as Ebola virus and Nipah virus), the team should be restricted to a minimum and only trained personnel with the proper equipment should perform the autopsy [20]. High-level isolation units are prepared in specific hospitals in Europe and in USA with detailed protocols for handling this kind of samples.

Protocols for PMM sampling in different clinical scenarios

We describe specific protocols for different clinical scenarios, including wide sampling of fluids, tissues and swabs, as most protocols generally advocate [12,21].

Although selection of specimens in PMM should be driven by the clinical context (symptoms, presentation, presumptive diagnosis), ante-mortem information is not always available in sudden death cases, and when available sometimes does not suggest an infectious COD. On such occasions samples should be collected if macroscopic findings suggest an infection [21].

The samples collected should be sent to the laboratory within 2 hours when stored at room temperature and within 48 hours when stored in refrigeration in adequate transport media [22]. When PMM is conducted to exclude infection, a minimal microbiological protocol depending on the conditions surrounding death, as reported by Fernández-Rodríguez et al. (12) should be applied, and conservation of snap-frozen tissues from different locations permits later molecular microbiological analyses if, eventually, an infection is confirmed or suspected as COD [23].

Thorough description of the specific microbiological techniques recommended for the different types of samples is outside the scope of this manuscript. It is important to note that retrieval of anaerobic microorganisms will not always be associated with a pathogenic role, and such findings should be considered in the context of post-mortem translocation of anaerobic flora from the gastrointestinal tract. Indications for anaerobic cultures are included in the tables.

Protocol for PMM sampling in SUDI and SUDC

The autopsy procedure in the investigation of any Sudden Unexpected Death in Infancy (SUDI) and in Childhood (SUDC) should include standard samples mainly from the respiratory and the central nervous systems, blood, spleen, heart, other tissues, and colon (Table 1) [10,24]. Additional samples should be procured whenever specific infections are suspected [12,21,25,26]. A wide variety of bacteria and viruses have been implicated in SUDI and the microbiologist or virologist will be able to advise on the available methods that best match the autopsy pathologist's suspicion [27–29].

Some neurotropic viruses such as human *Parechovirus* are responsible for a disseminated infection in infants. In such cases other organs, besides cerebrospinal fluid (CSF) and the brain, are involved in the infection and therefore the sampling should include cerebellum, lung, spleen, heart, bone marrow, adrenal gland, kidney, intestinal content, and swabs from the nasopharynx in order to confirm the infection [30,31].

Protocols for PMM sampling in sudden death with clinical symptoms at any age

These protocols are summarized in Tables 2 and 3. Table 2 includes the specimens to be collected when there is the suspicion of sepsis without an overt focus, and Table 3 shows those to be collected when there is suspicion of specific infections.

Sepsis

The autopsy diagnosis of sepsis is not always straightforward. Non-specific and non-localized presentations are frequent, particularly in the absence of fever. In such cases, a detailed history from family members and/or family doctors may help guide sampling and diagnosis.

As the possibility of sepsis is not always considered before starting the autopsy, aseptic measures are not always taken.

Table 1
Sampling protocol in sudden death in infancy and childhood [4,10,24]

Laboratory	Sample	Type of analysis
Bacteriology[a]	1. Blood culture – aerobic and anaerobic[b] 2. Cerebrospinal fluid 3. Nasopharyngeal swab 4. Swabs from any identifiable lesion 5. Lung 6. Spleen 7. Intestinal content	Direct bacterial culture[c]
Virology	1. Nasopharyngeal swab 2. Lung tissue 3. Intestinal content 4. Cerebrospinal fluid	Molecular analyses. If necessary viral cultures
Samples frozen at −80°C for further analyses	0.5 cm^3 of heart, muscle, liver, brainstem and kidney. Other tissues if relevant Serum	Further molecular analyses (viral or bacterial) as guided by histology Further serology if needed

[a] Additional samples such as middle ears and urine can also be taken.
[b] Anaerobic inoculation is specifically recommended in case of abscesses, abdominal surgery, intestinal pathologies or peritonitis.
[c] Direct bacterial culture includes antibiotic resistance studies.

Table 2
Post-mortem microbiology samples to be collected at any age for the investigation of sepsis without an overt focus [12,21]

Best timing of sample	Site of Sample	Quantity	Transport container or medium	Type of analysis Direct bacterial culture[b] Molecular analyses[e] Serology Antigenic analysis
Before the body is opened	Blood (peripheral vein or cardiac)	5–10 mL	Bottles for blood culture (aerobic and anaerobic)[a]	Direct bacterial culture
		3–5 mL	EDTA tube	Molecular analyses Virology
		5–10 mL (serum obtained by prompt centrifugation)	Sterile tube/serum activator tube	Serology and Antigenic analyses
	Cerebrospinal fluid (cisterna magna puncture)	3–5 mL	Sterile tube/container	Direct bacterial culture Antigenic analyses
		1–2 mL	Sterile tube/container	Molecular analyses Antigenic analyses
	Urine (suprapubic puncture)[c]	5–10 mL	Sterile tube/container	Direct bacterial culture
	Ascites (via right iliac fossa)	5–10 mL	Sterile tube/container	Cell count Direct bacterial culture (aerobic/anaerobic)
	Nasopharyngeal swab	1 flocked swab	Amies medium	Molecular analyses *Bordetella pertussis*-specific PCR
	Pharyngeal swab	1 flocked swab	Amies medium	Direct bacterial culture Antigenic analyses
	Skin petechiae	Fluid/1 flocked swab	Amies medium	Direct bacterial culture Molecular analyses
During evisceration	Lung[d]	1–2 cm^3 from each lower lobe or any obviously infected area	Sterile tube/container	Bacteriology including direct bacterial and fungal culture, rapid mycobacterial culture. Molecular analyses
	Liver[d]	1–2 cm^3	Sterile tube/container	Direct bacterial culture Molecular analyses
	Spleen[d]	1–2 cm^3	Sterile tube/container	Direct bacterial culture Molecular analyses
	Myocardium[d]	1 cm^3	Sterile tube/container	Direct bacterial culture, Molecular analyses
	Bile	3–5 mL	Sterile tube/container	Direct bacterial culture
	Adrenals	1–2 cm^3	Sterile tube/container	Direct bacterial culture, Molecular analyses
	Faeces	>5 mL	Sterile tube/container	Direct bacterial culture, Molecular analyses Antigenic analyses Direct parasitological examination
	Brain[d]	1–2 cm^3 (target abnormal areas if evident)	Sterile tube/container	Direct bacterial culture
		Alternative: meningeal surface	Flocked swab	Molecular analyses
During organ dissection	Brain Heart Lungs (all lobes) Liver Kidneys Spleen Pancreas Bone marrow Adrenal Thyroid Pituitary Any evident lesion	Representative samples	Histology cassettes in 10% neutral buffered formalin	Histopathology
Other less frequent samples	Vitreous humour			

[a] Inoculation in anaerobic bottles will be mainly performed under the suspicion of anaerobic infection. Bottles can be complemented with a blood tube with sodium citrate (3–5 mL).
[b] Direct bacterial culture includes antibiotic resistance studies.
[c] Under the suspicion of pyelonephritis, urine cultures should be performed immediately after the autopsy, and its easy contamination should be taken into account when interpreting the results.
[d] Macroscopically abnormal organs: lung, myocardium, spleen, liver, kidney, brain, skin petechiae biopsy, and adrenals (containing bilateral haemorrhage) can be processed for bacterial analyses, including molecular ones in case of sepsis. Lymph node sampling is useful in cases with lymphadenopathy.
[e] In tissues, when virology is requested apart from bacteriology, an additional recipient should be used.

Although this can hamper bacterial culture, PCR aimed at specific pathogens maintains its utility. The recommended samples include blood and at least four other tissues: spleen, heart, liver, brain, and both lungs [8,12,32] (Table 2). Skin petechiae [12,33] and vitreous humor, an isolated fluid resilient to contamination [33,34], may yield positive PCR results in *N. meningitidis* sepsis cases.

When viral haemorrhagic fever, such as that caused by Ebola or the Crimean Congo virus, is suspected, blood sampling from corpses can be used for virus detection [35,36]. Additionally, throat swabs and urine are recommended samples for Lassa; nasopharyngeal swabs, pleural fluid, urine and tissues for Bunyavirus; and oral swabs in viral transport medium for Ebola [37].

Invasive fungal disease

When invasive fungal disease (IFD) is suspected, tissue samples instead of swabs should be procured [38]. Fungal meningitis,

Table 3
Post-mortem microbiology samples to be collected for the investigation of other infections [12]

Pathology suspected	Samples for microbiological analysis	Quantity	Transport container or medium	Type of analysis Direct bacterial culture[a] Molecular analyses[b] Serology Antigenic analyses
Mandatory specimens in most aetiologies indicated below	Blood (to confirm bacteraemia)	5–10 mL	Bottle for blood culture (aerobic and anaerobic)[c]	Direct bacterial cultures
		3–5 mL	Tube with SPS/sodium citrate	Direct bacterial cultures
		3–5 mL	EDTA	Molecular analyses/virology
	Serum	3–5 mL	Centrifugation with clot activator	Serology and antigenic analyses
	Spleen	>1–2 cm^3	Sterile tube	Direct bacterial culture aerobic and anaerobic[c] Molecular analyses
Pneumonia	Portion of the zone affected of the lung. If not clearly affected, a portion of each lobe should be taken separately	>1–2 cm^3	Sterile container	Direct bacterial culture Mycobacterial culture when indicated Molecular analyses
	Urine	3–5 mL		Antigenic analyses: *Legionella, S. pneumoniae*
	Pleural exudate if empyema	>1–2 cm^3	Sterile tube/flocked swab	Direct bacterial culture Mycobacterial culture when indicated Molecular analyses
Other respiratory infections (tonsillitis, epiglottitis, bronchitis, bronchiolitis, laryngitis)	Swabbing the affected mucosae: pharyngeal, larynx, bronchial	2 (plus virus transport medium if necessary)	Amies liquid medium	Direct bacterial culture Molecular analyses If tonsillitis: antigenic analyses for *S. pyogenes* on pharyngeal swab.
Flu, viral respiratory infection	Nasopharyngeal swab (apart from lung tissue)	2 (plus virus transport medium if necessary)	Amies liquid medium	Antigenic analyses (IC and others) Direct bacterial culture Molecular analyses
Invasive fungal disease [d]	Lung, heart, brain, kidneys Large intestine CSF			Fungal culture and panfungal PCR Antigenic analyses for *Cryptococcus*
Bacterial peritonitis	Peritoneal fluid (ascites) in specific anaerobic container	5–10 mL	Sterile tube	Direct bacterial culture aerobic & anaerobic Molecular analyses
Gastroenteritis or colitis [e]	Faeces/Rectal swab	≥1 mL	Sterile tube Amies liquid medium	Direct bacterial culture specific to detect enteropathogens and *C. difficile* Molecular analyses Antigenic analyses (*C. difficile* GDH Ag and toxin)
Soft tissue infection	Aspiration with syringe of affected area and swabbing of lesions	As much as possible	Sterile tube Amies liquid medium	Direct bacterial culture aerobic and anaerobic Molecular analyses
	Portion of muscle affected	>1–2 cm^3	Sterile container	Direct bacterial culture aerobic and anaerobic Molecular analyses
Meningo-encephalitis and myelitis	CSF	2–3 mL	Sterile tube	Direct bacterial culture Molecular analyses Antigenic analyses
	Brain	>1–2 cm^3	Sterile container	Direct bacterial culture Molecular analyses
	Pharyngeal exudate [f]	Swab	Amies liquid medium	Direct bacterial culture Molecular analyses
		Middle ears swab (after opening of the petrous bones)		Direct bacterial culture, molecular analyses. If viral encephalitis suspected: molecular analyses for respiratory viruses
Endocarditis	Sample obtained of the friable vegetations	2 swabs/fragment	Amies liquid medium Sterile container	Direct bacterial culture Molecular analyses
Myocarditis	Ventricular walls/septum	5 mm^3	Cryotubes	Molecular analyses
Fulminant hepatitis	Liver	>1–2 cm^3	Sterile container	Molecular analyses (hepatitis viruses, CMV, EBV, toxoplasma)
Abscesses	Purulent exudate: Aspiration with syringe of affected area and swabbing of lesions	As much as possible/ swab	Sterile container. Amies liquid medium	Direct bacterial culture aerobic and anaerobic Molecular analyses
Pyelonephritis, cystitis	Urine, Kidney	≥1 mL >1–2 cm^3	Sterile tube Sterile container	In both samples: direct bacterial culture Molecular analyses
Genital infections	Cervical and vaginal samples Urine	2 swabs ≥1 mL	Amies liquid medium Sterile tube	Direct bacterial culture Molecular analyses

Table 3 (*continued*)

Pathology suspected	Samples for microbiological analysis	Quantity	Transport container or medium	Type of analysis Direct bacterial culture[a] Molecular analyses[b] Serology Antigenic analyses
Chorioamnionitis	Samples of membranes, ovular membranes, foetal lung,	2 swabs	Amies liquid medium	Direct bacterial culture aerobic and anaerobic
	Amniotic fluid	≥1 mL	Sterile tube	Molecular analyses
Congenital/perinatal infections: Toxoplasmosis, congenital CMV, congenital syphilis	CSF	2–3 mL	Sterile tube	All samples: Direct bacterial culture.
	Brain	>1–2 cm^3		Molecular analyses including TORCH pathogens (*Toxoplasma*, *Rubella*, CMV, Herpes simplex), syphilis, Coxsackie, Zika
	In the case of CMV, also urine and amniotic fluid (prenatal)	≥1 mL	Sterile container	Molecular analyses
	Serum		Serum separator tube	Serology (TORCH pathogens). If congenital syphilis: non-treponemal and treponemal assays in CSF/serum Molecular analyses
Tuberculosis	Tuberculoma	>1–2 cm^3	Sterile container	Mycobacterial culture and specific PCR
Malaria and other systemic parasitosis	Brain, liver, lung, myocardium, blood (as indicated before)	>1–2 cm^3	Sterile container	Specific parasite PCR In EDTA blood: antigenic analyses mainly for *Plasmodium spp.* and other systemic parasites
Botulism	Faeces	≥1 mL	Sterile container	Toxin detection, *C. botulinum* toxins PCR
	Exudates	2 swabs	Amies liquid medium	
	Tissues	>1–2 cm^3	Sterile container	

[a] Direct bacterial culture includes antibiotic resistance studies.
[b] Molecular analyses, including viral ones in case they are needed.
[c] Inoculation in anaerobic bottles will be mainly performed under the suspicion of anaerobic infection.
[d] Under the suspicion of *Histoplasma, Coccidioides*, or *Blastomyces* infection, the laboratory should be warned to take special precautions, since these are Hazard Group 3 pathogens. Panfungal PCR can be performed on paraffin-embedded tissues when there is no fresh tissue available [79].
[e] To track a food-borne contamination event back to its source, a microbiological analysis of the suspected food is compulsory.
[f] If meningococcal septic shock is suspected.

although uncommon, should always be considered in immunosuppressed patients with a history of travel to endemic areas. *Histoplasma* meningitis should be suspected in patients with meningitis or parenchymal lesions in whom no other pathogens are detected. Definitive diagnosis relies on the histological brain examination [39].

Meningitis and other neurological infections

CSF is the most useful sample to confirm or exclude meningitis. A small piece of brain tissue is also of use, allowing both culture and molecular analyses. When meningitis is identified at autopsy and CSF had not been collected prior to eviscerating the brain, swabbing the meningeal surface permits recovery of a meningeal exudate for culture and PCR. In cases of exudative meningoencephalitis both middle ears should be inspected and swabbed after opening the petrous bones.

Respiratory infections

Whenever a respiratory infection is suspected, multisite sampling including specimens from both the upper and lower respiratory tract is recommended [12], particularly in infants where there is not always macroscopic evidence of pulmonary infection.

At least, one portion of each lung should be taken prior to removal of the thoracic organs. A swab taken from the lower lobe bronchi immediately after separation of the main bronchi can complement this. In *Legionella* investigation, one or more lung tissue fragments (consolidated and necrotic areas) from the median aspect of the upper and lower lobes should be selected [40], not later than 48 hours after death. To perform culture they should be frozen at $-70 \pm 10^{\circ}C$ if not analysed on the day of the sampling. When legionellosis is suspected, it is important to prevent any contact of the lung with tap water to avoid environmental contamination and thus false positives. Complementary microbiological investigations on the putative water sources of *Legionella* and other environmental pathogens such as non-tuberculous mycobacteria or even *Pseudomonas aeruginosa* can be of paramount importance, particularly when there is a judicial suit under an outbreak scenario. To investigate respiratory viruses, swabbing of different locations from the upper respiratory airways is common practice [26]. Nasopharyngeal swabbing detects respiratory viruses [5,41–45] and *Bordetella pertussis*.

A sample of serum can be complementary for serology for some viruses and those bacteria, such as *Chlamydophila pneumoniae, Mycoplasma pneumoniae*, and *Legionella pneumophilia*, that are difficult to grow. However, in most cases, the lack of availability of an antemortem serum sample will harden the detection of rising antibody titres. The height of the antibody titre can give a clue in these cases.

Protocol for PMM sampling in sudden cardiac death in the young

Sudden death is defined as a natural, unexpected death occurring within 1 hour of the onset of symptoms [46]. Most sudden deaths are cardiac in origin. Here we focus on sudden cardiac death (SCD) in adults 18–35 years old. The SCD autopsy requires a multidisciplinary approach, with close collaboration between clinicians, autopsy pathologists and microbiologists.

In young adults, viral myocarditis is one of the leading causes of SCD, both during activity and at rest. Its prevalence has been underestimated because of the frequent lack of appropriate investigation. In the USA, it has been estimated that 9% of sudden deaths in young athletes in whom a confirmed cardiovascular event was documented are due to viral myocarditis [47]. In children between 1 and 16 years of age, 1.1% of all sudden deaths while sleeping were due to viral myocarditis (M.C. Cohen, unpublished data). Acute myocarditis is defined as myocardial necrosis with inflammatory infiltrates without evidence of coronary involvement [48]. Cardiotropic viral agents, mainly *Enteroviruses*, *HHV6* and *PVB19* can cause

acute and/or chronic myocarditis [49]. In viral myocarditis the heart may be macroscopically pale, floppy, and have tiny haemorrhages in the myocardium. Histology, immunohistochemistry, and molecular microbiology are valuable diagnostic tools.

For molecular techniques, and because viral myocarditis is characterized by multifocal clusters of inflammation and necrosis [50,51], the collection of five 5-mm^3 samples is recommended, including two from the left ventricular free wall, one from the septal wall, and two from the right ventricular myocardium. Samples should be placed in cryotubes and stored at −80°C. Samples of the spleen and EDTA whole blood may also demonstrate a more widespread presence of the virus in some cases of viral fulminant myocarditis [12].

In addition to virological molecular biology, real-time RT-qPCR for tissue mRNA interleukin 10, whose expression could be an indirect biomarker of viral heart infection can be useful [48,52–54].

The interpretation of molecular analyses is an important matter of concern, and the question often raised is whether the presence of viral agents *per se* indicates viral myocarditis as the COD. In our experience and according to published studies, the number of copies per microgram of total extracted nucleic acids is a good indicator of myocarditis [55,56]. For *PVB19*, the viral load threshold is considered to be 500 copies per microgram of total extracted nucleic acids. When the *PVB19* load is lower than this threshold this virus is unlikely to be the cause of sudden death [57]. For other cardiotropic viruses the viral load threshold remains undetermined so far. However, in some cases, the presence of a co-infection may explain the death despite moderate viral loads of each agent. In chronic myocarditis, peripheral blood should be analysed using the same viral molecular quantitative techniques as those used for cardiac tissues, in order to exclude contamination of cardiac or other tissues by a concomitant viraemia [58].

Bacteriological and parasitic causes of myocarditis and SCD are much less frequent. In endemic areas, Chagas disease (*Trypanosoma cruzi*) is a public health matter of concern. Toxoplasmosis, cysticercosis, and tick-borne diseases such as babesiosis are investigated in cardiac tissues and blood using specific monoplex or multiplex molecular techniques such as qPCR, PCR, or LAMP-PCR assays [59]. For bacteriology, myocardial and peripheral blood samples are cultured. For non-classical bacteriological causes, such as Lyme disease, tuberculosis, brucellosis, and syphilis, specific culture media, serology, and/or molecular techniques such as 16S ribosomal RNA PCR amplification followed by sequencing or next generation sequencing (NGS) in cardiac tissue have to be performed. Molecular techniques require classical RNA and DNA extraction from frozen samples taken at autopsy [59].

Microbiology sampling and the minimally invasive autopsy in adults and children

There is increasing demand for the development of minimally invasive autopsy (MIA) techniques for fetal, paediatric, and adult autopsies. In MIA, samples of organs, tissues, and body fluids are obtained via needle puncture and/or needle biopsy without opening the body [60]. For the most part, MIAs are performed using blind biopsy with a cutting needle. However, it has also been suggested that tissues might also be biopsied and fluids sampled at MIA using targeted image-guided biopsy/needle placement [60–65], thoracoscopy/laparoscopy [60,66,67] or endoscopy [60,67]. While magnetic resonance imaging (MRI) is currently the preferred imaging technique employed in fetuses, infants, and children, computed tomography (CT) is favoured in adults [68].

The procedure in adults involves the collection of CSF (obtained by cisterna magna puncture), blood (from the subclavian vein or heart), ascites, pleural effusion, liver, lungs, spleen, kidneys, bone marrow, and brain (trans-ethmoidal approach) in all cases plus the uterus in women of childbearing age, using a variety of biopsy needles [69–71].

When performing the MIA within 24 hours of death, Martínez et al. [71] were able to detect an aetiological agent in 89% of infectious deaths. Conventional invasive autopsy performed immediately after MIA identified the same causative microorganisms in 83.8% of 74 infectious deaths. As more than 50% of the patients from this study [72] were infected with HIV, it may be that microbiology sampling would have a lower diagnostic yield in populations with a lower rate of HIV infection.

The most recent studies focusing on paediatric MIA have successfully procured histology samples from all thoracic and abdominal organs as well as microbiology samples from the CSF, blood, nasopharynx, lung, urine, and colonic content using minimal invasion to the body through thoracoscopy and laparoscopy [73].

Quality considerations for PMM methods

PMM microbiology requires targeted sampling using aseptic techniques. The incorporation of molecular methods as part of routine of PMM represents a major diagnostic advance. Quality requirements according to NEN-EN-ISO 15189 (2013) or ISO 9001 (2015) are used in many laboratories. For instance, a workflow in facilities with separation of working areas for the bacteriology laboratory, the pre-PCR, PCR, and post-PCR settings is desirable. Laboratory methods require the use of internal controls to detect and minimize inhibition, and blank controls to track cross-contamination between samples.

Regarding molecular methods, many variants of PCR have shown to be useful, both pathogens targeted real-time PCR assays, including the multiplex (syndromic) molecular panels, and broad-range PCR assays, such as the 16S Sanger sequencing. However, the utility of the latter can be hampered as a consequence of post-mortem translocation, since apart from the potential pathogen other contaminating bacteria can be present, yielding an unreadable mixture of sequences. The implementation of NGS techniques and metagenomics will help to find the pathogen considered as responsible for the CODs in previous unsolved cases and can contribute to a deeper comprehension of the post-mortem events.

CE-IVD labelled assays often lack validation for post-mortem samples. This means the laboratory should validate these samples in-house.

Evolving techniques in PMM to help in the estimation of the post-mortem interval: post-mortem microbiome

The PMI is defined as the length of time between death and corpse discovery or autopsy performance. From the onset of death, the medical parameters used to estimate the PMI weaken, becoming less accurate as the interval increases. PMI estimation can be important in the investigation in some unexpected deaths. Several studies have shown that forensic microbiology may provide crucial information about the PMI.

Post-mortem microbiome succession is a foreseeable and time-dependent process resulting in predictable changes in internal organs microbiomes [74,75]. The microbiota from the nasal and ear canals have been proposed as sampling sites to estimate PMI [76]. Some authors propose regression models established on post-mortem microbial taxa turnover and shifts in community composition based on high throughput sequencing data, providing both spatial and temporal insight into the events surrounding death [77,78]. Considering the potential of the post-mortem microbiome to estimate PMI, standardizing guidelines for sample collection in real case scenarios would be valuable (Table 4) [76,78–85]. The development of metagenomics could aid PMM not only in the

Table 4
Sampling recommendations to determine the post-mortem interval

Sample/sample location	Sample[a] and reference	Quantity	Transport container or medium[b]	Type of analysis
Surface and cavities/ cadaver surface	Surface swabbing [83,84] Nostril and ear channels swabbing [76] [d]	Along >30 cm^2	Amies liquid medium Sterile phosphate-buffered saline	Molecular analyses[c]
Organs and tissues	Deep cuboidal sections [78,80,81]	1 cm^3	Sterile container	Molecular analyses
Blood	Blood obtained from heart and femoral veins [85] [c]	10 mL	Tube with EDTA	Molecular analyses
Soil to allow comparisons	Soil collected from the first 5 cm below the ground surface and sieved through 2-mm mesh [82]		Sterile container	Molecular analyses

[a] All samples must be ideally collected with sterile DNA/RNA free equipment.
[b] Specimens should be transported on dry ice, or stored at −20°C or −80°C.
[c] Molecular analyses include 16S rRNA gene amplicon bases sequencing approaches or detection by qPCR of specific groups of bacteria; DNA extraction should be performed with soil DNA extraction kits.
[d] To prevent or minimize the sampling of bacteria from the soil, the canals chosen for sampling are those on the side of the face facing away from the ground in corpses placed in a prostrate position.

estimation of PMI, but also, the accurate description of the thanatomicrobiome could help in the interpretation of post-mortem culture results.

Concluding remarks

Identifying an infectious COD using PMM is a shared aim by microbiologists and autopsy pathologists, which is also the common goal in clinical and forensic autopsies. Moreover, COD in the forensic setting may have legal implications. Therefore, PMM is an integral part in the evaluation of any sudden death.

An agreement in minimal requirements to collect samples at autopsy based on the proposed protocols described here should be the first step to improve the yield of PMM. Next steps should be recommendations for the type of analyses performed and for analyses interpretation. Long-term educational activities aimed at forensic and clinical pathologists can make them more aware of the need and usefulness of PMM and also encourage them to use it. The recent partnership agreement signed between the ESCMID and the ESP is already proving fruitful in improving the sampling and interpretation of PMM.

Transparency declaration

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