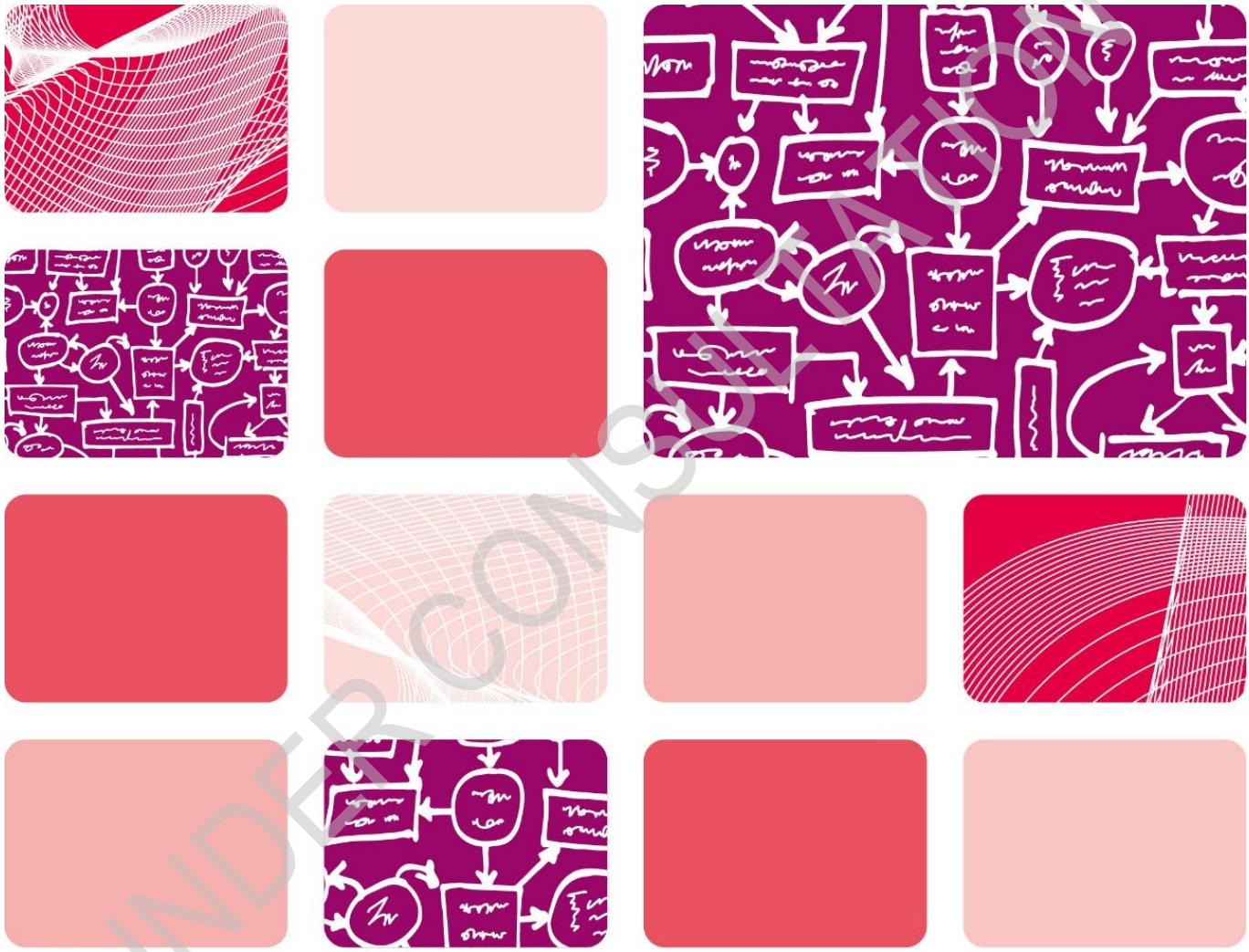
UK Health Security Agency

UK Standards for Microbiology Investigations

Postmortem Microbiology



Acknowledgments

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UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	-/dd.mm.yy
Issue number discarded	
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Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document may contain some distressing information and is therefore not intended for a lay audience. It provides a standardised approach to microbiological testing in postmortem examination (also referred to as autopsy), assisting in the investigation of the following examples (1,2):

- bacterial, viral, parasitic and fungal infections causing or contributing to death, especially in cases of sudden, unexpected or unexplained death
- emergent, novel or rare pathogens
- cases triggering public health and infection prevention and control interventions, due to antimicrobial resistance, outbreaks or notifiable diseases
- deaths requiring medicolegal investigation where infection may be the underlying cause of death, or microbiological testing may be of evidential value
- cases where the time elapsed since a person's death also known as the postmortem interval (PMI) is long or undetermined.

The UK SMIs focus on established, validated and accredited methods available to laboratories. Novel technologies are also reviewed and included when sufficient evidence for their performance is available. Therefore, microbiome succession and diversity analysis and postmortem interval (PMI) determination is highlighted in this document in terms of future development. However, it will not be extensively covered due to minimal evidence of routine use at the time of writing.

Documents with the UK SMI should be used in conjunction with other relevant UK SMIs and in alignment with current available guidance and those in development from relevant professional bodies, including the Royal College of Pathologists. To maintain this alignment, postmortem microbiology in congenital, perinatal and neonatal death will not be covered in depth in this issue. Only testing for *Treponema pallidum* (syphilis) and *Toxoplasma gondii* (toxoplasmosis) in infants to support accurate determination of cause of death will be covered, as established current guidelines are available.

This document provides microbiological testing guidance only. Guidance from legal and investigating authorities throughout the UK must be followed and will usually supersede this document in medicolegal cases.

4 Introduction

Postmortem microbiology can be a useful tool in identifying contributing factors to the cause of death (COD), but the efficacy and accuracy of the information obtained is dependent on ensuring various standards are met during all stages of the investigation. Adequate sampling, and the interpretation of results by both microbiologists and pathologists in context with the patient's macroscopic appearance, clinical history, biochemical test, radiology and histology results is imperative (3).

There are two forms of postmortem examinations in the UK. A hospital or consented postmortem can be requested by the patient's doctor after explicit consent from the next of kin. These are conducted either to investigate the illness, COD or to aid in the advancement of medical research (4).

However, most postmortems conducted in the UK are medicolegal (coronial) postmortems. These are requested by His Majesty's coroner in England, Wales and Northern Ireland or by the Procurator Fiscal in Scotland in deaths without a medical certificate of cause of death (MCCD) and in certain circumstances (5-7) including deaths that are/have:

- sudden, unexplained, unexpected or due to an unknown or not completely understood cause
- from an illness not diagnosed or (mal)treated by a medical practitioner
- violent or unnatural modes of death conducted by either non-forensic or forensic pathologists.
- occurred during an operation or before a person has recovered from anaesthetic
- a suggestion of industrial disease or industrial poisoning involvement.

If a non-UK resident dies in the UK, this must be reported to the local coroner or Procurator Fiscal, and they will use the same criteria above to determine whether a postmortem is required.

If an individual from the UK dies abroad, the country's local authorities may perform a postmortem. The standard and the time taken to complete this varies widely between countries. After local investigations are concluded, the person's body may be brought back (repatriated) to the UK. On return, a coroner (England/Wales), or the Procurator Fiscal (via referral by the Death Certification Review Service), may carry out another postmortem examination if the circumstances require it. Coroners in Northern Ireland do not have the authority to carry out an investigation into a death abroad (7). In these circumstances the body will usually need to be embalmed before repatriation. Embalming fluid contains alcohols and formaldehyde, which can significantly reduce the viability of microorganisms. Therefore, embalming alongside previous postmortem

activities may greatly affect the microbial information available to determine an infection related COD (8).

4.1 Investigations using postmortem microbiology

4.1.1 Sudden, unexpected or unexplained death

Multiagency guidelines jointly endorsed by The Royal College of Pathologists (RCPath) and The Royal College or Paediatrics and Child Health (RCPCH) in 2016 classifies sudden, unexpected or unexplained death in childhood and infancy as a death (or collapse leading to death) where there is no pre-existing medical cause, no immediately obvious natural cause and which would not have been reasonably expected to occur 24 hours previously. This includes deaths caused by accidents, suicides and possible criminal acts (9) .

Individuals under the age of 18

All UK nations define a child in line with the United Nations Convention of the Rights of a child, as anyone under the age of 18 (10-12). Sudden death in the young is divided into specific age ranges and modification of the postmortem examination correlates to these (9):

- sudden unexpected deaths in infancy (SUDI) from the early neonatal period (excluding stillbirths) to 12 months. This may include sudden infant death syndrome (SIDS), where an unexplained lethal episode occurs during sleep in children under 12 months of age, and remains unexplained even after a postmortem examination, review of the circumstances of death and the clinical history.
- sudden unexpected deaths in children (SUDC) from12 months up to age 18.

Infection and sepsis are leading natural causes of sudden death in childhood and infancy. Many pathogens can be responsible for infections leading to a fatal outcome aided by immune system immaturity in this age range. This includes primary infections causing sudden and rapid death such as *Neisseria meningitidis*, others causing pneumonia not previously detected during life, such as *Streptococcus pneumoniae* (13).

Viruses causing severe respiratory infections such as respiratory syncytial virus (RSV), or multi-organ failure due to disseminated infection of Herpes Simplex Virus (HSV-1 or 2) are also leading infectious causes of sudden death in childhood (14). Inflammation and tissue damage from such viral infections can lead to secondary bacterial infection, and the promotion of bacterial toxin production. Various studies have implicated a superantigen toxic shock syndrome toxin-1 (TSST-1) produced by *Staphylococcus aureus*, and α-Hemloysin (HLyA) producing serotypes of *E. coli* in SIDS (15,16).

Myocarditis has been found to represent almost 3% of SUDI and SUDC within the UK, with infectious causes in one retrospective study found to predominantly be caused by Parvovirus- B19 (PVB19) and Enterovirus infection (EV) (17). Complications of measles infection can cause myocarditis, which is even more of a consideration if vaccination rates continue to fall (18).

Individuals aged 18 and over

Sudden death in this group is attributed predominantly to sudden cardiac death (SCD) involving mostly ischaemic/atheroma heart disease, genetic cardiomyopathies and channelopathies in younger adults. Sudden adult/arrhythmic death syndrome (SADS) refers to a sudden cardiac death with negative toxicology, where no structural or morphological pathology is identified despite detailed postmortem examinations (19,20).

SCD by myocarditis in this age range has been shown in some studies to be predominantly caused by *Staphylococcus aureus*, Enteroviruses and Human Herpesvirus 6 (HHV6) (18,21). In immunocompromised individuals, influenza virus, fungal pathogens such as *Candida* and *Aspergillus* species, and protozoal infections by *Toxoplasma gondii* are also likely. In specific endemic areas, *Brucella species*, *Trypanosoma cruzi* and Mucorales can also be a cause of myocarditis and endocarditis (18).

Sudden non-cardiac death (SNCD) is a very broad category including respiratory, cerebrovascular, gastrointestinal and neurological diseases with or without associated infection (22). A Canadian study showed that community-acquired bacterial pneumonia, represented 43% of unexpected infectious death cases in Ontario, caused primarily by Gram-positive pathogens found in skin and oral flora including *S. aureus* (19%) and *Streptococcus pneumoniae* (25%) (23). A Spanish study of a series of sudden unexpected deaths due to infection in children and young adults found that respiratory infections were the most frequent COD, followed by cardiac infections, while neurological infections were infrequent (24). However, rapidly deteriorating (fulminant) meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Listeria monocytogenes* and meningoencephalitis from Herpes Simplex Virus 1 or *Plasmodium falciparum* infection are other examples of central nervous system SNCD. Gastrointestinal examples include, fulminant bacterial peritonitis, resulting from acute appendicitis, ruptured peptic ulcer, diverticulitis, or cholecystitis (25).

4.1.2 Congenital syphilis and toxoplasmosis in neonatal and infant deaths

Congenital infections (mother-to-child transmission of syphilis) due to *Treponema pallidum* (syphilis) and *Toxoplasma gondii* (toxoplasmosis) remain important and potentially under-recognised causes of stillbirth, neonatal death, and infant mortality, especially as official figures count live births only. Affected infants may be asymptomatic at birth or present with non-specific clinical features, meaning infection may not be suspected during life. In addition, reliance on maternal antenatal screening alone is insufficient, as testing may be incomplete, declined, incorrectly documented, performed early in pregnancy prior to seroconversion, or yield false-negative results.

Both syphilis and toxoplasmosis are associated with a spectrum of severe outcomes, including stillbirth, prematurity, hydrops fetalis, hepatosplenomegaly, central nervous system involvement, and ocular disease. Identification of these infections at postmortem has important implications for accurate determination of cause of death, informs recurrence risk counselling for future pregnancies, and enables appropriate referral for maternal treatment and public health follow-up where indicated. Consequently, targeted postmortem testing forms an essential component of comprehensive infant death investigation (26-28).

4.1.3 Emergent, novel or rare pathogens

Postmortem microbiology has a crucial role to detect emergent and novel pathogens not previously detected during life. The West Nile virus was first detected in USA in 1999 when performing ancillary microbiological autopsy analyses in some corpses suffering from encephalitis (29). Due to New York medical examiners insisting on this investigation, it was discovered that this virus had expanded to the USA.

During COVID-19 pandemic, autopsies were important not only to detect the virus in patients that have been undiagnosed during life, but also to have a deeper knowledge of the pathological mechanisms of the disease (30). More recently, microbiological investigations in specimens from forensic autopsies detected that the tick borne nairovirus responsible for incidences of Crimean-Congo haemorrhagic fever was spreading geographically in Spain, heightening the need for adequate surveillance (31).

Postmortem microbiology and microbial forensics have also been key in the detection, genetic characterization of biological agents used suspected for bioterrorism and crime, and the subsequent public health implications and criminal prosecution (3). In 2001, the ‘Amerithrax’ or anthrax letter attacks, microbiological testing and genotyping conducted from postmortem specimens assisted in identifying the Ames strain of *Bacillus anthrax*, linking the incidences to the letters sent to New Work, Washington D.C and Florida and then assisted in identifying the source. This work led to the development of whole genome sequencing analyses to help public health authorities

to determine whether possible outbreak infections are natural, accidental, or deliberate (32).

4.1.4 Cases triggering public health and infection prevention and control interventions, due to antimicrobial resistance, outbreaks and notifiable diseases

The rapid identification of infections, both antemortem and postmortem, is imperative for effective disease surveillance, the development of public health policy, and the implementation of robust infection prevention and control measures. This is exemplified by multiple meningococcal outbreaks in recent years.

In 2015–2016, an outbreak in Tuscany, Italy caused by a hyper-invasive *Neisseria meningitidis* serogroup C strain (MenC/cc11) led to several fatalities, followed by further cases in Lombardy in 2019–2020, including deaths in which closely related strains were identified from both antemortem and postmortem specimens. (33).

Similarly, the 2026 UK outbreak of meningococcal group B (MenB), including a fatal cluster in Kent described as the largest and fastest-growing recorded nationally, reinforces the critical role of rapid postmortem microbiology investigation in the identification of invasive infection as a cause of sudden or unexplained death, particularly in young, otherwise healthy individuals. The emergence of such outbreaks also highlights how timely microbiological confirmation after death can contribute directly to outbreak recognition and the implementation of population-level interventions such as a targeted 2026 MenB vaccination programme (34).

In England and Wales, notification of invasive group A streptococcal (iGAS) infection diagnosed through culture, molecular and rapid streptococcal antigen testing is mandatory. In the 2022/2023, 28 children's deaths in the UK were attributed to IGAS via notification of isolation from antemortem and postmortem specimens. Collaboration between public health authorities and Child Death Review (CDR) teams across each of the four UK nations used such data to rapidly review this outbreak, identifying patterns and mitigations to inform public health actions helping to prevent future deaths due to iGAS (35).

As of 2025, carbapenem-resistant *K. pneumoniae* bloodstream infections have surged in 23 EU member states, driven by the continued spread of multidrug-resistant high-risk lineages in hospitals. In addition, some *K. pneumoniae* lineages which are more likely to cause severe diseases have also become resistant to carbapenems, including the hypervirulent lineage *K. pneumoniae* ST23. Newly emerging carbapenem-resistant Enterobacterales (other than *K. pneumoniae*) are also spreading in hospitals. In certain cases of hospital associated deaths, performing antimicrobial susceptibility testing to relate the prescribed antibiotics with resistance patterns of isolated microorganisms from the postmortem examination, may be useful (36,37)

4.1.5 Medicolegal investigation

The necessary legal guidance for all these steps must be followed at all times and are outside the scope of this document.

Although a large percentage of postmortems conducted at coronial or Procurator Fiscal request are considered medicolegal, only a small fraction are forensic examinations conducted specifically to collect evidence for possible criminal proceedings. These criminal proceedings may be directly due to a suspicion of possible homicide as the COD or may be because evidence is required for investigation of a possible related crime, such as sexual assault or abuse or where neglect or medical malpractice is potentially involved.

There may be a wider selection of specimen types collected from forensic postmortems which may include trace evidence such as swabs from skin (i.e. vaginal, anal, bite marks). The tests requested may be more extensive than consented postmortems to assist in covering all investigative avenues, or very specific to the context of the forensic investigation.

Chain of evidence/ custody

As such specimens are considered evidence which may be admitted into court, it is essential, as far as possible, to maintain an unbroken step-by-step documentation of the chain of evidence/custody to ensure full traceability and integrity of the evidence. This requires comprehensive documentation covering all stages of the specimen's lifecycle, including collection, handling, transport, receipt, testing, storage, and final disposal. It should also include everyone who handles the specimen, the date, time, and location at each stage, along with signatures where required.

All microbiology laboratories must operate in accordance with the Royal College of Pathologists (RCPath) guidance G031-The retention and storage of pathological records and specimens, which applies routinely to all specimens, regardless of whether they originate from a postmortem or non-postmortem setting (38).

Where specimens are identified as postmortem, additional legal and regulatory requirements must be followed, including compliance with relevant Human Tissue Authority (HTA) postmortem guidance and legislation (39-42).

These requirements also apply in circumstances where a specimen is identified as being required for legal proceedings at a later stage. In such cases, chain of custody documentation must be established from the moment of becoming aware of the forensic importance.

Second postmortems

In certain cases, a second postmortem examination by another pathologist may be requested to the coroner or Procurator Fiscal, i.e. by the defence team in a legal case, or by family members. If permitted by the legal authority, new specimens may be

collected for testing, or requests for retesting of specimens received from the initial postmortem may be made.

The coroner/ Procurator Fiscal will seek expert advice from pathologists and microbiologists on the efficacy of testing or retesting specimens, but the final decision is solely made by the coroner/ Procurator Fiscal and only a judicial review in the High Court can challenge this authority. Therefore, all specimens received at coronial request should be tested and all results reported alongside any limitations which may affect the result (see section 7.2.1 and section 9.1).

If significant trauma and anatomical disruption, or delay in discovery of the victim occurs in such cases, the impact of environmental contamination and the postmortem interval will heavily affect postmortem microbiology interpretation.

4.1.6 The postmortem interval and associated postmortem microbial processes

The postmortem interval (PMI) is the time that elapses from the death of the individual until the discovery of their remains. This period is essential information in forensic science, criminal investigations and court proceedings related to homicides and unwitnessed deaths, including those in hospitals. It is also paramount in accurately interpreting the results of postmortem microbiology, i.e. assessing whether the potentially pathogenic microorganisms isolated or detected were present antemortem (before death) and not as part of the decomposition process and other postmortem events (43).

Forensic investigators use a combination of approaches to estimate the PMI due to the dynamic and complex nature of postmortem changes, beginning with the macroscopic appearance of the body and basic empirical measurements. Immediately after death, decomposition begins uniformly by the autolysis of cells by the cascading enzymic destruction of cell membranes and within this first 72-hour time frame other postmortem changes begin including (44) :

- the mortis triad
  - algor, the cooling of the body, measured often by rectal temperature
  - livor, the accumulation of the blood in veins and capillaries leading to a bluish- purple discoloration to the body
  - rigor stiffening of the muscles due to hypoxic decrease in ATP production
- the level of putrefaction i.e., the decomposition of body proteins and tissues by the diverse commensal microbiome communities in the body and external environmental microbes.

After 48-72 hours the mortis triad becomes less reliable, and as putrefaction develops, the stages are influenced by the composition of the body, i.e., fat can act as a preservative and therefore, higher fat content bodies decompose more slowly, and local climatic and environmental factors including the temperature, humidity, oxygen

levels, sunlight exposure, soil composition, submergence in water, and insect/ predator activity.

As the PMI lengthens by days to weeks to months, forensic entomology can be essential in the estimation of PMI, by assessing the successive stages of insect colonisation of the body, but this is also heavily influenced by geographical location, weather and seasonal variation. After years, most or all these indicators may not be available for PMI estimation. Therefore, many parameters and challenging variables need to be considered in the estimation of PMI, and the variability of the result is unavoidable (44).

These parameters and variables are also challenging in postmortem microbiology investigation. Invasion is used to describe a microorganism entering the bloodstream or organ when the patient is alive, leading to infection, bacteriaemia or sepsis and true positive results. However, false positive postmortem culture results from non-pathogenic or commensal flora can occur as described below (1,3,43,45-47) :

- Postmortem bacterial translocation (PMBT): The beginning of putrefactive decomposition involving movement of bacteria naturally present in mucosal surfaces (i.e., oropharyngeal and gastrointestinal) into the usually sterile tissues of the body after death. This leads to polymicrobial overgrowth involving *Escherichia coli*, *Klebsiella pneumoniae*, other Enterobacterales, *Pseudomonas aeruginosa*, *Enterococcus spp*., *Clostridia*, and *Streptococci*.
- Fungi of the genera Candida, Geotrichum, Malassezia, Trichosporon, and Trichophyton are part of the thanatomycobiome as commensals on mucosal membranes, especially in the skin and/or urogenital and gastrointestinal tracts. After death, such fungi are drivers of putrefaction leading to fungal overgrowth.
- Agonal spread: A theory that during the period just before death, referred to as the agonal period, mucosal damage caused by ischaemia or resuscitation allows bacteria to invade mucosal surfaces.
- Contamination: Microorganisms which are introduced into the tissue during inadequate sampling techniques at postmortem, such as the introduction of coagulase negative staphylococcus or fungi from the skin when taking peripheral blood specimens.

Generally, contamination is polymicrobial, whereas isolation of a single species from multiple sites is more likely to represent true bacteraemia/infection in life. However, even if adequate sampling protocols are used, a single pure organism isolated from multiple sites may possibly still represent contamination rather than infection.

The physical and atmospheric conditions of the location where the body is found, and the likelihood of contamination during moving and transportation the body, and then storage prior to postmortem, are also key to forming accurate conclusions in the COD (10).

Decreasing the incidence of environmental contamination at postmortem also ensures that organisms such as *Aspergillus fumigatus* complex or *Pneumocystis jirovecii* are correctly attributed to the COD in immunocompromised individuals.

In most articles and guidance PMBT is considered less problematic if specimens are obtained within 24 hours after death, or the body is appropriately stored at 4°C before autopsy (46,48).

See section 7.2.1 for more information on postmortem specimen degradation.

See section 9.1 for more information on the importance of considering PMI in the interpretation of postmortem microbiological testing results.

4.2 Histology and other diagnostics in postmortems

Histological investigation in parallel with microbiology testing verifies inflammation and cellular or tissue changes associated with the presence of pathogenic organisms. The presence of tissue inflammation is often the decisive factor in establishing a COD associated with an infection by a particular pathogen but may be the only sign of an infection if microbiology results are negative (45). Histology is also useful for identifying fungal elements, in particular hyphae invading tissue that can be indicative of invasive fungal disease, provided specimens have been obtained in a timely manner (49).

Conversely, postmortem microbiology typically cannot be interpreted in isolation and the correlation with the history of events leading up to death as well as other diagnostic tests, including histology, is essential for accurate interpretation (see section 4.4). The exception to this may be where death occurs rapidly due to an infection (e.g. due to toxic shock syndrome associated with bacterial toxins), there may be insufficient time for the development of inflammation related histological changes, and other more discreet changes must be considered (46).

Biochemistry is more often used to determine non-infectious COD such as diabetes, and anaphylactic shock. Determining the PMI from biochemical biomarkers in fluid specimens from closed compartments such as vitreous humour (VH) and cerebrospinal fluid (CSF) have been extensively investigated. These areas are contaminated more slowly after death, but inaccuracies arise from interpretating levels using clinical reference values established and validated only for plasma or serum and outside of the postmortem setting (50,51).

Postmortem toxicology can assist in determining both the cause and manner of death especially in coronial cases. However, several physiological and environmental factors occurring after death can affect the drug concentration, providing results which are not reflective of the levels present in life and can affect interpretation, including postmortem redistribution and tolerance. Moreover, the validation of assays on postmortem blood and/or body fluids has its known limitations (see section 7.2.1).

Therefore, dose estimations and acknowledgement of these limitations should be reported (52).

Postmortem cross-sectional imaging including multi-detector computed tomography (CT) and magnetic resonance imaging (MRI) is currently used in a small number of centres in the UK and generally alongside invasive postmortems. More frequently cross-sectional imaging is used without dissection in diagnosing specific causes of natural death, such as coronary artery disease and pneumonia. This is always in combination with the clinical history, external examination, and information about circumstances of the death. In other natural causes such as suspected sepsis and meningitis, imaging can be used to direct the focus of invasive postmortem or by image-guided needle sampling for histological, toxicological, and microbiological testing in minimally invasive autopsy (MIA) procedures (see section 4.5) (53).

4.3 Developments in forensic microbiology

4.3.1 Microbiomes and PMI

The variability in the estimation of PMI, especially in longer PMI cases, has led to the development of more accurate empirical approaches. These include next generation sequencing (NGS) and bioinformatics, which allow detailed investigation of the succession and diversity of microbial communities in cadavers, collectively known as the thanatomicrobiome or necromicrobiome.

During life, studies have shown that the succession of microbial communities especially in the gut dominated by *Bacteroides* species *and Lactobacillus* species, with smaller populations of Proteobacteria (including *Escherichia* species) and Actinobacteria (including *Bifidobacterium* species) are relatively stable, despite differences in the diet and lifestyle of individuals. However, after death the fermentation of the cellular byproducts produced during autolysis by these communities are believed to alter the composition of the substrates available, leading to the selection and succession of the microbiome populations. One study found that *Bacteroides* and *Lactobacillus* species exhibited repeatable and statistically significant exponential decay over time (54). There is also a marked shift from an aerobic community to an anaerobic microbial community including *Clostridium* and *Peptostreptococcus* species (55).

Figure 1. Summary of the changes in bacteria present within body sites over three stages of postmortem interval (50)

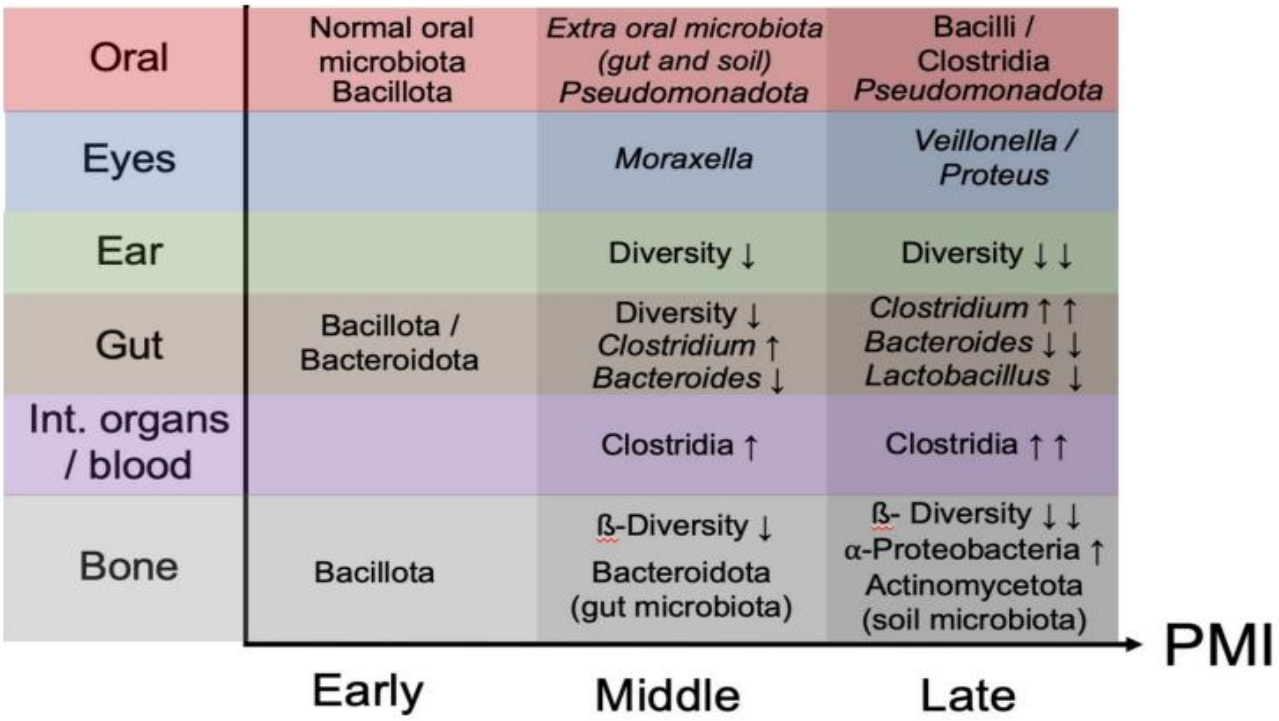
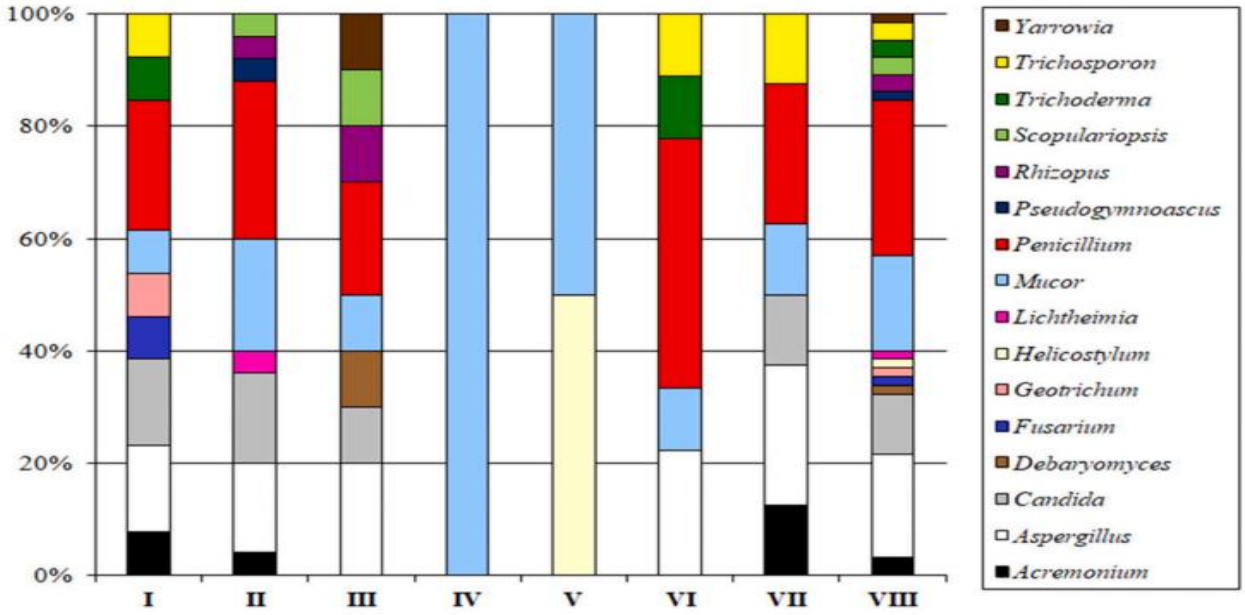


Figure 2. Percentage of the individual genus of fungi present in various stages of corpse decomposition:

I – bloated stage, II – putrefaction stage, III – putrefaction stage with mummification, IV – putrefaction stage with mummification and adipocere (waxy postmortem substance formed from anaerobic bacterial hydrolysis of fatty tissues), V – putrefaction stage with mummification and skeletonisation, VI – skeletonized stage (material from hair and bones), VII – skeletonised stage (material from nearby sites), and VIII –overall from all these cases (56).



Research is underway in human microbiomes in animal and human models to biometrically profile these changes in the microbial community to potentially establish a postmortem microbial clock and increase the accuracy of the PMI. This is by using a multitude of methods including next-generation amplicon sequencing of targeted taxonomic microbial DNA, gene markers such as 16S rRNA (bacteria) and 18S rRNA (fungi), internal transcribed spacer in fungi (ITS) alongside statistical and computational models (57).

Current evidence suggests that microbial succession follows broadly predictable patterns across multiple anatomical sites, including the abdominal cavity, skin, and surrounding soils in outdoor environments (54,58,59). Additionally, studies indicate that cadaver-derived microbial communities can integrate with surrounding soil ecosystems, with detectable signatures persisting for up to a year following decomposition. This has potential forensic utility, particularly in cases where a body has been relocated postmortem. In addition to bacterial succession, fungal communities also demonstrate time-dependent changes during decomposition, with distinct taxa associated with specific stages of decay, suggesting potential utility for PMI (60).

One study's model demonstrated an accuracy to within three days of error over a period of 48 days of decomposition (59), with another showing how microbial communities in cadavers tissue merge into one dynamic system with cadaver-derived microbes detected in the nearby soil for up to a year. This could be useful for estimating the PMI, even after the body has been removed from the location of death (60).

However, evidence relating to both bacterial and fungal succession remains in its early stages of development, with many studies based on small sample sizes, animal models, and variable environmental conditions; nevertheless, advances in sequencing technologies and increasing research interest are likely to improve reproducibility and support PMI determination and future forensic applications.

4.3.2 Microbiota and forensics

The unique combination of microorganisms (microbiota) found to exist in body sites/fluids, including the vagina, faeces, semen, saliva, skin and hair can also help towards identification of body fluids in forensic investigation. For instance, in cases of suspected sexual violence, by analysing the microbial community found in the vagina, which mainly consists of *Lactobacillus crispatus* and *Lactobacillus gasseri* species, vaginal secretions can be distinguished from other bodily fluids (33). Other studies showed that by using microbial community information it is possible to differentiate between menstrual, venous, nasal or skin wound blood at crime scenes (61).

Microbial analysis in forensic settings does have limitations, including the complexity of mixed specimens, i.e. those containing many body fluids, or originating from multiple people. The age and exposure of specimen to environmental factors which

can cause degradation and contamination in such forensic specimens, effects stability of microbial signatures, limiting accurate detection and increasing the yield of incomplete or misleading microbial profiles. Furthermore, the variability in microbial communities among individuals due to genetic, dietary and environmental factors can introduce uncertainty into the analysis.

The use of artificial intelligence (AI) is becoming more essential in managing the increasingly high-throughput, and complex community data obtained, reducing errors in sequence, and integrating data from the various methods used, i.e. metagenomics and transcriptomics. However, these AI models are limited by the availability of large amounts of high-quality, and diverse microbiome specimens, from environmentally diverse areas. Therefore, future rigorous validation under diverse conditions and an establishment of standardisation and repeatability in AI-driven microbiome analysis is required (62).

4.4 Minimally invasive autopsy

Conventional invasive postmortems are still preferred, but increasingly MIA techniques are being recognised as an alternative. This includes cases involving:

- a high risk of transmission of infection from the body, such as SARS-CoV-2.
- deaths in low- or middle-income countries with a lack of trained professionals
- religious or cultural sensitivities towards invasive postmortem, including fear of disfigurement in parents after foetal or paediatric death (1).

MIA involves collecting body fluid or small amounts of tissue from what are considered highly informative organs (i.e., brain and lungs) using fine needles using an endoscopic ‘keyhole’ technique, also known as minimally invasive tissue sampling (MITS) (63).

In another study it has been shown that in patients whose death followed a short history of respiratory illness, there is evidence that collecting an upper respiratory tract swab prior to invasive autopsy can rapidly indicate the COD (64). These swab results could also remove the need to dissect the body by directing the selection of tissue required for histology; this being especially beneficial in situations where views towards postmortem are distressing or problematic, i.e. neonatal death and due to religious beliefs (1).

5 Postmortem microbiology testing pathways

Pathologists undertaking sampling for postmortem microbiology should refer to local and national guidance, e.g. Royal College of Pathologist Autopsy guidelines series, when deciding on best practice for each individual case.

In all cases please refer to RCPath's G031-The retention and storage of pathological records and specimens (38), and relevant Human Tissue Authority (HTA) Postmortem guidance and legislation (39,40).

5.1 Testing principles and laboratory practice

This section concentrates on consented (hospital) postmortem microbiology.

Laboratory protocols should ensure that wherever possible postmortem microbiological investigations are targeted, interpretable, and adaptable to the context of each case, so that findings can differentiate between infections contributing to death and those that are incidental but informative of the individual's living conditions, vulnerabilities, or potential neglect.

Bidirectional discussions between microbiology consultants and pathologists are essential in determining the correct investigations required. After such communication additional specimens and relevant tests may be added to consented postmortem specimens.

Medicolegal postmortem specimens should be tested only for what is requested by the coroner or Procurator Fiscal. This may occur after initial testing has been completed. Please refer to section 7 for more information on specimen types.

Note: Please consider the information below whenever referring to the algorithms included in the document.

Sections 5.2 and 5.4 are based on a recommended set of specimens and tests to consider in consented (hospital) postmortem cases without specific clinical suspicion of infection.

Sections 5.3 and 5.5 include additional testing to be considered in the following circumstances:

- **if clinical information and/or antemortem clinical history is available**
- **if maternal clinical and immunological information is available after neonatal or infant death**
- **if indicated by macroscopic appearance**
- **if indicated by another available postmortem pathology result**
- **when sufficient specimen is available**

The pretest probability or the chance that the patient has an infectious disease should be assessed to guide specimen collection and testing using (when available) the clinical history, antemortem test results, symptoms, and the circumstances

surrounding the death. For example, a positive result from testing for pneumocystis pneumonia in an individual who in life did not present with a cough, could reflect colonisation rather than disease, potentially causing issues in interpretation and reporting the COD.

The tests conducted will also be dependent on:

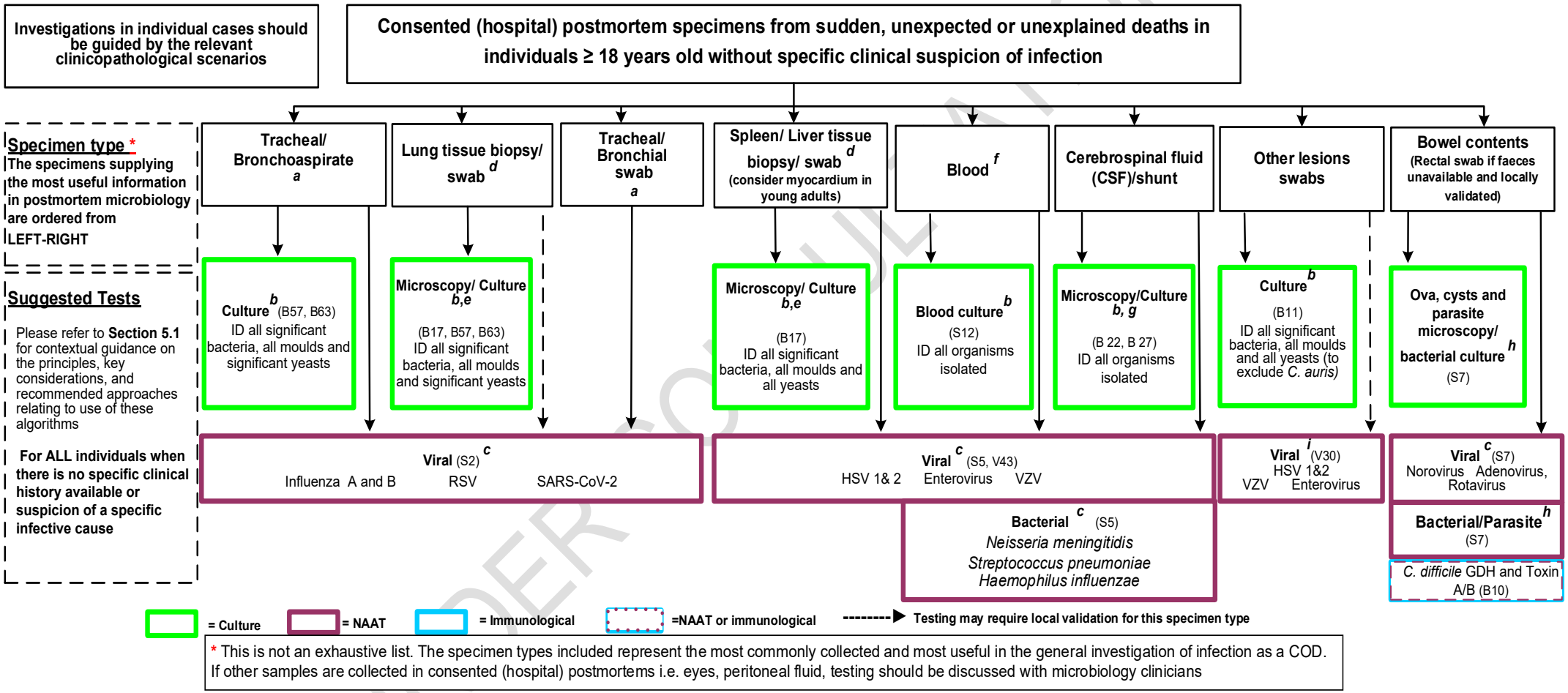
- the amount of specimen available. If the minimum amount of specimen received is less than stated in the table in section 7.1.2, bidirectional discussion between the consultant microbiologist and pathologist on the prioritisation of testing is required. The testing of specimens from other pathology departments, should also be considered whenever possible.
- The local workflow and capabilities of the laboratory. Referral to a specialist/ reference laboratory may be required.

Perinatal deaths with or without congenital infection are not currently within the scope of this document as alignment with other guidance under development is required before inclusion. However, section 5.6 highlights indications of testing for syphilis and toxoplasmosis from consented (hospital) postmortems in neonatal and infant death.

Section 5.7 - 5.14 and table 5.15 highlight compiled relevant testing and/or suggest additional specimen types and tests related to specific clinical suspicion of the COD.

Section 5.16 outlines how postmortem microbiology is adapted in complex cause-of-death scenarios, including emerging pathogens, public health concerns, medico-legal cases, and prolonged postmortem interval.

5.2 Suggested testing on specimens from consented postmortems in individuals ≥ 18 years old without specific clinical suspicion of infection



The following footnotes apply to algorithms 5.2-5.13 (excluding 5.6) and tables 5.3 and 5.5

Abbreviations:

Ag; Antigen

CMV; Cytomegalovirus

EBV; Epstein-Barr virus

HAV; Hepatitis A virus

HBV; Hepatitis B virus

HCV; Hepatitis C virus

HEV; Hepatitis E virus

HSV; Herpes simplex virus

HHV; Human Herpes virus

HIV; Human immunodeficiency virus

hMPV; Human metapneumovirus

RSV; Respiratory syncytial virus

a) The relevance of upper respiratory tract viruses detected in nasopharyngeal swabs and aspirates from cadavers is limited. Ideally lower respiratory tract specimens are more indicative of infection which may cause death. Where this is not possible, i.e. a nasopharyngeal swab or aspirate is acceptable.

b) Please refer to section 10 for guidance on antimicrobial susceptibility testing

c) Molecular kits used in routine primary testing may contain more extensive targets than included in algorithm 5.2, and all targets tested should be reported. The minimum targets for viral NAAT screen should be based on local laboratory assessments and capabilities and may include the suggested examples.

d) Collection and testing of lung tissue/ lung tissue swabs and bronchial aspirate specimens in association with the findings of upper respiratory samples such nasopharyngeal swabs can help to demonstrate passive migration of pathogen and provide overall context of possible infection. Spleen tissue/swab investigation is always complementary to cadaveric blood testing.

e) It is advisable to exclude the use of enrichment liquid medium such as Robertson's cooked media in postmortem specimens due to flora overgrowth in such specimens.

f) If immunological testing such as serology is requested, a discussion between the laboratory and the pathologist is advisable to ensure advice on testing options and limitations is delivered. The following factors should be considered:

- the time between death and collection of blood; the specimen should ideally be taken as soon as possible to avoid haemolysis which can cause non-specific binding to antigens/antibodies causing false positive and false negatives.
- the use of dried blood spots (DBS) made from a femoral stab instead of peripheral blood or recent antemortem specimens (if available)
- severely immunocompromised patients may not be able to raise an antibody response

g) Cell count in postmortem CSF may be inaccurate due to gradual increase of white and red blood cells after death, and the absence of postmortem specific ranges of interpretation (65).

h) Test for routine enteric bacterial pathogens. Consider testing for *Yersinia spp*. and other foodborne pathogens if a possibility of foodborne pathogen outbreak. Consider protozoa (*Entamoeba histolytica*, *Giardia, Cryptosporidium,* and *Cyclospora* species).

i) Viral studies should only be conducted in cases of obvious rash.

j) *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* and *Chlamydophila psittaci* investigation should ideally be conducted by NAAT, and preferred specimens are lower respiratory tract specimens or throat swabs. Serological diagnosis may be unreliable in individuals who are immunocompromised and cadaveric specimens. In this algorithm *Chlamydophila* species include *Chlamydophila pneumoniae* and *Chlamydophila psittaci*.

k) Detection of HHV-6 DNA may represent chromosomally integrated HHV-6 (ciHHV-6) and can result in persistently high viral loads without active infection. Results should therefore be interpreted with caution in postmortem cases.

l) Parvovirus B19 DNA may persist long after primary infection. Its detection by molecular methods should therefore be interpreted with caution in postmortem investigations, as it does not necessarily indicate active infection or causal involvement in disease.

m) After appropriate communication between pathology, microbiology and clinical teams, maternal history and available maternal test results should be considered where relevant and accessible.

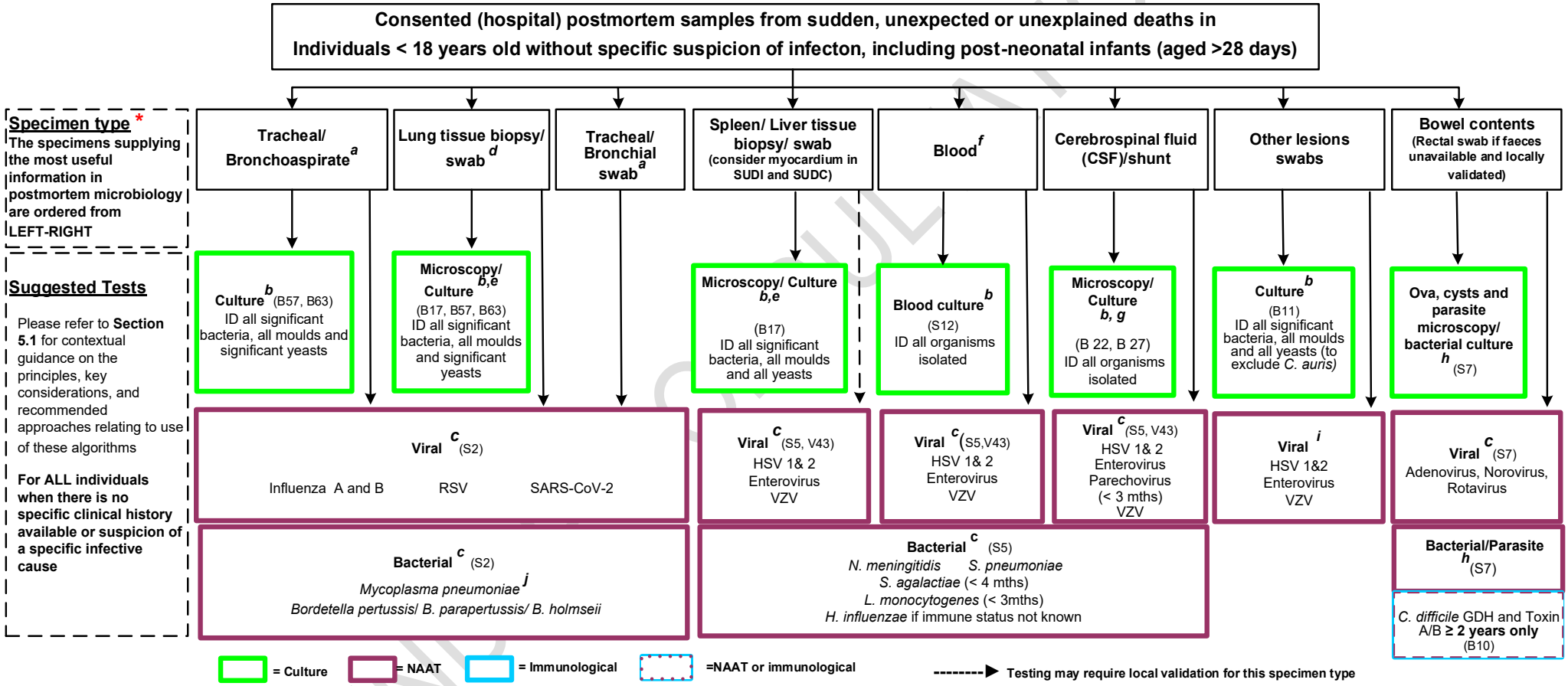
For infections that are primarily vertically transmitted, a documented negative maternal test during pregnancy makes infection in the infant unlikely. However, this should be interpreted with caution, considering the timing and completeness of maternal testing and the clinical context. Where maternal status is unknown, incomplete, or unreliable, targeted testing of the infant should be considered.

n) NAAT for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* should only be undertaken in consented (hospital) postmortems when there is a strong clinical suspicion of involvement in death and where appropriate consent and confidentiality considerations are addressed.

5.3 Suggested clinically guided additional testing on specimens from consented postmortems in individuals ≥ 18 years old

Specimen Type / Diagnostic Test	Tracheal/ Bronchoaspirate [a]	Lung tissue biopsy/ swab [d,e]	Tracheal/ Bronchial Swab [a]	Spleen Liver tissue biopsy/ swab [d,e]	Blood	CSF fluid/ shunt [e]	Other lesion swabs	Bowel content
Viral NAAT [c]	Adenovirus hMPV Parainfluenza virus (PIV 1-4) Rhinovirus RSV			Adenovirus CMV EBV HHV 6-8 [k] Parvovirus B19 [l]	Adenovirus, CMV EBV HAV, HBV, HCV HEV HHV 6-8 [k] HIV 1/2 Parvovirus B19 [l]	Adenovirus, HHV 6-8 [k] Parvovirus B19 [l] Parechovirus HIV 1/2 (only if serology positive), CMV, EBV		Astrovirus Sapovirus
Bacterial [c] NAAT	*Mycoplasma pneumoniae* [j] *Chlamydophila* species *Bordetella pertussis/ B. parapertussis/ B. holmesii* *Legionella pneumophila*				*Listeria monocytogenes* *Treponema pallidum* (only if serology positive)		*Treponema pallidum*	
Parasitic NAAT					*Toxoplasma gondii*			
Mycological NAAT	*Aspergillus* species *Pneumocystis jirovecii*			*Aspergillus* species *Candida* species	*Aspergillus* species *Candida* species Galactomannan	*Aspergillus* species *Candida* species		
Immunological testing [f]					Parvovirus B19 HIV 1/2 CMV Aspergillus Ag Cryptococcal Ag Galactomannan (to confirm histological evidence of invasive disease)	Cryptococcal Ag		

5.4 Suggested testing on specimens from consented postmortems in individuals <18 years old without specific clinical suspicion of infection including post-neonatal infants (aged >28 days)



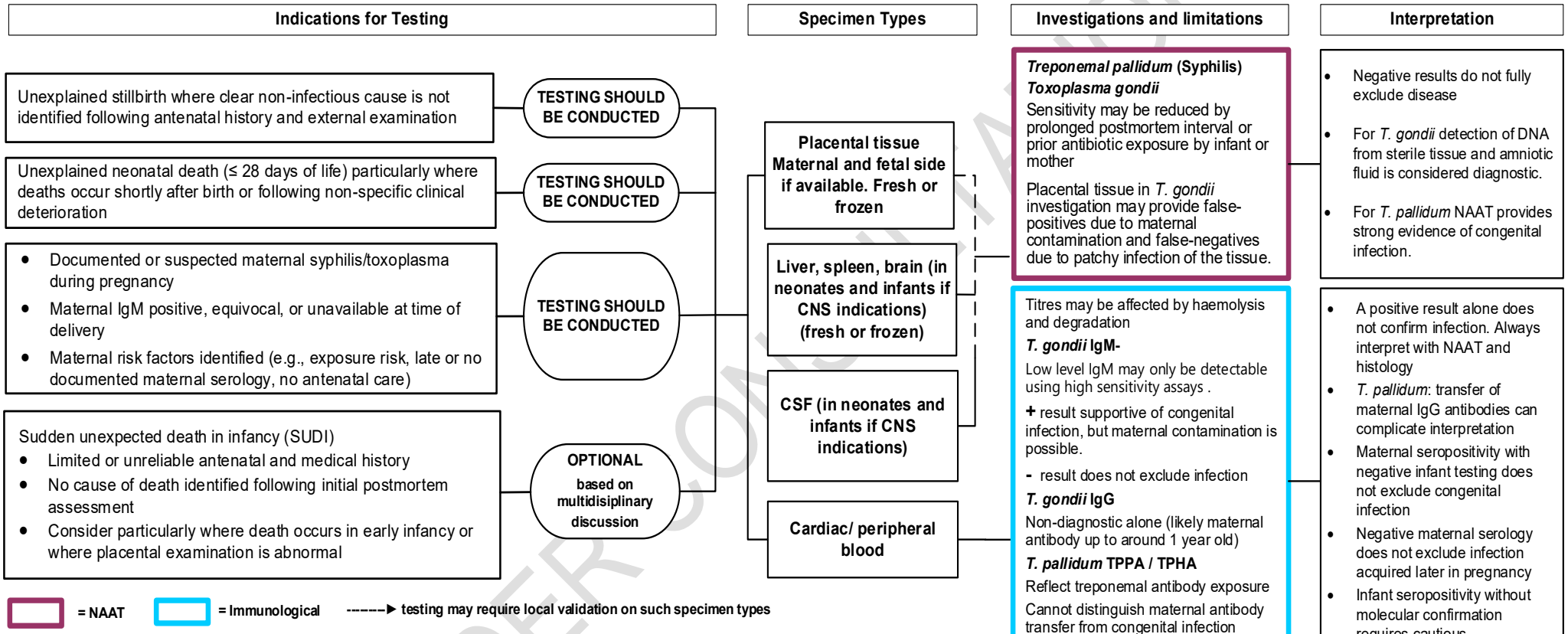
* This is not an exhaustive list. The specimen types included represent the most commonly collected and most useful in the general investigation of infection as a COD. If other samples are collected in consented (hospital) postmortems i.e. eyes, peritoneal fluid, testing should be discussed with microbiology clinicians

5.5 Suggested clinically guided additional testing on specimens from consented postmortems in individuals <18 years old including post-neonatal infants (aged >28 days)

Specimen Type / Diagnostic Test	Tracheal/ Broncho-aspirate *a*	Lung tissue biopsy/ swab *d,e*	Tracheal/ Bronchial swab *a*	Spleen Liver tissue biopsy/ swab *d,e*	Blood	CSF fluid/shunt *e*	Other lesion swab	Bowel contents (Rectal swab if faeces unavailable and locally validated)
Viral NAAT *c*	Adenovirus hMPV Parainfluenza virus (PIV 1-4) Parechovirus Rhinovirus			Adenovirus HHV 6-8 *k* Parvovirus B19 *l*	Adenovirus HHV 6-8 *k* **In infants** *m*: CMV, EBV HIV 1/2 HBV, HCV, HEV Parvovirus B19 *l*	Adenovirus CMV EBV HHV 6-8 *k*	*Treponema pallidum*	Astrovirus Sapovirus
Bacterial and parasitic NAAT	*Chlamydophila* species				**In infants** *m*: (see section 5.6) *Treponema pallidum* *Toxoplasma gondii* **Older children** *Treponema pallidum* (if serology positive)		*Treponema pallidum*	
Mycological NAAT	*Aspergillus species*			*Aspergillus* species	*Candida* species			
Immunological testing *f*					Aspergillus Ag Cryptococcal Ag CMV HIV 1/2 Galactomannan (to confirm histological evidence of invasive disease)	Cryptococcal Ag		

NOTE: In the context of emerging infections, outbreaks, or pandemics, testing strategies should be adapted accordingly. Where a circulating pathogen is associated with foetal, neonatal, or infant death (e.g. Zika virus), targeted testing should be prioritised based on current epidemiological guidance.

5.6 Consented (Hospital) postmortem testing for syphilis and toxoplasmosis in neonates and infants



* **Congenital syphilis:** Comparison of maternal and neonatal Rapid Plasmin Reagin (RPR) titres*; T. Pallidum* IgM in the blood of the neonate; VDRL in positive CSF specimens (suggestive of congenital syphilis)

Toxoplasma: Toxoplasma IgA as a marker of early infection in neonates

5.7 Suggested testing on postmortem specimens in individuals of all ages when there is a specific clinical suspicion of sepsis

Specimen type

Additional specimens to consider which provide the most useful information in postmortem microbiology for suspected sepsis.

Suggested tests

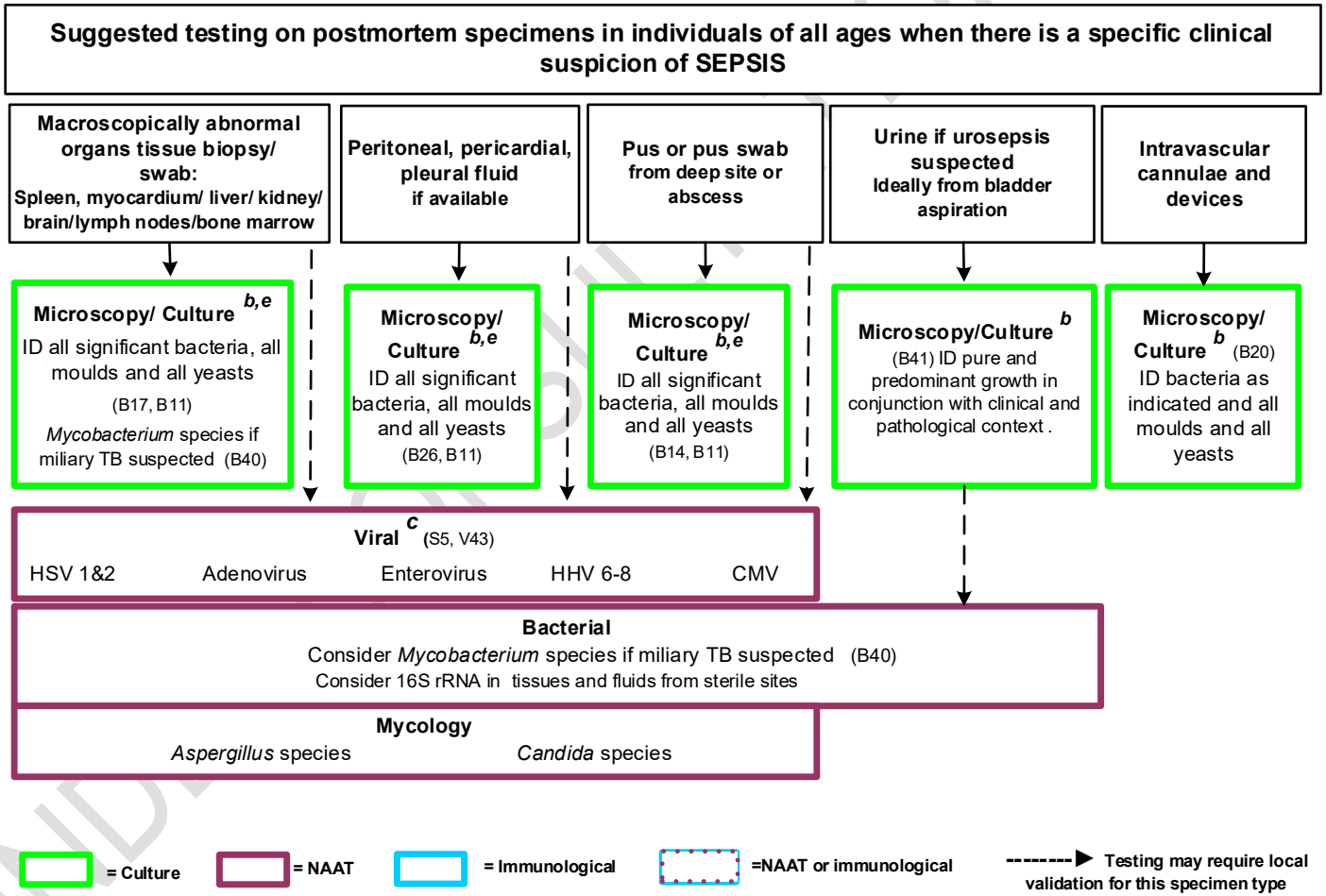
Please refer to Section 5.1 for contextual guidance on the principles, key considerations, and recommended approaches relating to use of these algorithms

If sepsis is suspected, these additional specimens and tests should be considered alongside those stated for:

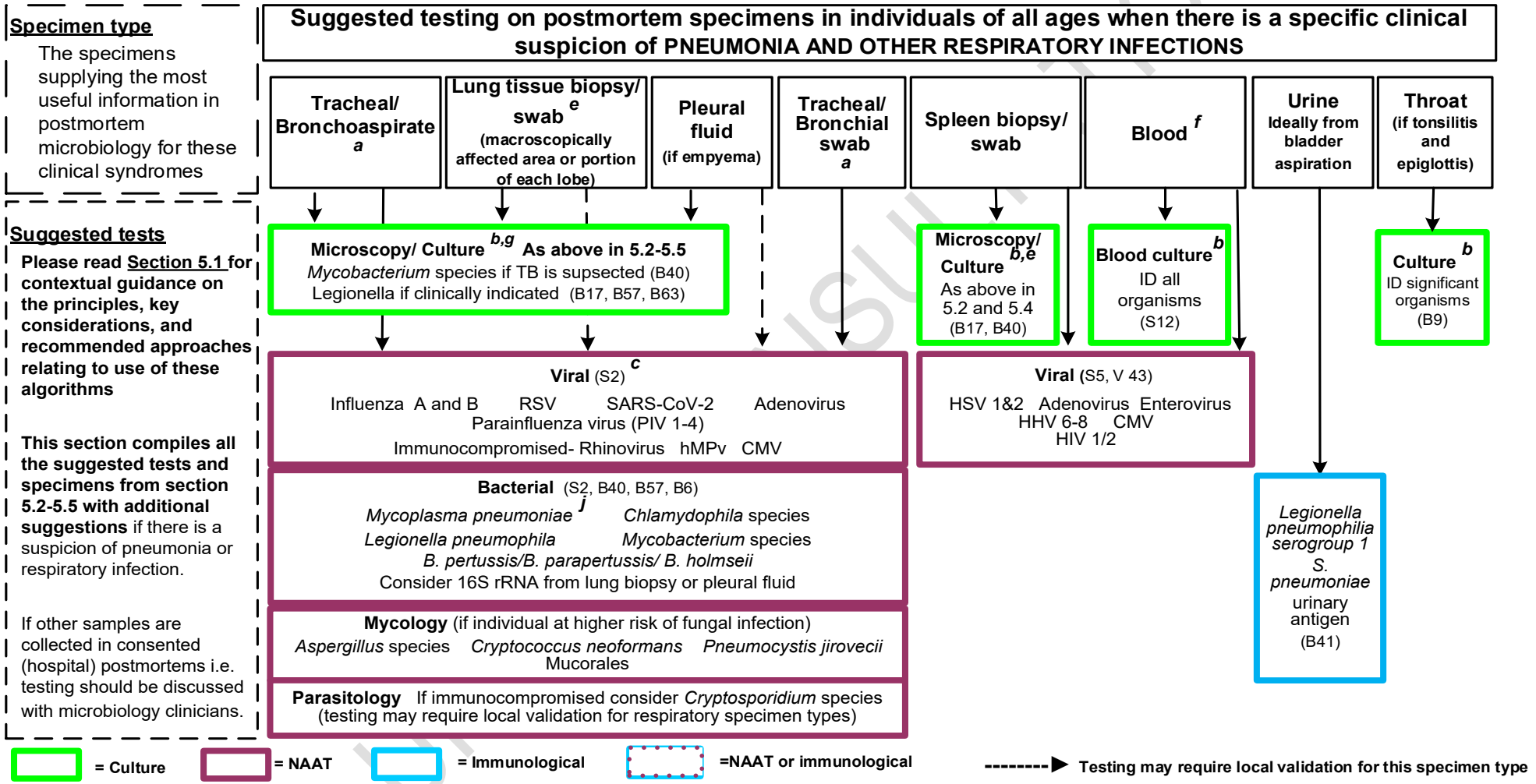
- **Blood**
- **Tracheal/Bronchoaspirate/ swab**
- **Lung tissue biopsy/ swab**
- **CSF**
- **Skin lesions/biopsy/swabs**

in sections 5.2 to 5.5 for the specific age range.

If other samples are collected in consented (hospital) postmortems i.e. testing should be discussed with microbiology clinicians



5.8 Suggested testing on postmortem specimens in individuals of all ages when there is a specific clinical suspicion of pneumonia and other respiratory infections



5.9 Suggested testing on postmortem specimens in individuals of all ages when there is a specific clinical suspicion of central nervous system infection

Specimen type

Specimens providing the most useful information in postmortem microbiology for suspected meningoencephalitis.

Suggested tests

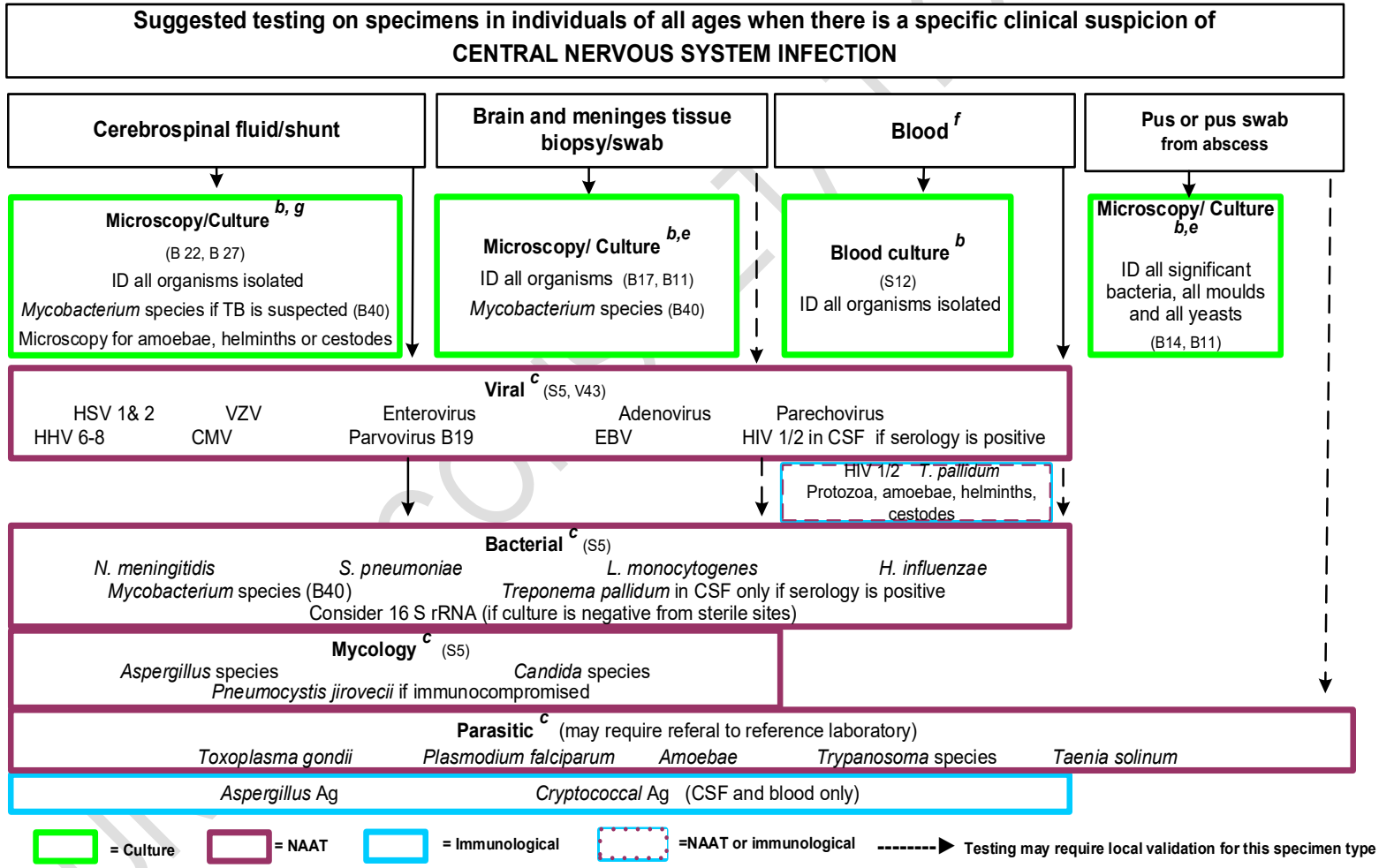
Please read Section 5.1 for contextual guidance on the principles, key considerations, and recommended approaches relating to use of these algorithms.

These sample types and tests should be considered alongside those stated for:

- **Tracheal/Bronchoaspirate/ swab**
- **Lung and spleen tissue biopsy/ swab**
- **Skin lesions/biopsy/swabs**
- **Urine**

in sections 5.2-5.5 and 5.7 for the specific age range if CNS infection is suspected.

If other samples not listed in this document are collected in consented (hospital) postmortems i.e. testing should be discussed with microbiology clinicians.



5.10 Suggested testing on specimens in individuals of all ages when there is a specific clinical suspicion of cardiac infection

Specimen type

Additional specimens to consider providing the most useful information in postmortem microbiology for suspected cardiac infection.

Suggested tests

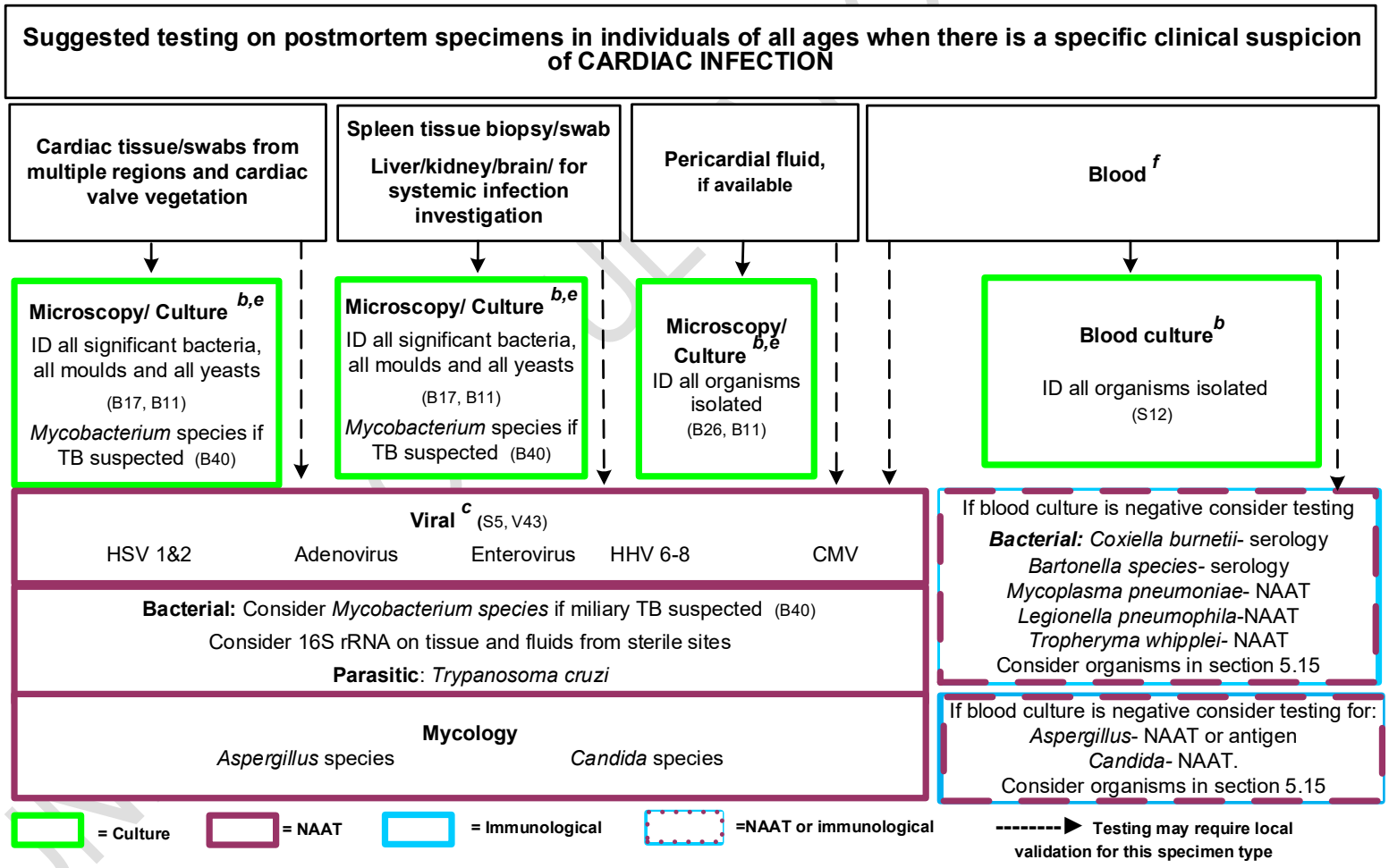
Please refer to Section 5.1 for contextual guidance on the principles, key considerations, and recommended approaches relating to use of these algorithms

These additional samples and tests should be considered alongside the tests for:

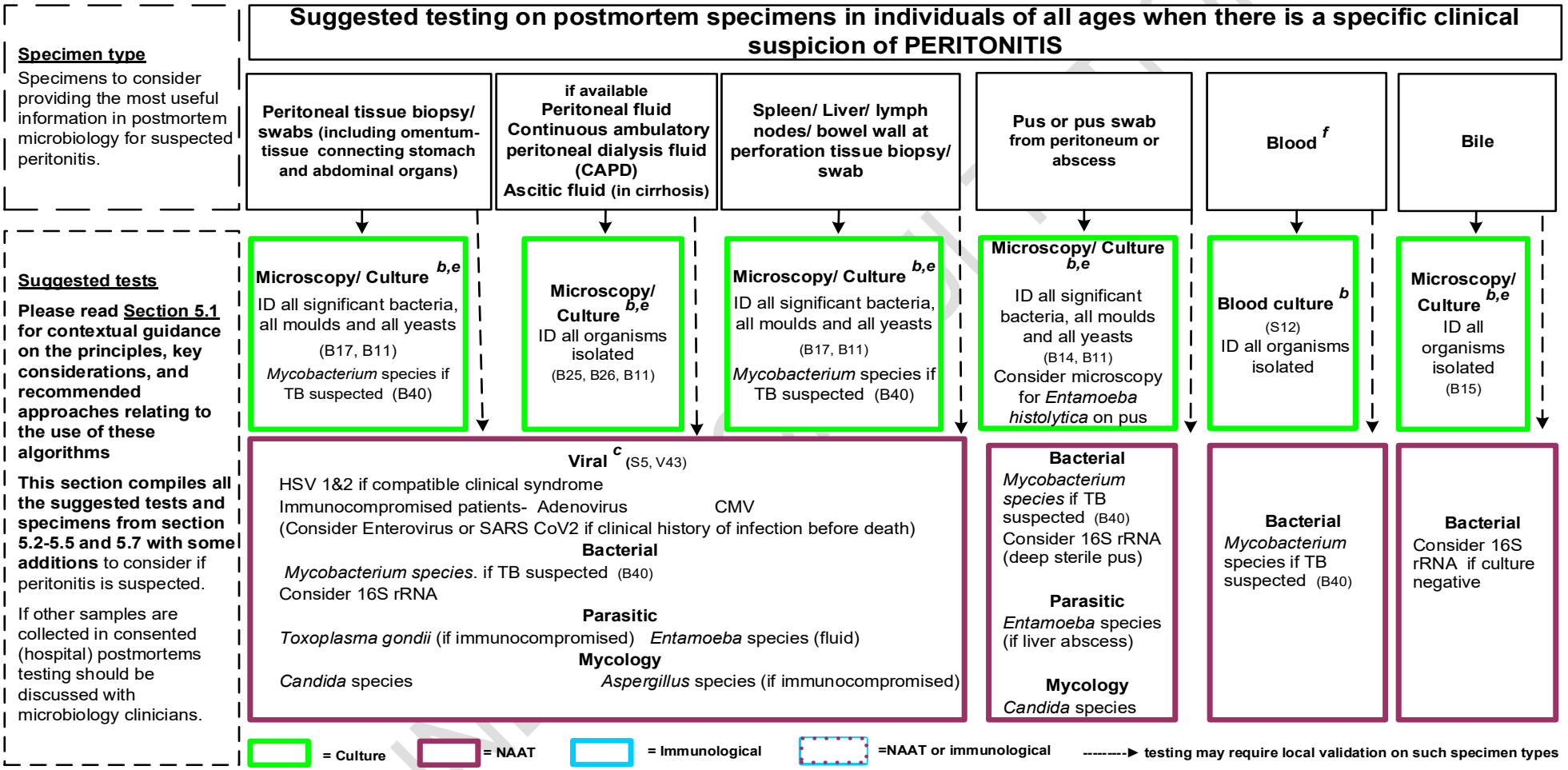
- **Blood**
- **Tracheal/Bronchoaspirate/ swab**
- **Spleen and lung tissue biopsy/ swab**
- **CSF**

in sections 5.2-5.5 for the specific age range and all in 5.7 if cardiac infection is suspected.

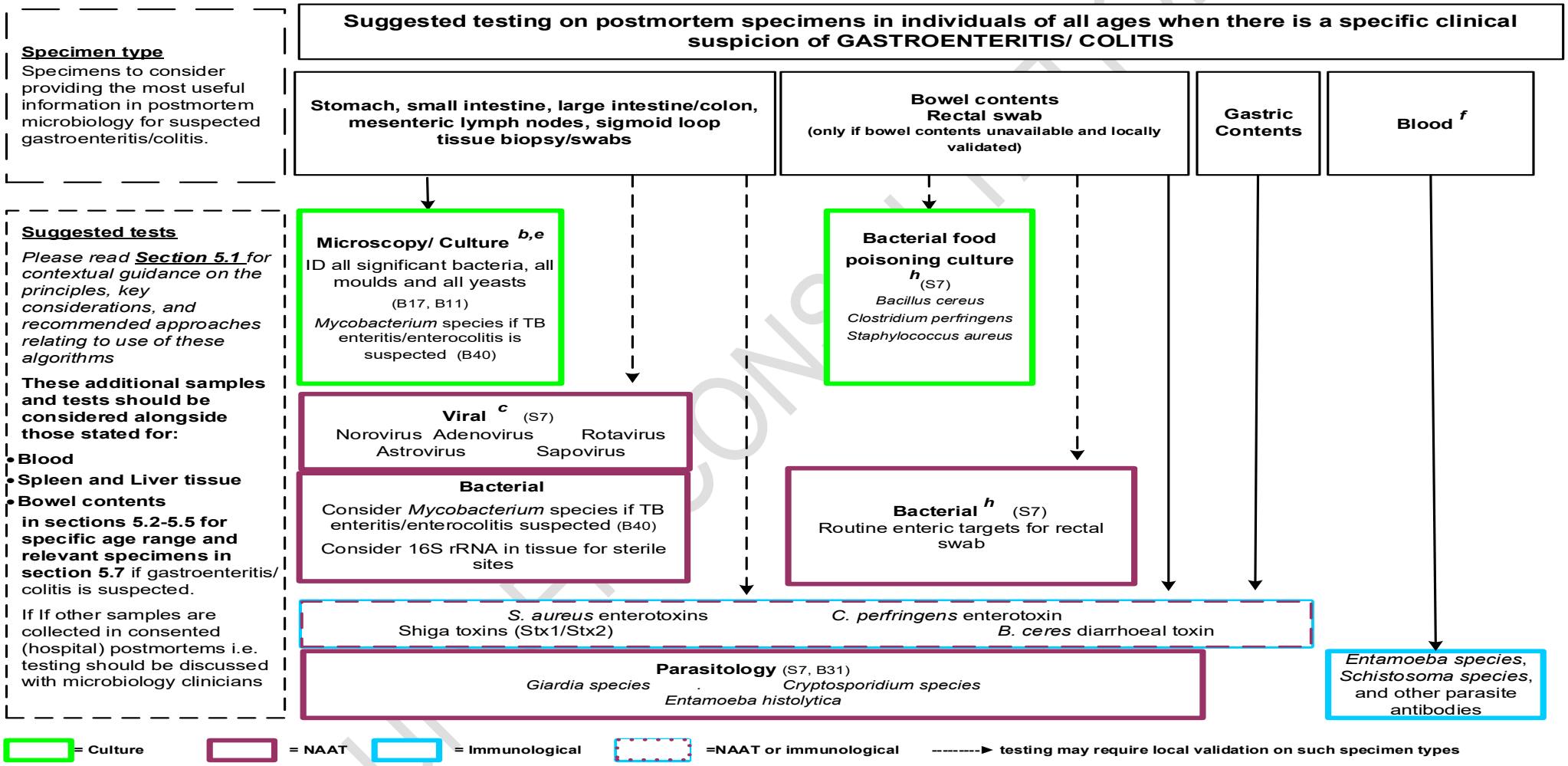
If If other samples are collected in consented (hospital) postmortems testing should be discussed with microbiology clinicians.



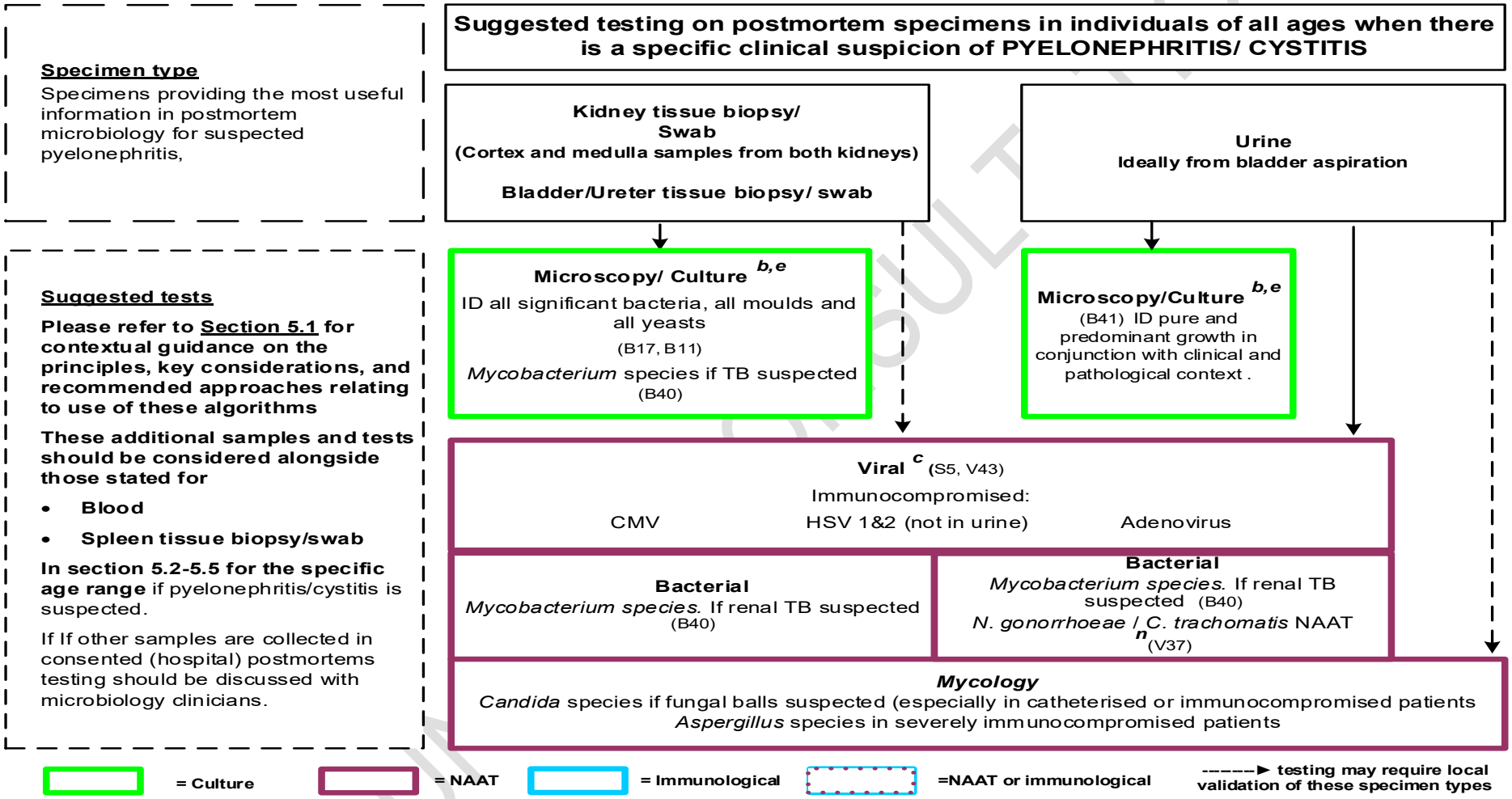
5.11 Suggested testing on specimens in individuals of all ages when there is a specific clinical suspicion of peritonitis



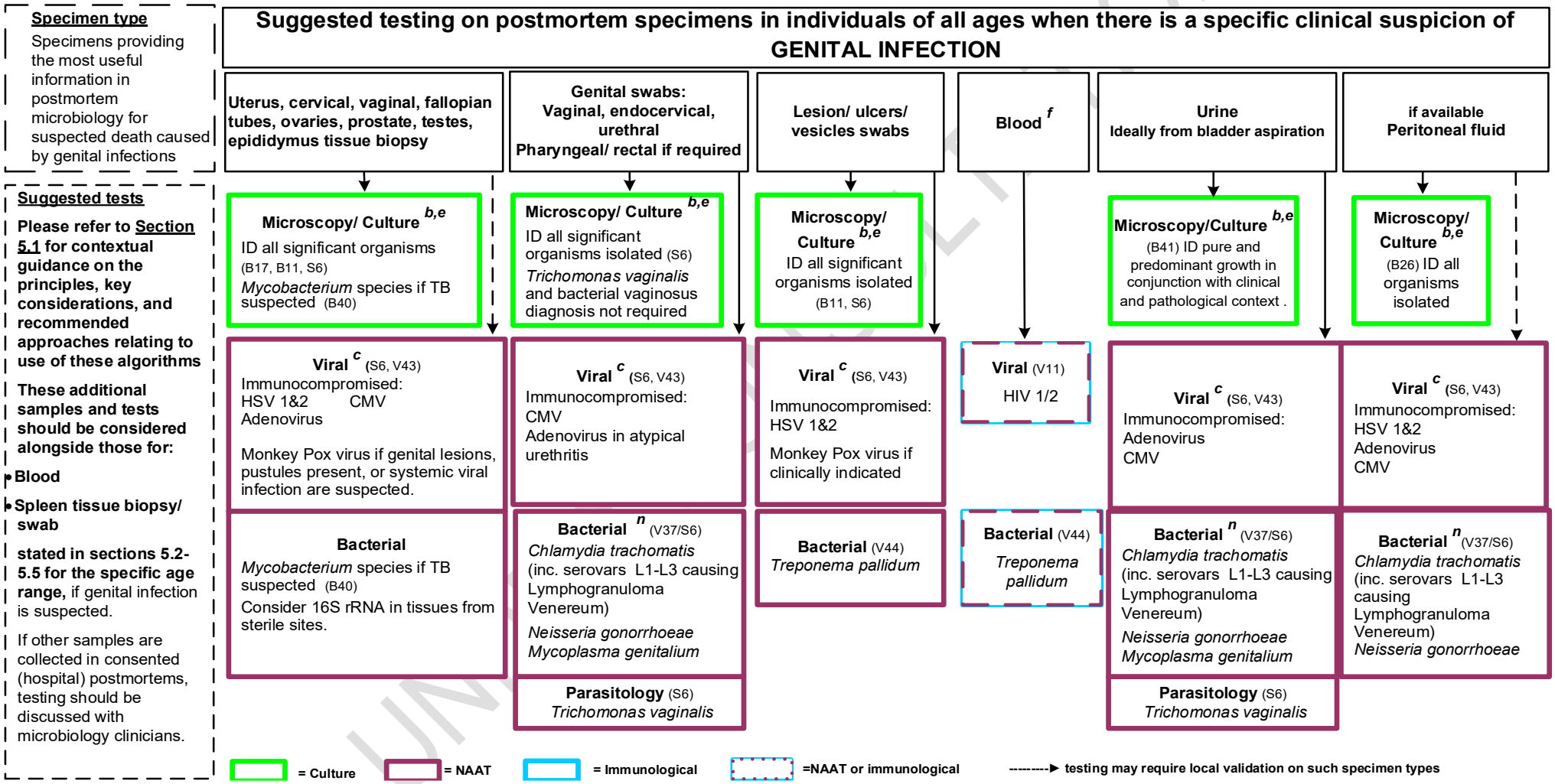
5.12 Suggested testing on specimens in individuals of all ages when there is a specific clinical suspicion of gastroenteritis/ colitis



5.13 Suggested testing on specimens in individuals of all ages when there is a specific clinical suspicion of pyelonephritis/cystitis



5.14 Suggested testing on specimens in individuals of all ages when there is a specific clinical suspicion of genital infection



5.15 Suggested testing on specimens in individuals of all ages when there is a suspicion of infection after international travel

The pathologist and infection specialist should discuss targeted testing for specific travel-acquired infection selecting the most direct specimen type to rule in or rule out a given infection.

This is not an exhaustive list. Most tests would be sent to the relevant reference laboratory (see section 11) and handled in accordance with HG3/HG4 safety protocols. G183-Precautions for high risk infectious autopsies. Refer to relevant reference laboratory user's manuals and ensure that it is clear that postmortem specimens are being sent, especially when blood is required.

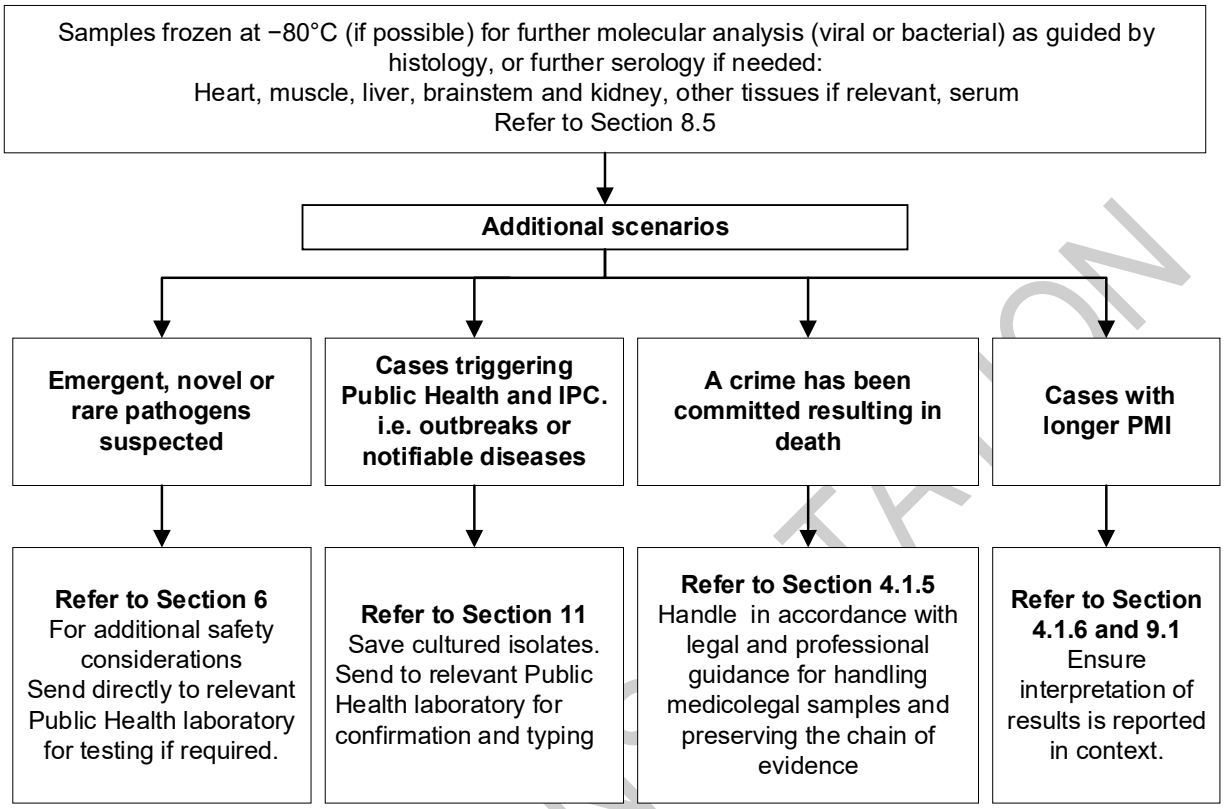
Suspected Organism	Typical exposure regions	Postmortem Specimens	Test
Viral investigation			
Crimean-Congo haemorrhagic fever virus (CCHFV)	Africa, Middle East, Balkans, Central Asia	Liver, spleen, lymph nodes tissue biopsy Blood	NAAT
Chikungunya virus	Africa, Asia, Americas	Liver, spleen tissue biopsy CSF/ brain tissue if encephalitis suspected Blood	NAAT NAAT Immunological
Ebola virus	West/Central Africa	Liver, spleen, lymph nodes tissue biopsy Blood Oral fluid swab	NAAT
Dengue virus	Tropics/subtropics worldwide	Liver, spleen tissue biopsy Blood	NAAT NAAT Immunological
Hantavirus	Americas-pulmonary syndrome (HPS) Europe/Asia-haemorrhagic fever with renal (HFRS)	Lung tissue biopsy (HPS) Kidney, spleen tissue biopsy (HFRS) Blood	NAAT NAAT Immunological
Hendra virus	Northeastern Australia	Lung, brain, and spinal cord tissue biopsy CSF Blood	NAAT NAAT Immunological
Japanese encephalitis virus	South/Southeast Asia	Brain/ spinal cord tissue biopsy CSF	NAAT NAAT Immunological
Lassa fever virus	West Africa	Liver, spleen tissue biopsy Blood	NAAT

Suspected Organism	Typical exposure regions	Postmortem Specimens	Test
Marburg virus	East/Central Africa	Liver, spleen, lymph nodes tissue biopsy Blood	NAAT
Middle East Respiratory Syndrome (MERS-CoV)	Arabian Peninsula	Lung, tracheal-bronchial tissue biopsy Tracheal/ nasopharyngeal swab Blood	NAAT NAAT Immunological
Nipah virus	Bangladesh, India, Malaysia	Lung, brain, spleen, liver tissue biopsy CSF Blood	NAAT NAAT Immunological
Rift Valley fever virus	East/Southern Africa, Arabian Peninsula	Liver, spleen tissue biopsy Blood	NAAT
Tick-borne encephalitis virus	Europe, Russia	Brain/ spinal cord tissue biopsy/ CSF Blood	NAAT NAAT Immunological
Yellow fever virus	Sub-Saharan Africa, South America	Liver, kidney tissue biopsy Blood	NAAT
Zika virus	Americas, Pacific, SE Asia	Brain tissue biopsy Blood Urine	NAAT
West Nile virus	Americas, Europe, Africa, Middle East	Brain/ spinal cord tissue biopsy Kidney, spleen, lung, pancreas in immunocompromised CSF	NAAT NAAT Immunological
Western/Eastern/ Venezuelan Equine Encephalitis viruses (WEE/EEE/VEE)	Americas (EEE/VEE)	Brain/ spinal cord tissue biopsy CSF Blood	NAAT NAAT Immunological
Parasitic Investigation			
Angiostrongylus cantonensis (rat lungworm)	Southeast Asia, Pacific Islands, Caribbean	Brain, lung tissue biopsy CSF	NAAT
Babesia species	USA (Northeast), Europe, China	Blood Spleen tissue biopsy Bone marrow	NAAT Microscopy

Suspected Organism	Typical exposure regions	Postmortem Specimens	Test
Echinococcus granulosus / multilocularis **(dog tape worm)**	Mediterranean, Middle East, China, Central Asia, South America	Liver/ Lung cyst contents Cyst wall tissue Blood	NAAT NAAT Immunological
Entamoeba histolytica	India, Mexico, Africa, Southeast Asia	Liver abscess contents Colon tissue biopsy Bowel/rectal contents Blood	NAAT Microscopy Immunofluorescence microscopy
Leishmania donovani / infantum /chagasi **(visceral leishmaniasis)**	India, Nepal, Sudan, Brazil, Mediterranean	Spleen/ liver tissue biopsy Bone marrow Blood	NAAT NAAT Immunological
Naegleria fowleri	Worldwide in warm freshwater	Brain tissue biopsy CSF	NAAT
Paragonimus sp.	East & Southeast Asia	Lung tissue biopsy	NAAT
Plasmodium falciparum (**Malaria)**	Sub-Saharan Africa, Oceania	Spleen, brain, heart tissue biopsy Blood	NAAT Microscopy Immunological
Other malaria species ***(P. vivax, P. ovale, P. malariae, P. knowlesi)***	Asia, Latin America, Africa	Liver, spleen tissue biopsy Blood	NAAT Microscopy NAAT
Schistosoma sp.	Africa, Middle East, Asia	Liver, intestine, bladder lung tissue biopsy Urine (*S. haematobium*) Blood	NAAT Microscopy Immunological
Strongyloides stercoralis **(hyperinfection)**	SE Asia, Africa, Latin America	Lung, intestine tissue biopsy Blood	NAAT NAAT Immunological
Taenia solinum **(neurocysticercosis)**	Latin America, Asia	Brain tissue biopsy	NAAT
Trypanosoma brucei **(African Trypanosmiasis)**	East & West Africa	Brain tissue biopsy	NAAT
Trypanosoma cruzi **(Chagas disease)**	Latin America	Spleen, heart tissue biopsy Blood	NAAT NAAT Immunological

Suspected Organism	Typical exposure regions	Postmortem Specimens	Test
Mycology Investigation			
Blastomyces dermatitidis	North America (Great Lakes, Mississippi/Ohio valleys)	Lung tissue, bone biopsy Skin	Fungal culture NAAT
Histoplasma capsulatum	Americas, Africa	Lung, spleen, liver tissue biopsy Bone marrow Tracheal/Bronchoaspirate/ swab	Fungal culture NAAT NAAT Fungal culture NAAT (aspirate only)
Paracoccidioides brasiliensis/lutzii	South America	Lung tissue and lymph node biopsy	Fungal culture NAAT
Talaromyces (T. marneffei)	Southeast Asia;	Liver, spleen tissue biopsy Bone marrow Skin	Fungal culture NAAT
Bacterial Investigation			
***Coxiella burnetii* (Q fever)**	Worldwide	Liver, lung, spleen tissue biopsy	NAAT Immunological
Brucella spp.	Middle East, Mediterranean	Liver, spleen tissue biopsy Blood	Culture NAAT Culture NAAT
***Burkholderia pseudomallei* (melioidosis)**	Southeast Asia, Northern Australia	Lung, spleen, liver tissue biopsy Blood	NAAT Culture NAAT
Francisella tularensis	Type A- North America	Spleen, liver, lung tissue biopsy Blood	NAAT Culture NAAT
***Leptospira spp.* (leptospirosis)**	Freshwater Worldwide	Kidney, liver tissue biopsy Blood	NAAT NAAT Immunological
***Rickettsia spp.* (typhus, spotted fever)**	Africa, Asia	Spleen, liver, skin tissue biopsy Blood	NAAT NAAT Immunological
Salmonella Typhi/Paratyphi	South Asia, Africa	Blood Spleen, liver tissue biopsy Bone marrow	Culture NAAT
***Yersinia pestis* (plague)**	Madagascar, Democratic Republic of Congo, parts of Asia;	Lung tissue, lymph node biopsy Blood	NAAT Culture NAAT

5.16 Additional scenarios for consideration in postmortem microbiology investigation



6 Safety considerations

The section covers specific safety considerations related to this UK SMI, and should be read in conjunction with the general safety considerations.

Recommendations regarding the performance of infectious postmortems are available from the RCPath's Autopsy Guideline Series, which includes information on high-risk Hazard Group 3 and 4 infectious postmortems and the Health and Safety Executive (HSE) Managing infection risks when handling the deceased (48)

Due to the possibility of suspected HG3 organism infection risk assessment of routine procedures with the primary clinical team and histopathology consultants is recommended.

If a biocrime is suspected at any point, the relevant government bodies should be contacted immediately before any further testing takes place. See section 11 and also refer to governmental guidance Chemical, biological, radiological and nuclear incidents: recognise and respond (66) .

All respiratory specimens should be processed in a microbiological safety cabinet in Containment Level 3 (CL3) conditions alongside all samples where infection with a

Hazard group 3 organism is suspected. This includes *Mycobacterium tuberculosis complex*, *Brucella* species, *Francisella* species, *Yersinia pestis*, *Burkholderia mallei*, *B. pseudomallei* or endemic fungi such as Histoplasma and Blastomyces.

If transmissible spongiform encephalopathies (TSEs) also known as prion diseases (including Creutzfeldt-Jakob Disease (CJD)) is suspected refer to public health guidance (67,68) .

Due to the severity of the disease, and the risks associated with generating aerosols, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been excluded (as must any laboratory procedure that may give rise to infectious aerosols).

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

If blood culture bottles are employed to provide an enrichment broth, then any consequential use and subsequent disposal of syringes and needles must comply with local safety protocols. Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI. The list of agents is regularly reviewed and updated by the Advisory Committee on Dangerous Pathogens (ADCP) and the resulting publication The Approved List of biological agents is issued by the Health and Safety Executive (HSE). Also view WHO fungal priority pathogens list to guide research, development and public health action.

The above guidance should be supplemented with local COSHH and risk assessments.

Refer to The Green Book for information on vaccinations relevant to laboratory staff.

7 Pre-laboratory processes

7.1 Specimen type

The specimen types included in section 5 represent the most useful in the general investigation of infection as a COD, but the specimens collected are determined by pathologists as below:

- **Consented postmortems in adults and older children-** the selection of specimens for postmortem microbiology should be determined by the antemortem information and clinical context if available.

In sudden death cases without antemortem or clinical information, the most informative specimens in microbiology are,

  - lung and spleen tissue/swabs
  - tracheobronchial aspirates/swabs
  - other tissues/swabs from areas where macroscopic findings suggest an infection (2)

  - blood specimen for blood culture
- **Consented postmortems in younger children-** specimens are taken as indicated in relevant clinical guidance. The extent of sampling is decided by the pathologist dependent on the case and using the circumstances of death or antemortem history if available (9) (69).
- **Medicolegal postmortems**- the legal and investigative authority determines the specimen types taken for investigation under medical advice.

This document suggests the postmortem samples which provide the most useful information in consented (hospital) postmortem microbiology.

The following information should also be considered in specimen selection:

General:

- Use of specific specimen types in individual assays is subject to local verification and validation requirements, alongside the manufacturer's instructions.
- Providing adequate specimen volume is essential for maintaining test sensitivity and accuracy, especially in a postmortem setting, as a repeat specimen is very likely to be not possible
- Wherever possible, antemortem specimens should be located (if in other departments) and tested preferentially.
- Cardiac blood is preferable, but peripheral blood is acceptable and should be collected from the subclavian vein wherever possible (3)
- Tissue specimens for culture are preferred over swabs. However, it is appreciated that in each case there may be circumstances which restrict the ability to collect the recommended specimen types. This may be because the site is severely damaged, decayed, or missing, the mortuary facilities restrict the option for storage and transport, or the family do not consent.
- Although a postmortem nasopharyngeal swab or aspirate is acceptable for most molecular platforms, it is important that correlating specimen types are also collected wherever possible, i.e. respiratory aspirates/swab and lung tissue swab. The correlation of pathogens isolated from such related specimens helps to interpret what is more likely an infection rather than contamination. This also applies to spleen tissue/swabs and blood cultures from cardiac blood, especially in the first 72 hrs postmortem
- If minimally invasive autopsy techniques have been used it is useful to indicate this on the test request as this could assist interpretation of results.
- If meningococcal septic shock is suspected, the investigation of throat swabs may be useful (2)
- Bacterial and fungal culture should not be conducted on respiratory swabs.

- Formalin fixed paraffin embedded (FFPE) tissue may be useful for molecular testing especially where specimens for microbiology were not taken initially at postmortem, and an infectious cause is suspected after the body has been released. Testing of this specimen type should be validated locally and is not recommended for fungal molecular testing (70).

Molecular testing

- When performing molecular testing EDTA blood tubes are required.
- Heparin tubes are inhibitory to NAAT testing.
- Citrate tubes can lead to Aspergillus NAAT false positivity.
- Knowledge of the detection range, sensitivity and specificity of a specific assay is required, along with an understanding of the limitations and risks of genomic amplification.
- Site-specific specimens (e.g., genital swabs, urine) generally provide the highest diagnostic yield, whereas aseptically obtained fluids (e.g. bladder aspirate) may reduce contamination but can have lower sensitivity due to low organism burden. This is particularly true for pathogens that primarily infect mucosal surfaces such as *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*.
- Low-level detection of multiple targets on highly multiplexed respiratory NAAT platforms may represent non-specific amplification arising from nucleic acid degradation, complicating interpretation and increasing the risk of false-positive results.
- Some multiplex molecular testing may give results for organisms not requested. Under these circumstances laboratories should follow local procedures. Follow the manufacturers' instructions and local testing laboratory user manual for appropriate transportation tubes and pots for sample collection.
- 16S and pan-fungal sequencing may be considered under infection specialist request but must be reported and interpreted with caution.

Biomarker testing

- Beta-D-glucan biomarker testing for invasive fungal infection is not recommended, due to high background levels caused by fungal overgrowth and cellular breakdown postmortem.
- Galactomannan biomarker testing of blood which may be taken for invasive aspergillosis may be helpful only in cases where fungal hyphae have been identified in histology sections.

Immunological testing

- Haemolysis of postmortem blood samples can cause non-specific binding to antigens/antibodies causing false positive and false negatives in immunological testing. Therefore, if serology is requested, it is strongly advised that a discussion between the laboratory and the pathologist is conducted to ensure advice on testing options and limitations is delivered.

  The following factors should be considered:

  - severely immunocompromised patients may not be able to raise an antibody response
  - antemortem immunological testing sample results should initially be considered if available
  - the time between death and collection of blood; the sample should ideally be taken as soon possible to avoid haemolysis.
  - It is acknowledged that this may not be feasible, but whenever possible either blood or a dried blood spot created from a femoral or cardiac stab taken on receipt of the cadaver into the morgue and prior to a full postmortem is preferred.

- If it is not stated that an assay's manufacturer has validated the assay for use with blood specimens from deceased patients, laboratories should aim to specifically validate these assays with such blood samples. *Appendix 19 of the European Directorate for the Quality of Medicines & HealthCare* guidance on the validation of infectious screening assays from postmortem blood describes three key factors to consider during validation which can lead to false negative results (71).

  - postmortem degradation of the sample with an associated fall in the detectable level of a target antigen.
  - accumulation of substances inhibitory to the assay due to postmortem changes to blood, i.e. red cell haemolysis, presence of precipitants/or byproducts created by cell death.
  - possible haemodilution of blood sample caused by the administration of blood components and/or colloids in the 48 hours preceding death due to severe blood loss and circulatory arrest. This may cause a decreased detectability of the antibodies or antigens in the blood, possibly leading to false-negative results. (72,73). If validation has not been conducted, results must be reported with an associated disclaimer.

- The specific choice of sample container may be subject to local validated testing methods and platform.
- If a sub-optimal volume is provided (perhaps due to dilution) this must be reported.

7.2 Specimen collection and handling

7.2.1 Sampling in postmortem

Please refer to the relevant guidelines available from the RCPath's Autopsy guidelines series for disinfection of the body and dissection.

A postmortem should ideally be conducted 24 hours after death, but if not possible, the body should be transferred as soon as possible and stored at 4°C.

The duration of refrigeration before postmortem specimens are collected is very useful information when interpreting microbiology results and should be included on test requests wherever possible.

Specimens should be collected aseptically and transferred to the appropriate sterile CE/ UKCA marked leak proof containers and transported in sealed plastic specimen bags.

The specific choice of specimen container may be subject to local validated testing methods and platform.

Ideally separate specimens should be sent if multiple tests are required.

Heparin or oxalate (anticoagulant) or fluoride (preservative) in blood sampling tubes is toxic to many microorganisms and should be avoided.

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Table 4: Recommended specimen types and associated stage of postmortem collection, container type and volumes.

The specific choice of sample container may be subject to local validated testing methods and platform.

Stage of postmortem	Specimen types	Container type	Recommended volume/size
Beginning of postmortem before opening the body	Blood cultures, ideally from peripheral or cardiac vein	Aerobic and anaerobic (aerobic only if only a small volume is available) bottle Paediatric bottle Consider specific bottles for mycological and mycobacterial culture	Adult: 40-60 mL Paediatric bottle 5 mL Please see UK SMI S12: Sepsis and systemic or disseminated infections
	Blood, ideally from peripheral or cardiac vein Femoral or cardiac stab if serology is required (see above text for limitations)	Whole blood- with clot activator to be centrifuged for serum	3-5 mL
		EDTA	5-10 mL
	Cerebrospinal fluid (via cisterna magna puncture)	Universal/ container or tube	> 1 mL
	Ascites (via right iliac fossa)	Universal/ container or tube	5-10 mL
	Urine (via suprapubic puncture)	Universal (with or without additives i.e. boric acid dependent on local policy)	5-10 mL
	Nasopharyngeal/ Tracheal aspirates/exudates	Universal/ container or tube	5-10 mL
	Nasopharyngeal swabs	Solid or liquid Amies medium for culture For viral studies, transport medium or use of dry swabs as per local testing platform and verification/validation	N/A
	Tracheal swabs		
	Skin swabs		

Stage of postmortem	Specimen types	Container type	Recommended volume/size
During evisceration (removal of organs from body cavity)	Bile	Universal/ container or tube	3-5 mL
	Pus	Universal/ container or tube/syringe	As much as possible
	Bowel contents/ faeces	Universal/ container or tube	> 5 mL
	Tissue from: Lung (from each lower lobe or obviously infected area) Spleen Liver Brain	Universal/ container or tube	Minimum specimen size will depend on the number of investigations requested.
	Swabs of tissue	Solid or liquid Amies medium for culture For viral studies, transport medium or use of dry swabs as per local testing platform and verification/validation.	N/A
During organ dissection (once organs are removed)	Other tissues: Brain (inner sections) Heart Kidneys Pancreas Adrenal Thyroid Pituitary Any other evident lesion	Universal/ container or tube	5-10 mL

Specimens taken during a forensic postmortem and part of a chain of evidence must be dealt with in accordance with regional/national protocols.

Early and bidirectional communication between the infection clinical team and the postmortem team. should be conducted whenever guidance on the most relevant specimen type for testing is required.

7.3 Specimen transport and storage

Specimens should be transported and processed as soon as possible. Specimens stored at ambient temperature should be sent within 2 hours including those in viral transport medium, and within 48 hours when taken in adequate transport media and stored at 2 and 8 °C.

For specimens from medicolegal postmortems i.e.by coronial or Procurator Fiscal request, all stages of specimen handling and transport by must be documented in accordance with the chain of custody and with the relevant legal guidance.

Naïve specimens stored for prolonged periods at ambient (non-refrigerated/frozen) conditions may be prone to nucleotide degradation.

Ideally separate specimens should be sent if multiple tests are required.

Specimens for virus detection should be frozen at –80°C if testing is delayed greater than 48 hours (74) .

Note: Plasma specimens for viral NAAT can be stored long-term at - 20° or - 70°C to minimise RNA loss (75).

FFPE tissue should be transported at ambient temperature in appropriately labelled, leak-proof containers. Samples can be stored at room temperature in a dry environment.

Compliance with postal, transport and storage regulations are essential (76,77).

This guidance should be supplemented with local COSHH and risk assessments.

Specimen transport information and storage consideration related to this UK SMI should be read in conjunction with the scientific information on the UK SMI webpages.

7.4 Clinical information required on test request

Full clinical details of the presentation and patient history where possible should be provided with requests. The following information should be included as a minimum:

- that the individual is deceased
- specimen date and time of collection
- site of specimen collection
- type of infection suspected
- type of swab/specimen sent to the laboratory

Whenever possible the following should also be included:

- the date of death (ideally the PMI)
- the date of refrigeration
- whether death occurred abroad, and the body embalmed

8 Laboratory processes

For safety considerations refer to section 6.

Wherever possible, it is advised that laboratories incorporate a method to identify postmortem specimens in their laboratory information management system (LIMS), to ensure appropriate sample processing, interpretation, reporting and retention is conducted.

8.1 Molecular testing and rapid methods

The decision to use molecular and other technologies to investigate postmortem specimens should be made locally and are dependent on the local workflow and capabilities of the laboratory.

If molecular testing is conducted on a unvalidated specimen type this must be clearly indicated on the final report.

Rapid organism identification systems such as DNA microarray, nested Polymerase Chain Reaction (PCR) and amplicon rescue multiplex PCR on direct specimens such as positive blood cultures is not routinely recommended for postmortems specimens. This is because rapid identification will no longer affect treatment. However, in outbreak scenarios, such platforms may still be helpful in rapidly indicating the presence of resistance genes such as mecA, although the presence of a gene does not necessarily relate to its expression and phenotypic characterisation may still be required.

8.2 Microscopy

Refer to relevant technical UK SMIs and to UK SMI TP 39 – Staining procedures.

Cell counts and differentials are required on all CSFs and ascitic fluids as described in UK SMI B 27 – Investigation of cerebrospinal fluid and UK SMI B 26 - Investigation of fluids from normally sterile sites.

Note: Cell count in postmortem CSF may be inaccurate due to gradual increase of white and red blood cells after death, and the absence of postmortem specific ranges of interpretation (65).

8.3 Culture

Postmortem specimens should be cultured as recommended in the relevant technical UK SMIs, However, due do the possibility of postmortem microbiome changes, and

contamination in such specimens, consideration should be taken before using enrichment liquid medium such as Robertson's cooked media or extending incubation of culture plates likely to increase the quantity of polymicrobial overgrowth and contamination, such as anaerobic culture.

8.4 Identification

As the same organisms are of significance in infection in antemortem and postmortem, reference to individual UK SMIs is advised and clinically significant isolates should be identified to species level.

However, due to PMI and the processes described in section 4.1.6 and section 4.3.1, caution and additional steps may be needed to separate polymicrobial overgrowth and contamination from significant isolates requiring identification. This may include subculture to selective or chromogenic agar.

Discussion with an infection specialist or microbiologist is advised whenever it is unclear which organisms may be significant in a specimen.

The use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) for identification of positive cultures from most clinical specimens and sites has greatly reduced the time from isolation of a positive culture to its identification and antimicrobial susceptibility testing (if required).

The MALDI-TOF procedure for the identification of filamentous fungi is not yet optimised and MALDI-TOF databases may only contain limited fungal species. Please see UK SMI TP 40- MALDI-TOF MS test procedure.

8.5 Storage of postmortem specimens

8.5.1 Primary testing specimens

If specimens are received directly for postmortem microbiological testing (primary specimens), storage of remaining specimens MUST be in line with the relevant Human Tissue Authority Postmortem guidance and legislation (49)(50). Please also refer to RCPath's G031-The retention and storage of pathological records and specimens (48). It is however suggested to store all significant isolates from postmortem specimens wherever possible for a period decided by local policy.

8.5.2 Secondary testing specimens

If specimens are received from other departments for secondary testing, i.e. from histology, it is advised that any remaining specimen be returned as soon as testing is complete.

9 Post-laboratory processes

9.1 Reporting and interpretation of results

Interpretation of postmortem microbiology results requires an understanding of their significance in the context of other findings. This includes correlation with results from other antemortem and postmortem specimens, the patient's clinical history, circumstances surrounding death (including the PMI), treatments received, and associated biochemical, toxicological, and histopathological findings.

To clearly stress the postmortem origin of specimens and to support consistent interpretation, a standardised comment should be included on all postmortem microbiology culture reports as shown above. For example:

"Postmortem microbiology results should be interpreted with caution due to the potential for contamination from commensal internal and external flora. Results should be considered in conjunction with clinical history, findings from other antemortem and postmortem specimens (where available), and other pathological investigations."

Interpretation should be undertaken within a multidisciplinary framework involving infection specialists, microbiologists, the consultant histopathologist, and the postmortem team, with appropriate communication between all parties.

Where feasible, results should be considered collectively at a case level to support integrated interpretation and reporting, particularly in cases with legal or forensic implications. It is recognised that automated release of negative results may limit opportunities for collective reporting in some laboratory settings.

Additional factors which may also influence interpretation

Sampling method:

Specimens obtained during minimally invasive autopsy may be subject to reduced contamination, which should be considered when interpreting results.

Upper respiratory tract findings (paediatric context):

Detection of respiratory viruses in upper respiratory tract samples (e.g. nasopharyngeal swabs or aspirates) may have limited direct relevance to COD. However, in paediatric cases, such findings may provide insight into mucociliary dysfunction and increased susceptibility to secondary infection (e.g. Streptococcus pyogenes with toxin-mediated disease), even in the absence of lower respiratory tract involvement.

Forensic considerations:

In cases with potential forensic relevance, microbiological findings (including fungal organisms) may reflect environmental exposure, postmortem change, or contamination rather than true infection.

9.1.1 Reporting molecular, immunological and rapid test results

Report viral DNA/RNA and serological results as indicated in specific UK SMIs.

Consider adding locally derived interpretative comments and add a disclaimer if the specimen type tested is not validated for the molecular test used.

9.1.2 Reporting microscopy results

Report organism and life cycle stage(s) or specific fungal elements seen.

For fungal microscopic investigation please refer to the British Society for Medical Mycology Best practice guidelines (78).

Refer to: the following for microscopy for *Mycobacterium* species UK SMI B 40: Investigation of specimens for *Mycobacterium* species and for parasites UK SMI B 31: Investigation of specimens other than blood for parasites.

9.1.3 Reporting culture results

Ensure that it is stated clearly that specimen have originated from a postmortem and utilise a standard comment as suggested in section 9.1.

Non-significant growth

Negative culture results in postmortem microbiology should be reported factually (e.g. “no significant growth”) and interpreted with caution.

A negative result does not exclude the presence of infection, as culture yield may be affected by factors such as PMI, prior antimicrobial therapy, and sampling limitations.

Significant

The significance of cultured organisms is strengthened where there is concordance with isolates from multiple sites, recovery from normally sterile tissues (e.g. blood, spleen), or correlation with histopathological evidence of infection.

The semiquantitative presence of microorganisms should be reported using locally determined terminology, such as ‘Heavy’ ‘Moderate’, Light’ or ‘Scanty’ growth. This provides information to understand relative growth and thus significance in specimens taken from different areas of the deceased, i.e., does the quantity of organisms isolated from an upper respiratory specimen correlate to that isolated from a lung aspirate/ tissue specimen?

In contrast, mixed growth or common commensals, especially in low amounts or from a single site, should be interpreted carefully. Overgrowth may obscure clinically relevant organisms, so results should be considered alongside microscopy, other laboratory findings, and the overall clinical context.

In some specimen types, deviation away from the technical UK SMIs may be required when reporting.

Incubation times can be reported if decided locally.

9.1.4 Reporting time

Postmortem samples should be processed and reported according to standard laboratory workflows, except where high-risk organisms or infection control concerns necessitate alternative procedure such as interim or urgent reporting.

10 Antimicrobial susceptibility testing

As the significance of organisms in postmortem specimens is complex, the relevance of routine antimicrobial susceptibility testing is limited. Therefore, conducting antimicrobial susceptibility testing in postmortem specimens should only be considered in the following situations:

- when specifically requested by the pathologist and/or infection clinical team
- in bacterial meningitis cases
- in sepsis cases being treated with antimicrobial therapy at the moment of death
- if multi-drug resistance organisms are suspected
- in outbreaks with multi-drug-resistant pathogens

For interpretation of susceptibility testing results, laboratories should test and interpret according to the EUCAST breakpoints; refer to EUCAST guidelines for breakpoint information.

Alternatively, the Clinical and Laboratory Standards Institute (CLSI) method along with the corresponding CLSI breakpoints can be used: Susceptibility Testing Subcommittees (clsi.org)

Note: Laboratories must ensure that the appropriate methodology and corresponding breakpoints are used consistently. EUCAST and CLSI methods are not interchangeable, and combining elements from both may lead to inaccurate interpretation. Laboratories should validate or verify all methods used, as appropriate

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

10.1 Reporting of antimicrobial susceptibility testing

Laboratory AST results should be reported in a clear and clinically relevant manner to support assessment of whether infection, and specifically antimicrobial resistance, contributed to death.

Only resistance patterns that are likely to have influenced clinical management or outcome should be emphasised. If local laboratory workflow dictates AST testing in more than the scenarios listed in section 10, all antimicrobial susceptibility results should be suppressed on the final report, unless specifically requested by the infection specialist, pathologist, coroner or Procurator Fiscal.

AST findings should be interpreted in conjunction with clinical, microbiological, and pathological information, and should inform, but not determine in isolation, the recording of antimicrobial resistance on the Medical Certificate of Cause of Death (37).

11 Referral to reference laboratories

Any notifiable disease or causative agent should also be notified to the relevant body. If a case is a suspected urgent notifiable disease, or possible intentional release of pathogen (biocrime), the local UKHSA health protection team must be informed within 24 hours to discuss the next steps.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms.

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for specimen submission:

England

Wales

Scotland

Northern Ireland

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required and notify causative agents, primarily for public health purposes e.g. routine *Cryptosporidium* detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of specimens may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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