Post-mortem Lodox® as a potential substitute for histologic confirmation of pneumonia in sudden infant death investigation

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Plagiarism Declaration

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Abbreviations

FPS	Forensic Pathology Service
FMP	Forensic medical practitioner
SRM	Salt River Mortuary
FP	Forensic Pathology
Lodox®	Low dose digital x-ray
SUID	Sudden unexpected infant death
SIDS	Sudden infant death syndrome
WHO	World Health Organisation
CR	Conventional radiology
CT	Commuted tomography
MRI	Magnetic resonance imaging
LIP	Lymphocytic interstitial pneumonia
CI	Confidence interval
HREC	Human Research Ethics Committee
AP	Anterior-Posterior
H&E	Haematoxylin and eosin
κ	Weighted kappa
PPV	Positive predictive value
NPV	Negative predictive value

Abstract

Background: Lodox®, low dose digital x-rays, may be used as a cost-effective screening tool in developing countries for the post-mortem diagnosis of pneumonia in the medicolegal investigation of sudden unexpected infant death, avoiding the need to perform an internal examination. However, interpretation of the Lodox® is not standardised, and this modality has not yet been validated or tested against the recognised best standard in autopsy practice, histologic examination.

Objective: This study aimed to evaluate whether interpretation of a post-mortem Lodox® scan is sufficient to diagnose pneumonia in alleged sudden infant deaths, without the need for confirmatory histology.

Methods: A retrospective cross-sectional study was performed. Lodox® scans and lung histology from 100 infant decedents less than 1 year of age, admitted to Salt River Mortuary between 2011-2021, were independently interpreted according to standardised guideline by a senior forensic pathology registrar (100 cases), radiologist and anatomical pathologist (25 cases each). The data were captured in a Microsoft Excel® spreadsheet and subjected to statistical analysis.

Results: Fifty-seven male infants (57%) and forty-three female infants (43%) were included in the study. The majority of infants were 1 to 5 months of age (67%) with an average age of 3.25 months and the most frequent age encountered being 2 months old. More than half of the Lodox® scans and lung histology were categorised as minor/non-specific changes. Lodox® categorisation was mostly inconsistent with histology categorisation (weighted-k: 0,14; CI: -0,02-0,29) with a negligible relationship between the two modalities (Pearson co-efficient: 0,14 (p:0,16) and Spearman co-efficient: 0,15 (p:0,13)). A strongly positive relationship between registrar and anatomical pathologist histology categorisations was observed (Pearson co-efficient: 0,4 (p:0,05) and Spearman co-efficient: 0,42 (p:0,04)). Fair inter-rater agreement was noted between the registrar and radiologist categorisations; however, these findings displayed broad confidence intervals. (Lodox® weighted-k: 0,27; CI: - 0,09-0,59; histology weighted-k: 0,29; CI: -0,03-0,49). Moderate correlation noted between registrar and radiologist Lodox® categorisations was statistically insignificant (Pearson co-efficient: 0,35 (p:0,09) and Spearman co-efficient: 0,31 (p:0,11)). The Lodox® displayed a low sensitivity of 32% (17%, 51%) with a low positive predictive value of 42% (22%, 63%). Conversely, a high specificity of 80% (68%, 88%; p:0,21) in addition to a high negative predictive value of 72% (61%, 82%; p:0,21) was observed.

Conclusion: The potential utilisation of the Lodox® scan as a substitute for histologic diagnosis of pneumonia in sudden infant death would make a vast difference in forensic practice in lower income countries, where caseloads are high and access to sophisticated imaging is limited. The small sample size in this study (n:100) did not yield statistically significant information to conclude that a relationship exists between Lodox® and histology categorisation. Nonetheless, results suggest that the diagnosis of pneumonia on Lodox® may be unreliable, resulting in failure to identify unnatural causes of death that may not be apparent on history, Lodox® and external examination, as well as compromise public health interventions and cause of death data. Further studies are required to properly evaluate post-mortem Lodox® as a potential substitute for internal examination and histologic diagnosis of pneumonia in sudden infant death investigation.

Chapter 1: Background and literature review

This chapter introduces medicolegal death investigation in South Africa, focusing on sudden infant deaths. Two of the ancillary tools used to determine cause of death in these cases are discussed, with specific reference to the diagnosis of pneumonia.

BACKGROUND

Medicolegal death investigation and South African law

South African law mandates the medicolegal investigation of deaths which may be other than natural, according to the Inquests Act (58 of 1959). Deaths which should be deemed unnatural are defined in the Regulations of the National Health Act (61 of 2003), with one of the categories being deaths which are sudden and unexpected, or unexplained, or where the cause of death is not apparent.

In terms of the Inquests Act, a forensically trained doctor employed by the state Forensic Pathology Service (FPS) shall examine the body of a deceased individual, if available, in order to ascertain the cause of death with greater certainty. The Regulations Regarding the Rendering of Forensic Pathology Service (2018) further stipulate that this post-mortem examination may include the submission for analysis of any tissue, fluid, object, or thing related to the body, for the purposes of establishing the cause and circumstance of death.

The Inquest Act makes provision in section 3(2) for the extent of the medicolegal examination to be at the discretion of the Forensic Medical Practitioner (FMP) assigned to the case. Furthermore, The National Code of Guidelines for Forensic Pathology Practice in South Africa (2018) states that, "*If a partial dissection reveals that the death was unquestionably due to natural causes, further dissection is not essential.*" In short, the FMP assigned to the case will decide how far to investigate a case that proves to be natural. Implicit in this statement is that the post-mortem examination may be terminated as soon as sufficient evidence has been found to determine the cause of death. The amount of information required varies with individual practice and depends on the complexity of the case and the FMP's level of expertise. Thus, in South Africa there are no standard operating procedures to direct the nature of a particular examination (Du Toit-Prinsloo et al, 2011).

Sudden infant death investigation at Salt River Mortuary

Salt River Mortuary (SRM) was a medicolegal facility serving the west metropole of the city of Cape Town, in the Western Cape province of South Africa from 1957 to 2023. In November 2023, SRM was replaced by a new facility, the Observatory Forensic Pathology Institute (OFPI). In the region served, approximately 4000 forensic post-mortem cases are processed per year (Western Cape Government, n.d). In order to relieve the service load, decedents are screened on admission by a specialist Forensic Pathologist (FP). This involves reviewing the history provided, performing, and interpreting a full body Lodox® Xmplar-dr digital x-ray (Lodox®) and conducting an external post-mortem examination. Based on this, if the FP is convinced that the death was natural, they complete a death notification form that specifies the most likely cause of death, without an internal examination of the body.

Sudden unexpected infant death (SUID) cases represent a significant percentage of the cases processed by SRM without an internal autopsy examination. During the period of assessment of a study undertaken at SRM (2013 to 2017), approximately 6.76% of admissions were categorised as SUID on admission (Heathfield, Martin and Ramesar, 2020). These are infants less than 1 year of age, whose death is alleged to have occurred suddenly and unexpectedly. SUID is a catch-all term, with deaths better categorised into particular causes after evaluation, including external and internal examination, histological, toxicological, microbiological, virological and genetic investigations. The study done at SRM found that 64,18% of SUIDs were classified as being due to natural causes, 27,30% due to unnatural causes, and 8,52% remained under investigation after post-mortem examination (Heathfield, Martin and Ramesar, 2020).

Some of the cases that remain under investigation are those that meet the criteria for sudden infant death syndrome (SIDS). This is distinct from SUID and is defined as the death of an infant less than 1 year of age where the cause of death remains unexplained following death scene investigation, review of the clinical history and a full post-mortem examination with specified ancillary investigations as per the San Diego classification of SIDS (Krous, 2003). Importantly, a SUID case can only be categorised as SIDS once a full detailed examination has excluded other causes.

Investigation to exclude SIDS is time-consuming, expensive and in an environment with a very high case burden, the need for expediency favours avoiding a full internal autopsy. However, death notification forms are used by Statistics South Africa for public health and monitoring purposes, hence the importance of accurate cause of death formulation. The World Health Organisation (WHO) has previously categorised South Africa as having low

quality cause of death data (Mathers, 2005). According to the Burden of Disease Unit at the Medical Research Council of South Africa, this has been upgraded to medium quality; nevertheless, a higher standard of cause of death data is necessary for monitoring health status and disease burden (Bradshaw, 2010).

At SRM, cause of death was recorded as lower respiratory tract infection[1] for over half of SUID cases between 2013 and 2017, making it the most common disease stated. Furthermore, 64% of SUIDs had external examinations only (Healthfield, Martin and Ramesar, 2020). It can be deduced that the cause of death determination was based on the clinical information, external examination, Lodox® scan interpretation although the quality of the information gathered from this combination of investigations is uncertain.

The methods employed to determine cause of death should be evidence-based. Various combinations of clinical history, radiology, external and internal examination have been used to diagnose pneumonia post-mortem. The Lodox® Xmplar-dr full body digital x-ray imaging device has been successfully used in medicolegal death investigation, specifically in detecting skeletal injuries and radio-opaque foreign bodies (Bateman, 2008). The interpretation of soft tissues such as the lung fields on the Lodox® scan has however not been proven to be a valid investigation to use when compared to histological examination of lung tissue, which is the best current standard (Quarrie, 2015).

Training in Lodox® scan interpretation and histologic examination

Correct interpretation of the Lodox® is vital if it is to be used with confidence in cause of death formulation. To ensure the requisite quality of interpretation, adequate training and experience is essential. Forensic registrars are a subset of FMPs specialising in forensic pathology. It is important to note that the FMPs performing and interpreting the Lodox® scans in the local setting have no formal training in radiology. Interpretation of conventional x-rays is taught at medical school, based on scans from living individuals performed in the upright position. These skills are refined in clinical practice and the knowledge directly transferred for use in the post-mortem setting. FMPs do receive technical training from the Lodox® Systems (Pty) Ltd to perform Lodox® scans; however, no formal training in interpretation of the scans is undertaken in the forensic pathology specialisation program. Post-graduate courses are available for post-mortem radiology internationally, but are

[1] Lower respiratory tract infection is an umbrella term encompassing various conditions such as acute bronchitis, pneumonia, and acute exacerbations of chronic lung diseases like COPD or bronchiectasis.

prohibitively expensive for South African forensic doctors, and limited financial assistance is made available to attend such courses.

Regarding histologic examination, during the forensic pathology specialisation, doctors receive one year of dedicated training in surgical histopathology. An examination including written and practical components has to be passed as part of the Forensic Pathology Fellowship program. Although this training entails examination of specimens that differ from those encountered in autopsy practice, the training focuses on the approach to histologic examination of tissue slides and gaining experience in observing and reporting tissue and cell morphology.

LITERATURE REVIEW

Child mortality and pneumonia

The total number of child deaths under the age of 5 years was 5.0 million worldwide in 2020, with Sub-Saharan African countries accounting for the highest infant mortality. Pneumonia is a leading cause of death globally (WHO, n.d) and in South Africa, it is the second most common cause of death in infants under the age of 1 year (Maluleke, 2016). The South African infant mortality rate was 23.57 per 1000 live births in 2023, decreasing by 3.02% from 2022 ("United Nations – World…", 2022). It has been observed that although child mortality is decreasing in South Africa, a substantial percentage (50.9%) of child deaths occur outside of healthcare facilities, with pneumonia remaining one of the leading causes of these deaths (Bamford, 2018).

Pneumonia is defined as inflammation of the lungs with consolidation and may be classified according to causative organism or location in the lungs ("Pneumonia" 2003:1463). It may have a viral, bacterial, or fungal aetiology; while consolidation may involve a single lobe or multiple lobes in lobar pneumonia or may involve and be concentrated around the airways in bronchopneumonia ("Pneumonia" 2003:1463). Consolidation is a radiologic sign that refers to an area of homogeneous increase in lung parenchymal attenuation that obscures the margins of vessels and airway walls. Histologically, the type and location of inflammatory cells in the lung correspond with the aetiologic cause of pneumonia. For example, in bacterial pneumonia, neutrophils are found in the alveoli and/or around the airways, while in viral pneumonia, lymphocytes, may be found in the interalveolar septae. There are certain

viral pneumonias, like cytomegalovirus, that may also show intra-alveolar haemorrhage (Weisenberg, 2020).

Antemortem diagnosis of pneumonia

There is no current gold standard for the antemortem diagnosis of pneumonia. Diagnosis in the paediatric population may be reached based on symptomatology such as cough, shortness of breath and fever, together with examination signs including increased breathing rate, rales/crackles, rhonchi and wheezes, dullness to percussion and pleural friction rub (Gamache, 2020). Nasopharyngeal swabs or tracheal aspirates may be obtained from paediatric patients to identify causative organisms. However, there is difficulty in distinguishing whether organisms have merely colonized the respiratory tract or whether they are the true causative agent of the respiratory tract infection (Rodrigues & Groves, 2018). Histologic examination of the lung tissue in life is rarely performed, due to the invasiveness of acquiring lung tissue through needle biopsies, and the fact that there are relatively adequate clinical correlates with which to infer the diagnosis.

Various less invasive radiologic modalities are able to detect a pneumonic process in the lungs. These include conventional radiology (CR), ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) (Zar, Savvas & Nicol 2017;358:2739). Of these, CR is the most freely available and commonly used. It is relatively cheap, fast, and simple to perform, and it exposes both the patient and radiographer to relatively low doses of radiation. Although widely used, there are differing opinions on the clinical use of chest x-rays in community-acquired pneumonia: some believe that they should be performed routinely, while others suggest that they only serve a purpose in individuals that are hospitalised or have severe symptoms (Wooton & Feldman, 2014). Importantly, interpretation of chest x-rays is dependent on the quality of the image and the expertise of the interpreter (Swingler, 2001).

Another challenge is that there are limited data on inter-observer agreement in interpretation of plain film x-rays, even amongst specialists. Voigt et al found that there is poor to fair interrater agreement between radiologists and paediatric radiologists in the interpretation of community acquired pneumonia, but that 'chance corrected interrater agreement was highest for pleural effusions, infiltrates, and consolidations and lowest for interstitial patterns and peribronchial thickening' (2021). Similarly, Johnson and Kline found moderate to poor inter-observer agreement between radiologists and senior- and junior paediatric emergency physicians (2010). Despite its limitations, conventional radiography is still the most accessible modality, especially in low to middle income countries such as South Africa.

A variation on CR scans is the Lodox® scan, developed in South Africa. This is a low-dose, whole body x-ray scan that allows for interpretation of skeletal and soft tissues such as the lung fields. It consists of a C-arm that emits a fan-beam of x-rays from one end with an x-ray detector on the opposite end. A table slots into the machine. A full body scan of an adult is possible in approximately 13 seconds. The images, which are of moderate quality (Beningfield et al, 1999), are then available immediately. The images are digitised, enabling real-time viewing on a monitor and storage in a digital database. This digital format allows manipulation, including colour contrast enhancement, brightness and zooming capabilities. Printed hard copies of the scans can then be generated if required. In the trauma setting, Chen et al showed that although the Lodox® scan was less sensitive than conventional x-rays and CT scans in detecting lung pathologies such as pneumothorax and haemothorax, those pathologies that were not detected were not clinically significant and did not alter the management of patients (2010). It can be inferred then that the Lodox® scan typically does not miss life-threatening diagnoses that are usually detected by conventional x-ray films, including lung field infiltrates.

The only published antemortem paediatric Lodox® research is a small pilot study with 33 participants which was undertaken at Red Cross Children's Hospital, Cape Town, in 2009. Erect Lodox® chest x-rays and CR films were assessed in terms of technical aspects and image quality. A paediatric radiologist and a paediatrician with experience in chest radiology independently and blindly assessed the scans. There was 58% agreement between the Lodox® and CR scan diagnoses, 'the highest for mediastinal pathology and the least for diffuse interstitial pulmonary disease' (Daya, 2009:83). The authors noted that there are diagnostic limitations in Lodox® use, with 42% false negative findings and 86% false positive findings (Daya, 2009:83). The high percentage of false positive findings was thought to be related to movement artefact and chevron exposure artefact from rotating the C-arm to perform an erect scan, both of which are not of concern in the supine scans performed in a post-mortem population.

Due to the controversy around the need for x-ray in the clinical diagnosis of pneumonia, together with the varying inter-observer variability in interpretation (Sarria et al, 2003), it has been problematic to use radiological diagnosis in clinical research. For this reason, the WHO formed a group that developed a standardized tool as a guideline for the radiological diagnosis of pneumonia in children in epidemiologic studies (WHO/V&B/01.35, 2001). This tool is aimed at use within vaccine trials and provides clear definitions of significant pathology, end-point consolidation, other (non-endpoint) infiltrates and pleural effusion in

the antemortem diagnosis of paediatric pneumonia (WHO/V&B/01.35, 2001:24-26). The criteria for diagnosing end point pneumonia are tabled below as per the WHO standardized tool (*Table 1*).

TABLE 1 STANDARDISED TOOL FOR THE RADIOLOGICAL DIAGNOSIS OF PNEUMONIA IN CHILDREN IN EPIDEMIOLOGIC STUDIES (WHO/V&B/01.35, 2001).

Categorisation	Findings on chest x-ray
Normal scan	No infiltrates
Minor/non-specific changes	Lung inflation normal to increased, minor patchy infiltrates not of sufficient magnitude to constitute primary endpoint consolidation/small areas of atelectasis
Pathologic changes	Presence of consolidation, infiltrates, or effusion, linear and patchy (interstitial infiltrate) in a lacey pattern involving both lungs, featuring peribronchial thickening and multiple areas of atelectasis.

More recently, a deep learning model[2] for interpretation of paediatric chest x-rays was trained using the above WHO standardized methodology. Pretraining of the model was achieved through the use of datasets, including 224 314 adult and 4 172 paediatric chest x-rays, and compared against interpretations provided by radiologists and paediatricians. The study found that the deep learning model classified paediatric pneumonia similarly to specialists in the radiology and paediatric fields (Chen et al, 2021). The study noted that these findings show the potential of including computer-aided interpretations in future studies using the WHO standardized methodology.

Post-mortem diagnosis of pneumonia

Recent research in the field of post-mortem radiology has predominantly focused on modalities such as CT, MRI, and ultrasound. It has been shown that post-mortem CT is useful in diagnosis of fractures, specifically rib fractures, and post-mortem MRI has shown

[2] Bhatt et al., in their article "The State of the Art of Deep Learning Models in Medical Science and Their Challenges," define **artificial intelligence (AI)** as a process that allows a machine to mimic human behaviour. It creates a functional model of the human brain capable of making decisions based on its learning. A subset of AI, known as "**machine learning**," uses statistical methods to enable machines to evolve through experience and learning. **Deep learning**, a further subset of machine learning, is inspired by the human brain's information processing methods (2021).

promise in the diagnosis of traumatic injuries; however, it is unclear whether disease states such as pneumonia can confidently be diagnosed (Arthurs et al, 2017). It is important to note that these modalities are expensive and difficult to justify in a resource-constrained setting. Currently in South Africa, there are no dedicated post-mortem CT scan machines. Consequently, routinely available imaging resources in South Africa consist of conventional radiology and Lodox®.

The specific imaging modality notwithstanding, post-mortem artefacts may interfere with the radiologic diagnosis of pneumonia. These include increased opacification due to pulmonary oedema developing as an agonal occurrence, post-mortem lividity with gravity dependent settling of the blood, and decompositional changes such as increased radiolucency from post-mortem gas production and weaker soft tissue contrast (Elifritz et al, 2014:3453). Gravity dependent settling of the blood situated toward the back of the body if the deceased is lying flat (supine) may be misinterpreted as consolidation, the radiologic sign for possible aspiration, oedema, atelectasis, or pneumonia. With regard to putrefaction, increasing post-mortem interval may result in difficulty differentiating soft-tissue emphysema and air embolism from decompositional gas (Levy & Harcke, 2011).

In view of the challenges posed by post-mortem artefacts in radiology, histologic diagnosis of pneumonia is still considered the best standard in the autopsy setting. Despite concerns around intra- and inter-observer variability (Hunt et al, 1995, Corley et al, 1997), direct visualization of an infective process in the lung tissue, together with a history of antemortem clinical symptoms, allows a reasonably certain diagnosis of pneumonia as the cause of death.

This can be further refined when the type and distribution of inflammation in the lung tissue and airways are considered. Pneumonia is understood to be viral if there is increased interstitial lymphocytic inflammation in the lung tissue, known as lymphocytic interstitial pneumonia (LIP); bacterial if acute inflammation with neutrophilic infiltration is seen in the alveoli or around/involving the airways; and fungal or tuberculous if necrotizing or non-necrotizing granulomas are visualized (Kumar, Abbas, and Aster, 2015:702-711). As is often the case, there is overlap in the histologic patterns due to initial viral infection with superadded bacterial infection (Kumar, Abbas & Aster, 2015:702-711).

A group of paediatric pathologists and forensic pathologists have developed a consensus document, 'Consensus on diagnostic criteria for the exclusion of SIDS', in which agreement was reached on the diagnoses of particular neurologic, cardiac and lung pathologies (Rognum, 2003). This document provides a tool/framework that may be used to standardise histologic diagnosis in general. The consensus document separates lung histology findings

into those with non-existent or minor changes (consistent with SIDS); those with pre-existing congenital disorders, clinical symptoms and/or post-mortem findings which are not severe enough to explain the cause of death (borderline SIDS); and those categorised as "explained infant death" where the cause of death is explained according to clinical information and/or the results of the post-mortem examination. The findings are interpreted using the anatomical structures of the lungs as seen in *Table 2*.

TABLE 2 HISTOLOGIC FINDINGS IN SIDS, BORDERLINE SIDS AND EXPLAINED INFANT DEATH TAKEN FROM CONSENSUS ON DIAGNOSTIC CRITERIA FOR THE EXCLUSION OF SIDS (ROGNUM 2003).

Anatomical Structure	SIDS	Borderline SIDS	Explained infant death
Alveoli	Occasional neutrophils present: but fewer than 10 alveoli contain ≥ ten cells	Not markedly consolidated, but at least ten alveoli, each containing ten or more neutrophils.	Marked, obvious pulmonary consolidation.
Interstitial Tissue	Occasional lymphocytes scattered diffusely, or a few widely scattered, moderate-sized foci of infiltration.	Moderate lymphoid infiltrates present in several sections.	Widespread lymphoid infiltrates in the walls of the alveoli in all sections (interstitial pneumonia)
Bronchi and peribronchial tissue	Mild or moderate peribronchial lymphoid infiltrates with insignificant neutrophil component; no pus cells in lumen	Neutrophil infiltration in wall, but insufficient pus in lumen to obstruct larger bronchi. Lymphoid cell infiltrates form heavy cuffs around bronchi or bronchioles in more than one section.	Purulent exudate fills lobar bronchi and larger branches of bronchial tree, atelectasis distant to obstruction. In addition: Specific lung diseases that can cause death (congenital, allergic, and inflammatory; also, bronchiolitis, obstructive).

The agreement between post-mortem radiological and histological diagnosis of pneumonia has been relatively under-researched. A retrospective study performed at Salt River Mortuary (SRM) reviewed the Lodox® findings that were documented over a period of one year in the autopsy reports of children aged 2 years and below. These were compared to the stated cause of death. The authors concluded that the Lodox® interpretations were consistent with the pathologic findings (Douglas et al, 2012). It was, however, not clarified whether these pathologic findings were macroscopic only, or both macro- and microscopic. Furthermore, no standard guidelines were used for the interpretation of the Lodox® scans, and the documented findings could well have been influenced by the macroscopic or

microscopic appearances. Indeed, this study makes conclusions based on the unverified premise that the forensic doctor made the correct diagnosis, either radiologically, pathologically, or both. The authors concede these limitations and the need for a prospective study to compare autopsy and radiologic findings.

In contrast, De Lange et al found poor radiologic-pathologic correlation for soft tissue diagnosis in the post-mortem paediatric population (2007). In this study, lungs are referred to as soft tissue. The study was a case series over a 4-year period in Norway. Four predominant patterns were found in the lung fields including normally aerated lung, reduced aeration to opaque lungs, diffuse patchy densities and localised densities in addition to pneumothorax and pneumopericardium. The study focussed on radiology and did not provide details about the histologic exam. Similar to Douglas et al, no methods of standardisation were used in either radiologic or histologic interpretation. Interestingly, it is mentioned that although soft tissue findings are frequently found microscopically, they seem to be of little or no importance to the cause of death and may partly be due to post-mortem artefacts. Again, more research was recommended to clarify the relationship between the two modalities.

In keeping with paediatric populations, poor correlation between the post-mortem conventional radiology and pathologic findings was found in an adult population in the Western Cape, South Africa. In this study, Lodox® scans were interpreted, and macro- and microscopic examination of the lung tissues performed and documented using a standardised format. The most common findings on the Lodox® scan that correlated with respiratory pathology were consolidation and ground glass opacities, yet these were not strongly associated with specific respiratory pathologies (Quarrie, 2015). Kappa coefficients ranged from –0.12 to 0.16 when Lodox® was compared to histologic evidence of acute or chronic inflammation (Quarrie, 2015). There were several methodological flaws in this research. No reference sources were provided to support the standardised tools for histologic and radiologic reporting that were developed for use. Furthermore, a specialist radiologist interpreted the Lodox® scans in the study, whereas it is forensic doctors that interpret the scans in everyday practice in South African forensic mortuaries. Regarding the histology, although diagnoses were made by consensus between the two investigators involved, their years of experience was not mentioned. Additionally, adequate quality assurance methods were not used, for example, Lodox® interpretation and autopsy findings were not blinded, possibly introducing interpretation bias. The questionable conclusions of this study are further compromised by the fact that they cannot necessarily be extrapolated to apply to a

paediatric population, where both radiologic and histologic features of pneumonia differ from those of adults. As such, the results of this study cannot be used to inform standard practice in forensic investigation of SUID.

A final method of post-mortem diagnosis of pneumonia is the verbal autopsy, which involves interviewing the deceased's caregivers regarding the circumstances of death and symptoms preceding death. The WHO states that, "Verbal autopsy is an essential public health tool for obtaining a reasonable direct estimation of the cause structure of mortality at a community or population level, although it may not be an accurate method for attributing causes of death at the individual level" (2007). Regarding pneumonia as a diagnosis, information about the aetiological agent is seldom available if this method is widely used. A study undertaken in Namibia to evaluate the validity of the verbal autopsy showed that '…it may be used to determine the relative public health importance of leading causes of death. The level of misclassification, and especially the low specificity for some diagnoses, will make assessment of trends in cause-specific mortality more difficult' (Mobley, 1996).

RATIONALE FOR THE STUDY

Research involving Lodox® is relevant because most developing countries do not have regular access to post-mortem CT or MRI scans, whereas Lodox® is a relatively cost-effective and currently available diagnostic modality. The post-mortem radiology field is a new and relatively small discipline and through collaboration, new relationships will be fostered that may benefit future research in this field and enlarge the South African medical technological footprint.

By reducing the number of internal investigations required for post-mortem examinations, the workload of Forensic Pathology Service could be alleviated. The associated benefits could include: cost reduction (less burden on taxpayers); improved efficiency and service delivery; greater capacity for training and development; enhanced morale, welfare, and retention of staff. These benefits are likely to be amplified in resource-constrained countries such as South Africa.

These advantages aside, a non-invasive autopsy technique involving clinical history review, external examination, and Lodox® scan alone would be more acceptable to certain cultural groups in South Africa, whose beliefs may conflict with legally mandated invasive post-mortem procedures. Avoidance of an internal examination would likely be similarly

attractive to parents of infant decedents in general. That said, confidence in the accuracy of the assigned cause of death is also important for family closure.

Currently there is a dearth of evidence supporting the scientific validity of non-invasive post-mortem examinations in making certain diagnoses post-mortem, such as pneumonia in infants. Indeed, forensic pathologists may be erroneously assigning a cause of death to cases which would have been more correctly classified as SIDS or borderline SIDS if they had undergone an internal examination. This is further problematic as cause of death data informs public policy and rational resource allocation.

We anticipated that the outcomes of the study could inform or be incorporated into a standard operating procedure guiding the investigation of these cases. If adopted at a provincial or national level, this may eventually impact the everyday practice of forensic doctors in the South African setting. Study results have the potential to enable more evidence-based use of Lodox®, ensuring that defensible evidence is supplied for the medicolegal death investigation, as well as satisfying the communities within which the Forensic Pathology Service functions.

Chapter 2: Methods

This chapter describes the methodology of this retrospective cross-sectional study. The aims, objectives, study design, inclusion- and exclusion criteria, sample size, case selection, blinding, standardisation of interpretation, collection of data and ethical considerations are explained. One hundred Lodox® cases of SUID were reviewed and interpreted using the WHO standardised protocol and captured using a Google form (*Annexure 4*). The histology slides from these cases were assigned different study numbers and were reviewed and interpreted using the standardised protocol (*Annexure 5)* based on the SIDS consensus document (Rognum, 2003). Every 4th scan and every 4th histology case were selected (25 scans and 25 histology cases were selected), then reviewed, and interpreted by the respective moderators. Subsequently, the interpretations derived from the Lodox® scans (n:100) were paired with the histological interpretations of the same case for statistical evaluation. This analysis incorporated a total of 100 cases, all of which were interpreted by the forensic registrar.

Aims

This study aimed to evaluate retrospectively whether interpretation of a post-mortem Lodox® scan is sufficient to diagnose pneumonia in alleged sudden infant deaths, without the need for confirmatory histology.

Objectives

(1) To view and interpret the Lodox® scans, with specific reference to the chest/lung fields, of infants admitted as sudden unexpected deaths where cause of death was determined to be a lower respiratory tract infection or remained under investigation.
(2) To broadly categorize the Lodox® findings into normal scan, minor/non-specific changes, or pathological changes.
(3) To view and interpret the histologic tissue sections from the lungs of these infants.
(4) To broadly categorise the histology findings into normal or minor changes, non-specific changes, or pathological changes.
(5) To statistically evaluate the agreement between the Lodox® scan interpretation of the chest and the histologic findings of the lungs.

(6) To attempt to statistically evaluate the validity of the Lodox® scan as a tool to diagnose pneumonia in the post-mortem setting in alleged sudden infant death admissions.
(7) To assess the accuracy of non-specialist interpretation of the Lodox® scan and histology of lung tissue in SUIDs by evaluating the interrater agreement between a forensic pathology registrar and specialists in the fields of radiology and histology.

Study design and setting

A cross-sectional study was conducted on SUID cases which underwent post-mortem examinations at Salt River Mortuary between 2007 and 2021. The data collected represent a single point in time: death. Retrospective samples were used for prospective data analysis. The materials required for analysis were the Lodox® scans and tissue slides, which were obtained as part of the medicolegal death investigation as per the Inquests Act. Interpretation of the Lodox® scans and histology slides was conducted by the investigator (forensic registrar, Dr Tracy Leanne Cook) and moderators (paediatric radiologist, Professor Owen Arthurs, and anatomical pathologist, Dr Marco Otto).

Inclusion criteria

Infants admitted to Salt River Mortuary as alleged SUIDs where cause of death was determined to be a lower respiratory tract infection or remained under investigation.

Exclusion criteria

- Infants younger than 1 day or older than 1 year.
- Infants that were possibly stillborn.
- Infants that were admitted for reasons other than alleged sudden death, such as homicide, accident, or procedure-related deaths.
- SUIDs in which another clear non-respiratory cause of death was found at autopsy.
- SUIDs in which the preliminary cause of death was stated as "under investigation" due to suspicion of foul play or because more investigative information was required.
- Infants that showed evidence of decomposition on macroscopic examination, including bloating, skin slippage, marbling of the skin, or notable tissue autolysis.

- Cases where Lodox® scans were not available or were of a less than acceptable quality.
- Cases where histologic slides were not available, processed by the National Health Laboratory Services, or were of less than acceptable quality.

Sample size

Professor Martin Kidd, a professional statistician, assisted with the sample size calculations. Following review of the literature regarding radiologic and histologic agreement for post-mortem diagnosis of pneumonia in SUID, no relevant data on which to base assumptions for sample size on were found. Assumptions of the percentage of cases that may fall into the categories seen in *Table 3* (3x3 contingency table) were made based on experience in the department and mortuary. In order to conduct a statistical analysis using Kappa statistics, certain assumptions were made about the frequencies or proportions of the different categories in Table 3. Given the lack of prior research on SUID with these specific results, the assumptions were based on the experiences of forensic pathologists from the UCT Division of Forensic Medicine and Toxicology and SRM. This provided an estimated distribution of cases across the categories in the contingency table. The results are with respect to Lodox® scans.

TABLE 3 CONTINGENCY TABLE WITH HEAT MAP OF EXPECTED LODOX® AND HISTOLOGY DATA

	Non-existent/ minor histologic changes	**Non-specific histologic changes**	**Frank histologic pathology**
Normal Lodox®	Lodox®/Histology agree. True Negative **3%**	Lodox®/Histology disagree. False Negative **5%**	Lodox®/Histology disagree. False Negative **2%**
Non-specific Lodox®	Lodox®/Histology disagree. False Negative **5%**	Lodox®/Histology agree. True Positive **55%**	Lodox®/Histology disagree. False Negative **5%**
Obvious radiologic pathology	Lodox®/Histology disagree. False Positive **0%**	Lodox®/Histology disagree. False Positive **20%**	Lodox®/Histology agree. True Positive **5%**

***Heat Map Key**: Green - Radiologic and histologic results concordant (True positive); Orange – Discordant results (False negative); Red – Discordant results (False positive results)*

Calculations undertaken by a statistician found that based on these estimations, a sample size of 1400 would be required to obtain the following percentage errors, with a confidence interval (CI) of 95% (*Table 4*):

TABLE 4 ESTIMATED PERCENTAGE ERROR FOR 1400 CASES

Combination of Lodox® and histology results	Estimated percentage error	95% Confidence interval
Normal Lodox® & frank histologic pathology	17%	11% - 23%
Non-specific Lodox® & frank histology pathology	42%	34% - 50%
Obvious radiologic pathology & normal histology	0%	0% - 3%
Obvious radiologic pathology & non-specific/minor changes	25%	22% - 28%

A confidence interval is a range of values that most likely contains the true result with a certain degree of confidence ("What is confidence…", 2023). The true result should fall within the confidence interval with 95% confidence.

This was a pilot study, and due to work obligations and time constraints 1400 cases were not practical, therefore only 100 cases were included. This changed the confidence intervals for the estimated percentage errors, particularly for the false negative and false positive cases (*Table 5*):

TABLE 5 ESTIMATED PERCENTAGE ERRORS FOR 100 CASES

Combination of Lodox® and histology results	Estimated percentage error	95% Confidence interval
Normal Lodox® & frank histologic pathology	17%	2% - 48%
Non-specific Lodox® & frank histology pathology	42%	15% - 72%
Obvious radiologic pathology & normal histology	0%	0% - 31%
Obvious radiologic pathology & non-specific/minor changes	25%	16% - 36%

Case selection

Office autopsies, a Division of Forensic Medicine and Toxicology database of the cases that have undergone medicolegal examination at Salt River Mortuary (Office Autopsies HREC: R036/2014), was accessed by the investigator for the years 2007 to April 2021. The databases were filtered by age (>1 day and < 1 year), admission category (unexpected or unexplained sudden death), Lodox® scan (performed), tissue sections for histological examination (retained), and cause of death (lower respiratory tract infection/pneumonia/bronchopneumonia or 'under investigation'). In this manner, a total of 1114 cases were identified from the Office Autopsies databases.

The completed post-mortem reports of these cases were accessed via LiveLink, FPS's electronic content management system. The reports from 2007 to 2011 and 2014 (507 cases) were not available on the system and were thus excluded from the study, leaving 607 cases for potential inclusion. These reports were inspected to confirm cause of death, Lodox® scan availability and whether tissue sections were retained for histology. Cases with suspicion of foul play or a non-respiratory cause of death were excluded. If cases were still under investigation as per the post-mortem report but no foul play was suspected, the case was included.

The data collected and analysed in this study were radiological scans and histological slides that were previously performed as part of routine service delivery as per the Inquests Act (58 of 1959). They fall under the registered and approved-for-research Forensic Radiology Registry and Forensic Histology Repository (HREC:037/2014).

Lodox® scans performed in the anterior-posterior (AP) position were viewed using the DVS Lodox® Software for DICOM images. This software was made available to the investigator by the Lodox® Systems (Pty) Ltd. A digital search of the Forensic Radiology Registry for the scans of the filtered cases was performed using the mortuary reference number and the images were saved in a password protected file. The images were reviewed for appropriate quality using the criteria below *(Table 6)*, taken from the radiology quality criteria for Lombok radiographs (Benson, Steinhoff, 1999).

TABLE 6 INCLUSION CRITERIA FOR LODOX® SCAN

LODOX® QUALITY CRITERIA	YES	NO
Clavicles and ribs approximately equidistant to the spine		
Vascular shadows seen in the lung periphery		
Large lower vessels and thoracic vertebrae seen through the cardiac silhouette		
Background outside deceased's silhouette is black, not grey		
Bones and tissues easily distinguishable from soft tissue		

A Google form was created using the above criteria (*Appendix 2*) and the answers were automatically exported to a Microsoft Excel® spreadsheet saved on Google Drive. If all five answers were 'yes', then the case satisfied the Lodox® quality criteria for inclusion.

To ensure that there would be enough scans for a buffer in the event that a scan or the associated histology slides had to be excluded, a total of 221 Lodox® scans were downloaded. Of the 221 downloaded scans, 132 met the criteria for inclusion. Seventy-seven cases were excluded on the basis that the clavicles were not equal distance from the thoracic vertebrae; 1 case was excluded on the basis that vascular shadows were not seen in the periphery of the lungs; 8 cases were excluded on the basis that the background was grey and not black; and 4 cases did not meet 2 Lodox® criteria for inclusion (*Figure 1*). In total

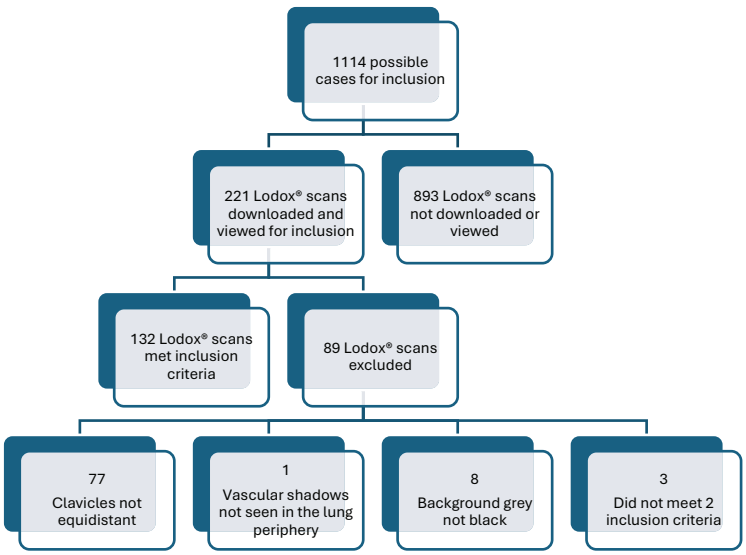


FIGURE 1 INCLUSION/EXCLUSION OF LODOX® SCANS

The corresponding histology slides for the cases that met the Lodox® criteria were requested from the histology repository. If the tissues had not yet been processed for the case or if the post-mortem examination was performed by anatomical pathology registrars, these were excluded. A Google form (*Appendix 3)* was created using the criteria in *Table 7* and the haematoxylin and eosin (H&E) stained histology slides of each case were reviewed for quality.

TABLE 7 INCLUSION CRITERIA FOR HISTOLOGY SLIDES

HISTOLOGY SLIDE QUALITY	YES	NO
Number of tissue sections: Five or more tissue sections represented (one per lung lobe)		
Portions of the lobes represented: Peripheral and central lung seen		
Staining quality and decompositional change: No loss of cellular detail No large gas bubble formation		

A deviation from these planned inclusion criteria was that fewer than five tissue sections was accepted, provided the FMP had sectioned larger pieces of lung tissue that encompassed both peripheral and central portions of the lungs, ensuring adequate lung representation. HREC was informed of this deviation.

One hundred of the 132 cases that then satisfied the histology quality criteria were included in the study and spanned years 2011 to 2019[3], excluding 2014 (*Figure 2*).

[3] The study's timeframe for included cases (2011-2019) predated the initiation of COVID-19 testing at SRM that commenced in March 2020. Given that COVID-19 may cause viral pneumonia, such cases would not have been specifically excluded.

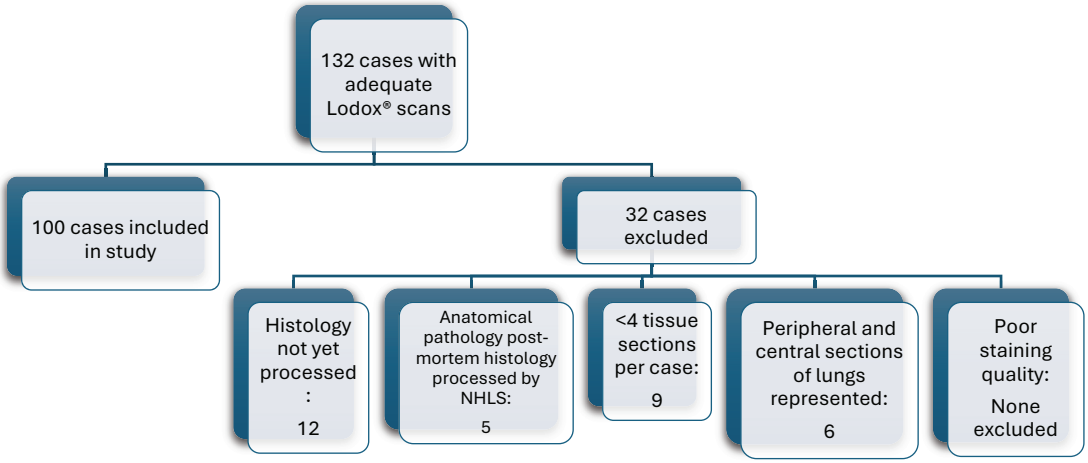


FIGURE 2 INCLUSION/EXCLUSION OF HISTOLOGY CASES

Minimisation of bias

This study involved blinding of the investigator and moderators during data interpretation, but not the investigator when statistical outcomes were calculated. Blinding was used to prevent bias in the interpretation of the Lodox® scans and the histology slides, which were delinked from both the mortuary reference numbers and each other. Furthermore, moderators were blinded to the primary investigator's initial radiology and histology interpretations.

To achieve this, each case was assigned two unique study numbers using a freely available online random number generator (www.randomizer.org). One study number was used for the scan and another for the tissue slides. The study numbers and the associated mortuary number were placed into separate documents to be used as keys, linking histology to radiology interpretations before data analysis.

Lodox® engineering staff assisted in de-identifying the radiological images prior to interpretation. This was done by transferring the Lodox® scans to a secure Google Drive folder along with the document that linked the Lodox® mortuary reference number to the study number. A specialist forensic pathologist in the Division of Forensic Medicine and Toxicology took custody of this document. The Lodox® engineer removed the mortuary reference number as well as the date the Lodox® scan was performed and added the study number to the image. The de-identified scans were saved in the Google Drive folder and the original file containing the scans labelled with mortuary reference numbers was deleted.

Similarly, the chief histology technologist ensured de-identification of the histology slides before interpretation. An opaque sticker marked with the histology study number was placed over the mortuary reference number that the slides were already labelled with. The chief histology technologist took custody of the key that linked the mortuary reference numbers to the histology study numbers.

In addition to the blinding described above, potential sources of cognitive bias were considered and managed. When viewing the histology slides, confirmation bias was minimized by ensuring that any additional stains originally ordered were not automatically issued with the case. These slides were specifically requested by the investigator after the initial assessment, to confirm a particular diagnosis. For the same reason, cases selected for moderation were submitted to the specialist radiologist and anatomical pathologist without being accompanied by the investigator's initial interpretation.

Lodox® and histology interpretation

The Lodox® scans were interpreted based on the WHO standardization of diagnosis of pneumonia in children framework (WHO/V&B01.35). Although the purpose of conventional x-ray diagnosis of pneumonia in epidemiologic studies differs from that in forensic practice, the WHO framework provided a basis for standardized interpretation of Lodox® scans in the post-mortem setting.

The histology was interpreted based on '*Consensus on diagnostic criteria for the exclusion of SIDS*' (Rognum, 2003). This allowed for examination of the lung histology based on findings that were agreed upon by a group of paediatric pathologists and forensic pathologists. The criteria for the histologic diagnosis of pneumonia in the consensus article are fairly well defined.

The investigator was a forensic registrar (trainee) with 4 years' experience in Forensic Pathology who had completed the 1-year anatomical pathology rotation required for training. The Lodox® scan interpretation was moderated by a specialist paediatric radiologist with experience in post-mortem radiology. The histology slides were moderated by a specialist anatomical pathologist with 13 years' experience in anatomical pathology and a special interest in foetal-perinatal pathology.

The objective of the moderation process was to evaluate the accuracy and validity of the diagnoses made by the forensic registrar and as a form of triangulation. For both the Lodox®

images and the histology slides, every 4th case was moderated by the specialists using the same type of data collection form used by the investigator. When instances of disagreement arose, the moderators' interpretation was taken as correct, and the discrepancy noted. Thereafter, the investigator and moderators discussed the discrepant cases. This was done as learning experience for the forensic registrar. In the local setting (SRM), forensic medical practitioners do not necessarily have access to a radiologist or anatomical pathologist with whom to discuss cases.

Collection of Data

During June and July 2023, data were collected from cases dating from 2011 until 2019. Three digital data collection forms were used: Demographic data form (*Appendix 1*), Lodox® data collection form (*Appendix 4*) and Histology data collection form (*Appendix 5*). The data collected on the demographic data collection form were used for descriptive purposes and the Lodox® and histology data collection forms were used for descriptive purposes and statistical analyses. The table below (*Table 8*) details the variables that were documented for each case:

TABLE 8 STUDY VARIABLES

Variable Name	Variable Type	Method of Data Capture
Study number	Descriptive Numerical continuous	Range (0-xxx)
Gender	Descriptive Categorical nominal	Coding 1 – Male 2 - Female
Age	Descriptive Categorical nominal	Range (0-12) months
Lodox® interpretation	Categorical nominal	Coding 1 – Normal 2 – Minor/Non-specific changes 3 – Pathologic changes
Histology findings	Categorical Nominal	Coding 1 – Normal 2 – Minor/Non-specific changes 3 – Pathologic changes

In addition to these variables, both the investigator and moderators had the option to provide a written description of their findings on the Google forms for both Lodox® and histology. However, only the investigator chose to utilize this option. Data recorded by the investigator and moderators on the data collection forms was automatically exported to a

Microsoft Excel® spreadsheet and downloaded onto a laptop. The data were cleaned prior to analysis and managed according to the data management plan (dmp.lib.uct.ac.za/plans/3612).

The keys for unblinding the cases were then provided to the investigator, allowing the Lodox® and histology interpretations for each case to be linked and saved. The raw data was emailed to a statistician for statistical analysis.

Statistical Analysis

Statistical analysis of the collected data was conducted using TIBCO Statistica® 14.0.0 with the assistance of a professional statistician, Professor Martin Kidd.

No studies focusing on the use of post-mortem Lodox® for diagnosis of pneumonia in infants were found in the literature, therefore the validity of Lodox® as a post-mortem diagnostic test was unknown. In the absence of reference values from the available literature, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for the diagnosis of pneumonia by Lodox® were calculated from the data gathered in this study.

To statistically evaluate the dataset, a null hypothesis was formulated, namely that there is no agreement between interpretation of Lodox® images diagnosed as pneumonia and interpretation of lung tissue histology diagnosed as pneumonia in sudden infant deaths.

Agreement between Lodox® interpretation and lung histology interpretation (current best standard test) was sought. Agreement refers to the degree of concordance between two or more sets of measurements/variables. It quantifies concordance between two variables or between investigator and moderator, also known as inter-rater variability (Ranganathan, Pramesh, Aggarval, 2022). The weighted kappa (κ) test was used to calculate agreement between the categorical (nominal) variables. Values for weighted κ range between -1 to 1. There are two methods of interpreting the results; a weighted κ less than 0.6 indicates a significant level of disagreement. The second method is shown in *Table 9*.

TABLE 9 INTERPRETATION OF WEIGHTED KAPPA (K) RESULTS

Weighted κ	Interpretation of result
< 0	Less than chance agreement
0	Zero agreement equivalent to chance
0.10-0.20	Slight agreement
0.21-0.040	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 - 0.80	Substantial agreement
0.81 - 0.99	Near perfect agreement

A negative weighted κ result indicates that the agreement between the test results is less than chance agreement.

Contingency tables (2x2 and 3x3 tables) were generated to evaluate the agreement between Lodox® scan interpretations and lung tissue interpretations as well as the investigator interpretations and moderator interpretations of the Lodox® scans and lung tissue histology.

Both the chi-squared test and Fisher's exact p-values were calculated and the conventional value for statistical significance ($p<0.05$) was used. The Fisher's exact p-value had more relevance than the chi-squared test in this study because the sample size was small.

Positive predictive value of the Lodox® was calculated to assess the probability that a pathologic diagnosis interpreted on Lodox® scan was consistent with the histology interpretation. Negative predictive value of the Lodox® was calculated to assess the probability that a non-pathologic Lodox® interpretation was consistent with histologic interpretation. This could only be analysed for the investigator's findings since the moderators did not assess the Lodox® and histology for the same cases due to blinding.

Correlation coefficients were calculated to determine the strength of association between investigator Lodox® and histology results, and between investigator and moderator results. Results for correlation coefficients range from -1 to 1. The Pearson test uses raw data variables in the calculation whereas the Spearman test uses ranked order variables in the calculation. The correlation coefficient results may be interpreted according to *Figure 3*.

Pearson and Spearman correlation coefficients	Interpretation of relationship between Lodox® and histology results and inter-rater results
0	No relationship
0.1 to 0.19	Negligible relationship
0.2 to 0.29	Weak positive relationship
0.3 to 0.39	Moderately positive relationship
0.4 to 0.69	Strongly positive relationship
> 0.7	Very strong positive relationship

FIGURE 3 INTERPRETATION OF CORRELATION COEFFICIENTS

Calculating estimated percentage error for the collected data was not possible. In order to initially calculate a sample size, assumptions were made based on experience and time in the mortuary. These assumptions were not based on scientific evidence and therefore cannot be used to determine the percentage error for the results of the study.

Ethical considerations

The data in this study were retrospective interpretations of radiological scans and histological slides that were obtained as part of routine service delivery as per the Inquests Act (58 of 1959). They fall under the registered and approved-for-research Forensic Radiology Registry and Forensic Histology Repository (HREC:037/2014) respectively; therefore, informed consent was not sought from the next-of-kin. Considering the next-of-kin, obtaining informed consent might have put the families of the deceased at risk of experiencing additional trauma, therefore informed consent was not requested.

Privacy, confidentiality, and anonymity was maintained at all times. The data were aggregated and anonymised. Individual cases are not identifiable in the dissertation and the investigator did not know the identity of individuals in the study. Only mortuary reference numbers were captured and used as a method of identifying cases and tracking case files, and only de-identified, anonymised relevant data were used for subsequent data analysis. The Lodox® engineers did not work in the public health sector; instead, they were employed by Lodox® Systems (Pty) Ltd. They provided free assistance for this project and gave no

undue pressure to draw specific conclusions about the applicability of the Lodox® scan in this setting.

The collected data were stored on a password protected laptop computer in the Division of Forensic Medicine and Toxicology, University of Cape Town. The project was conducted in compliance with the Protection of Personal Information Act (4 of 2013) and was not discussed with anyone other than the research team. Once the study was completed, the collected data were given to the supervisor of the project, who will store the data electronically with password control for as long as she is employed at the University of Cape Town.

Ethical approval

The study was reviewed for scientific rigor and ethical standards by the Department of Pathology's research committee. Ethical approval was sought from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee.

The study commenced once ethical approval was granted (HREC study number 728/2022). The study was executed in accordance with the declaration of Helsinki (1964) as amended by the 64th WMA General Assembly, Fortaleza, Brazil, in October 2013.

Chapter 3: Results

The research data collected, and subsequent statistical analyses are presented in this chapter, beginning with a brief demographic description of the cases included. For Lodox® and histology respectively, the forensic doctor's interpretation is followed by the specialist interpretation, which are then compared to one another. Finally, case-matched Lodox® and histology results are compared by examining agreement and correlation of the forensic doctor's interpretations as well as specialist inter-rater variability and correlation.

DEMOGRAPHICS

According to the inclusion criteria, a total of 100 cases were included in the study and comprised 43 female infants (43%) and 57 male infants (57%) (*Figure 4*).

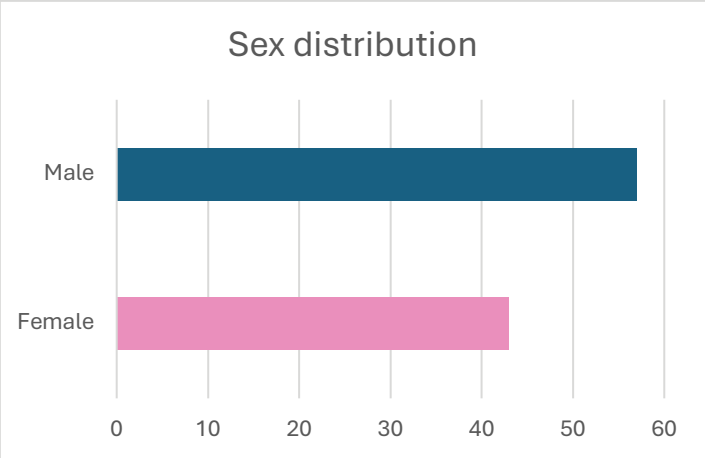


FIGURE 4 SEX DISTRIBUTION

The majority of infants (n:67, 67%) were between the ages of 1 and 5 months, with an average age of 3.25 months and 2 months being the most common age. Of the cases, 18 (18%) were younger than one month, and 15 (15%) were between six and twelve months old (*Figure 5*).

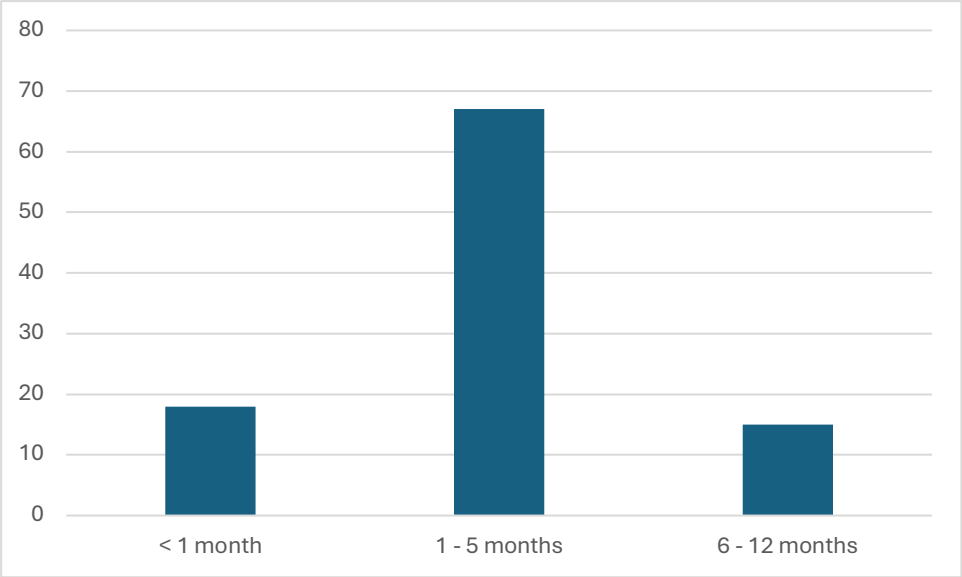


FIGURE 5 AGE DISTRIBUTION

It was observed that there were more males than females between the ages of 1 and 12 months, while there were more females in the age group of 1 day to 1 month (*Figure 6*).

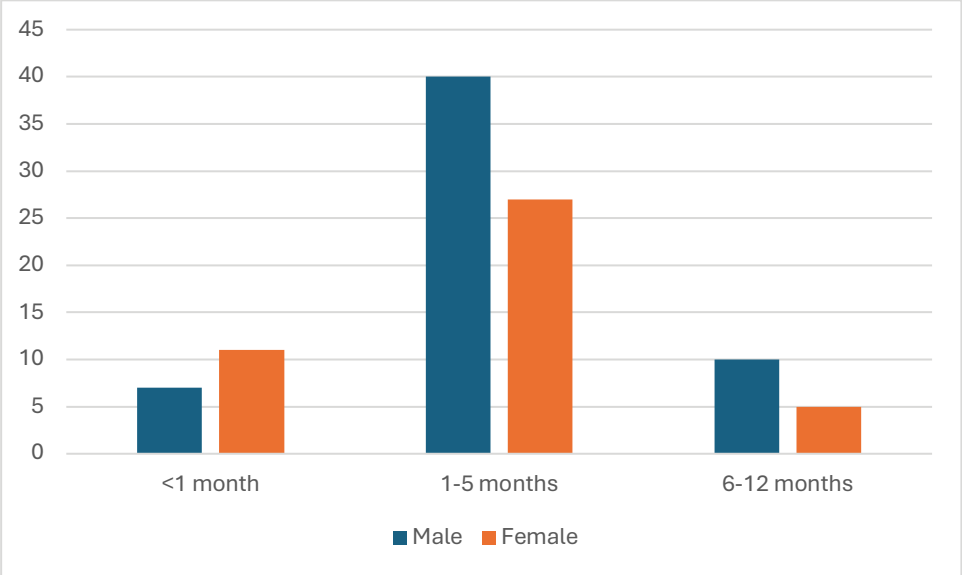


FIGURE 6 SEX DISTRIBUTION IN RELATION TO AGE RANGES

LODOX® RESULTS

Forensic registrar (investigator) Lodox® results

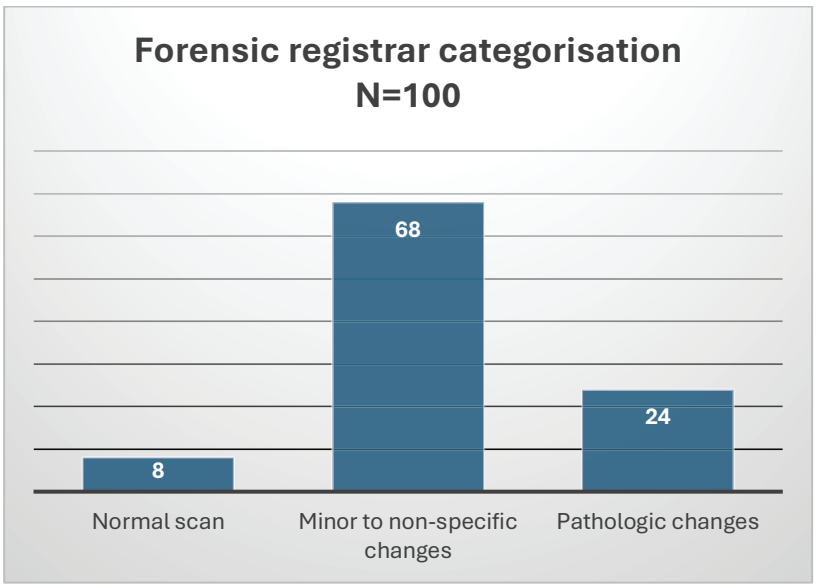


FIGURE 7 DISTRIBUTION OF FORENSIC REGISTRAR LODOX® CATEGORISATION.

One hundred cases were reviewed by the forensic registrar. Eight cases (n:8, 8%) were categorised as normal. Most Lodox® scans (n:68, 68%) were categorised as minor/non-specific changes. This is in line with the preliminary sample size assumptions (*Table 3*). Twenty-four cases (n:24, 24%) were categorised as pathologic changes consistent with pneumonia (*Figure 7*).

Radiologist (moderator) Lodox® results

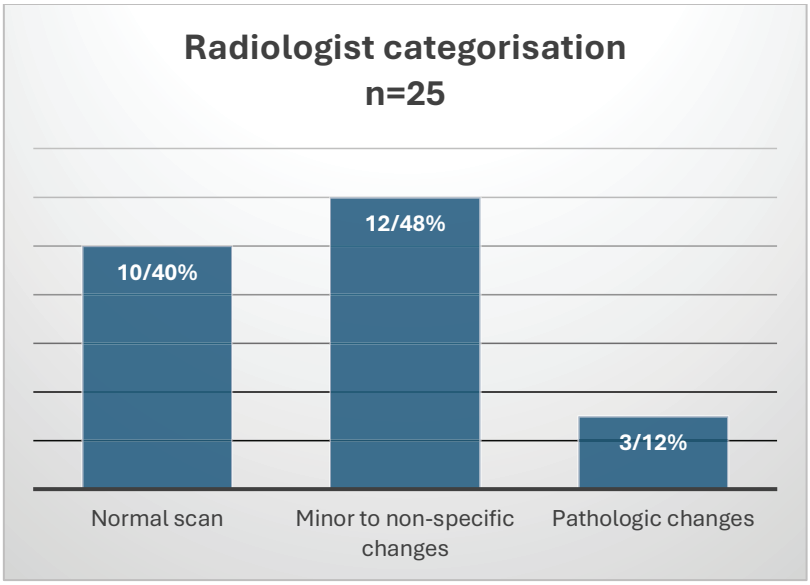


FIGURE 8 DISTRIBUTION OF RADIOLOGIST LODOX® RESULTS

Every fourth Lodox® scan (n = 25) was reviewed by the radiologist. Ten (10, 40%) cases were categorised as normal. The majority of cases (n:12, 48%) were classified as non-specific changes. The radiologist classified 3 scans (12%) as pathologic (*Figure 8*).

Comparison between forensic registrar and radiologist Lodox® results

TABLE 10 CONTINGENCY TABLE OF INVESTIGATOR AND MODERATOR LODOX® RESULTS

	Chi-square(df=4) =6.70, p=0.15 Fisher Exact (r x c) p=0.17						
	Radiologist: Normal Lodox® scan	**Column%**	**Radiologist: Minor to Non-specific changes**	**Column%**	**Radiologist: Pathologic changes**	**Column%**	**Row totals**
Forensic registrar: Normal Lodox® scan	2	20%	0	0%	0	0%	2
Row %	100%		0%		0%		
Forensic registrar: Minor / Non-specific changes	6	60%	10	83%	1	33%	17
Row %	35%		59%		6%		
Forensic registrar: Pathologic changes	2	20%	2	17%	2	66%	6
Row %	33%		33%		33%		
Totals	10		12		3		25

Objective 7 involved evaluating interrater agreement between the forensic registrar and the radiologist in order to assess the accuracy of non-specialist interpretation. To that end, a subset of 25 cases were moderated. *Table 10* is a contingency table that compares the interpretations made by the forensic registrar to those made by the radiologist for the 25 moderated cases. The radiologist categorised 10 cases as normal, while the forensic registrar categorised six (6, 60%) of these cases as minor to non-specific changes and two (2, 20%) cases as pathologic. There was concordance in the interpretation of two (2, 20%) normal scans. The radiologist categorised 12 cases as minor to non-specific changes, while the forensic registrar categorised none of these as normal and two (2, 17%) cases as pathologic. There was concordance in the interpretation of ten (10, 83%) minor to non-specific cases. The radiologist categorised 3 cases as pathologic, while the forensic registrar categorised none of these as normal and one (1, 33%%) as minor to non-specific changes. There was concordance in the interpretation of two (2, 67%) cases.

The histograms below show the forensic registrar's categorisation compared to the radiologist's interpretation of the 25 moderated Lodox® scans (*Figure 9-11*).

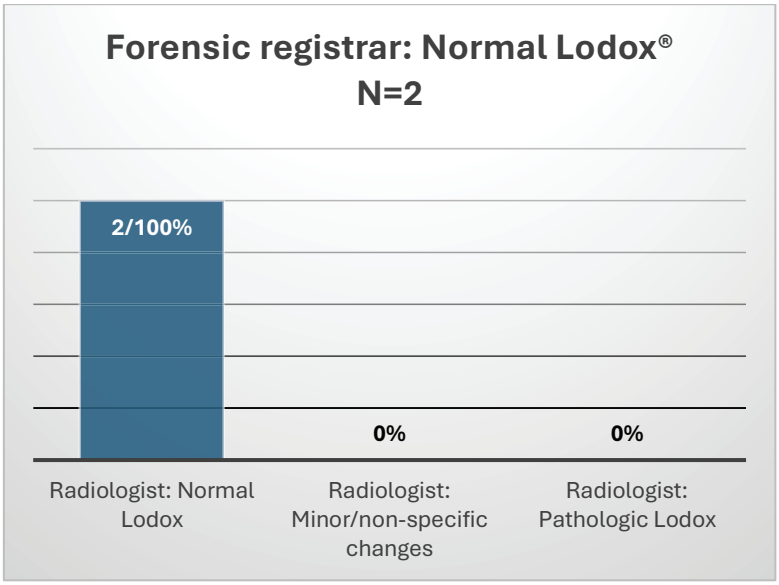


FIGURE 9 HISTOGRAM: FORENSIC REGISTRAR NORMAL SCANS VERSUS RADIOLOGIST INTERPRETATIONS. CHI-SQUARE(DF=4) =6.70, P=0.15 FISHER EXACT (R X C) P=0.17, KAPPA = 0.28 (0.01-0.54)

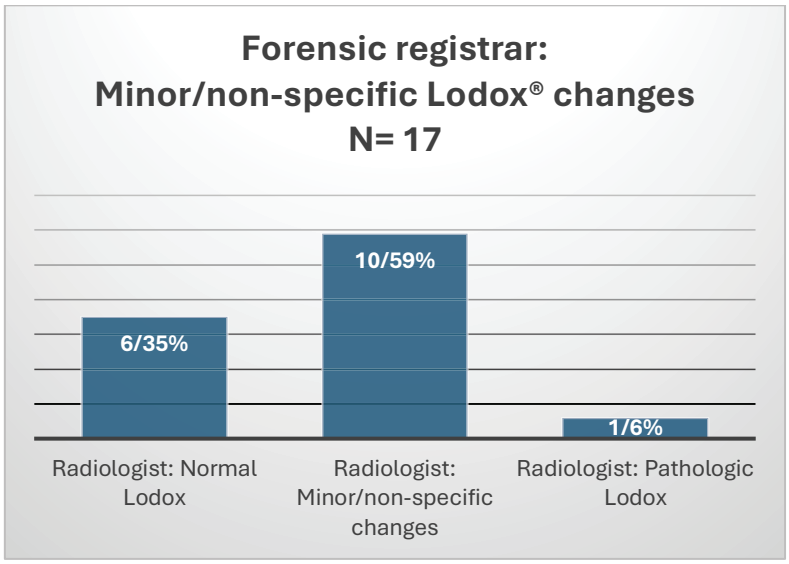


FIGURE 10 HISTOGRAM: FORENSIC REGISTRAR MINOR TO NON-SPECIFIC LODOX® CHANGES VERSUS RADIOLOGIST INTERPRETATIONS. CHI-SQUARE(DF=4) =6.70, P=0.15 FISHER EXACT (R X C) P=0.17, KAPPA = 0.28 (0.01-0.54)

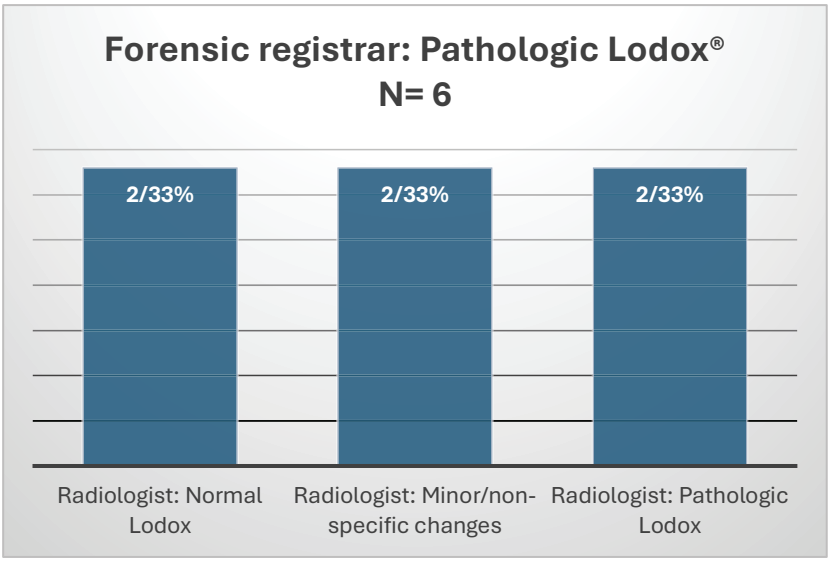


FIGURE 11 HISTOGRAM: FORENSIC REGISTRAR PATHOLOGIC SCANS VERSUS RADIOLOGIST INTERPRETATIONS. CHI-SQUARE(DF=4) =6.70, P=0.15 FISHER EXACT (R X C) P=0.17, KAPPA = 0.28 (0.01-0.54)

The sample size of this study was too small to draw the conclusion that there was a statistically significant relationship between the radiologist and forensic registrar Lodox® categorisations, as indicated by the p-value of 0.10, obtained using the Fisher Exact test.

HISTOLOGY RESULTS

Forensic registrar (investigator) histology *results*

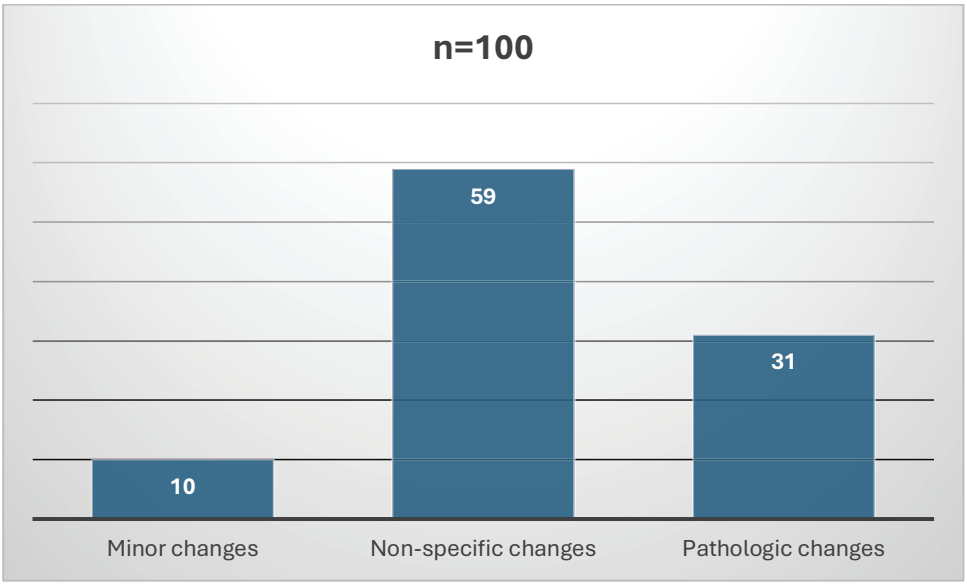


FIGURE 12 DISTRIBUTION OF FORENSIC REGISTRAR HISTOLOGY RESULTS

One hundred cases were reviewed by the forensic registrar. Ten cases were classified as minor changes. The majority of cases (59 cases, 59%) were categorised as non-specific changes and 31 cases (31%) were deemed pathologic (*Figure 12*).

Anatomical pathologist (moderator) histology results.

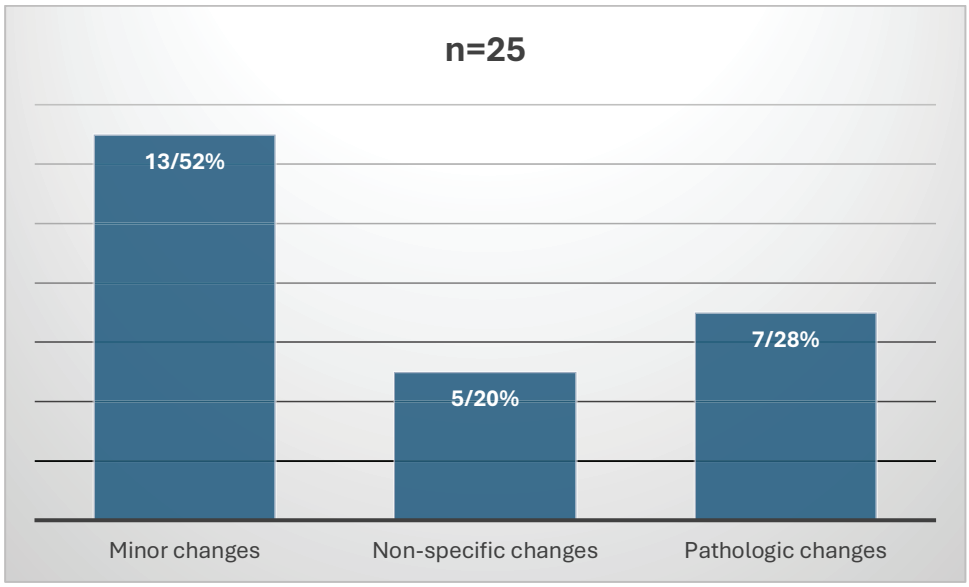


FIGURE 13 DISTRIBUTION OF ANATOMICAL PATHOLOGIST HISTOLOGY RESULTS

Every fourth histology case (n = 25) was examined by the anatomical pathologist. The bulk of the anatomical pathologist cases (13 cases, 52%) were categorised as non-existent/minor changes. The anatomical pathologist classified 5 of the remaining cases (20%) as non-specific changes and 7 cases (28%) as pathologic (*Figure 13*).

Comparison between the forensic registrar and anatomical pathologist histology results

TABLE 11 COMPARISON OF FORENSIC REGISTRAR AND ANATOMICAL PATHOLOGIST HISTOLOGY RESULTS

	Chi-square(df=4) =10.21, p=0.04 Fisher Exact (r x c) p=0.01						
	Anatomical pathologist: Minor changes	**Column%**	**Anatomical pathologist: Non-specific changes**	**Column%**	**Anatomical pathologist Pathologic Changes**	**Column%**	**Row %**
Forensic registrar: Minor changes	0	0%	1	20%	0	0%	1
Row %	0,00%		100,00%		0,00%		
Forensic registrar: Non-specific changes	12	92%	2	40%	3	43%	17
Row %	70,59%		11,76%		17,65%		
Forensic registrar: Pathologic Changes	1	8%	2	40%	4	57%	7
Row %	14,29%		28,57%		57,14%		
Totals	13		5		7		25

Objective 7 involved evaluating interrater agreement between the forensic registrar and the anatomical pathologist in order to assess the accuracy of non-specialist interpretation. To that end, a subset of 25 cases were moderated. *Table 11* is a 3x3 contingency table that compares the categorisations made by the forensic registrar and the anatomical pathologist for the 25 moderated cases. The anatomical pathologist categorised 13 cases as minor changes, while the forensic registrar categorised twelve (12, 92%) of these as non-specific changes and one (1, 8%) case as pathologic. There was 100% concordance for the minor results category as none of these cases were interpreted as such by the anatomical pathologist and forensic registrar. The anatomical pathologist categorised 5 cases as non-specific changes, while the forensic registrar categorised one (1, 20%) of these cases as minor changes and two (2, 40%) cases as pathologic. There was concordance in the interpretation of two (2, 40%) non-specific cases. The anatomical pathologist categorised 7 cases as pathologic,

while the forensic registrar categorised none of these as minor changes and three (3, 43%) as non-specific changes. There was concordance in the interpretation of four (4, 57%) pathologic cases.

The histograms below show the forensic registrar's categorisation compared to the anatomical pathologist's interpretation of the histology slides (*Figure 14-16*).

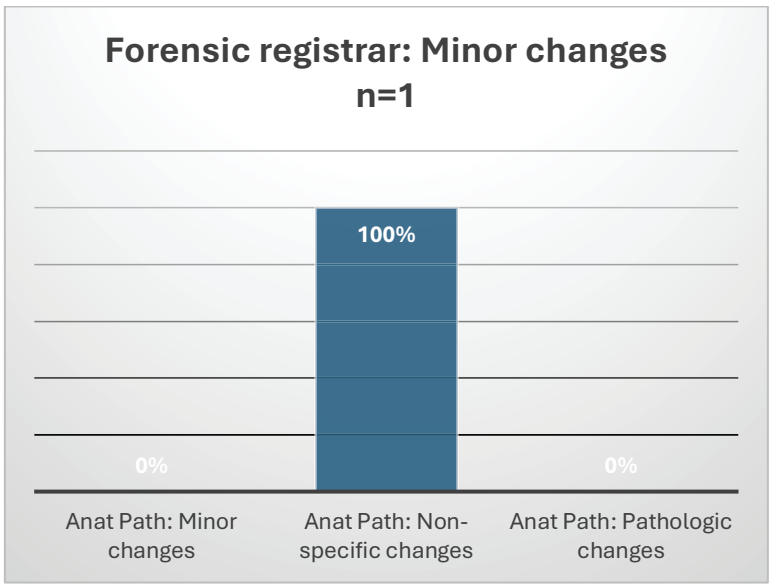


FIGURE 14 FORENSIC REGISTRAR MINOR CHANGES VERSUS ANATOMICAL PATHOLOGIST INTERPRETATIONS. CHI-SQUARE (DF=4) =10.21, P=0.04 FISHER EXACT (R X C) P=0.01, KAPPA=0.01

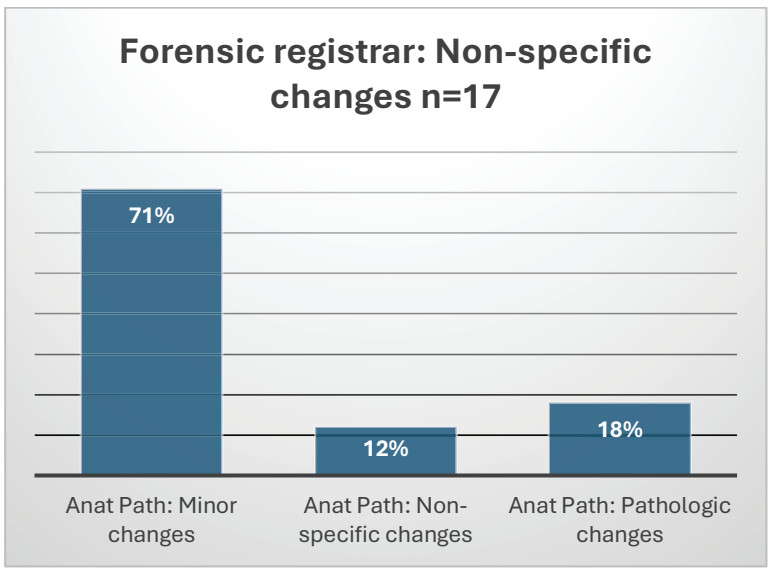


FIGURE 15 HISTOGRAM: FORENSIC REGISTRAR NON-SPECIFIC CHANGES VERSUS ANATOMICAL PATHOLOGIST INTERPRETATIONS. CHI-SQUARE (DF=4) =10.21, P=0.04 FISHER EXACT (R X C) P=0.01, KAPPA=0.01 (-0.22-0.21).

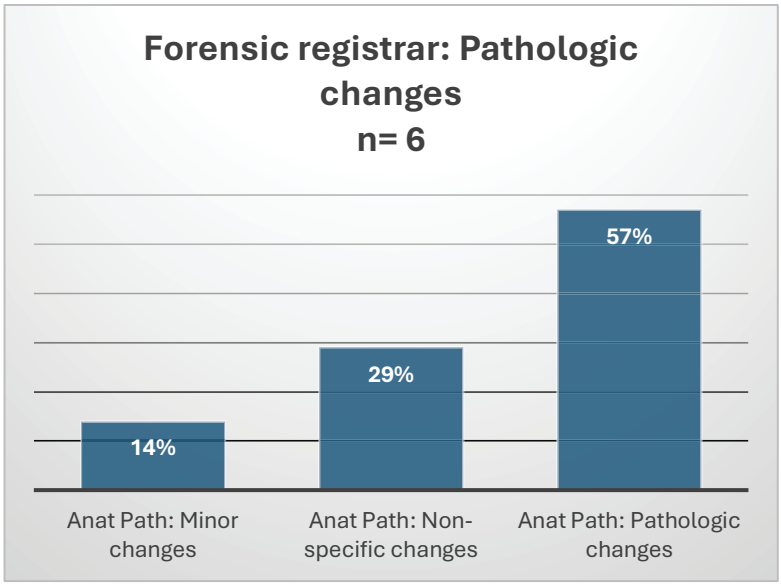


FIGURE 16 HISTOGRAM: FORENSIC REGISTRAR PATHOLOGIC FINDINGS VERSUS ANATOMICAL PATHOLOGIST INTERPRETATIONS. CHI-SQUARE (DF=4) =10.21, P=0.04 FISHER EXACT (R X C) P=0.01, KAPPA=0.01 (-0.22-0.21). ANAT PATH= ANATOMICAL PATHOLOGIST.

COMPARISON BETWEEN LODOX® AND HISTOLOGY RESULTS

TABLE 12 CONTINGENCY TABLE SHOWING FORENSIC REGISTRAR LODOX® AND HISTOLOGY RESULTS

Forensic registrar Lodox® Categorisation n=100	Marked cells have counts > 10. Chi-square(df=4) =9.89, p=0.04 Fisher Exact (r x c) p=0.15 Forensic registrar Histology categorisation n=100						
	Minor changes	Column%	Non-specific changes	Column%	Pathologic changes	Column%	Totals
Normal scan	0	0%	8	14%	0	0%	8
Row %	0%		100%		0%		
Minor or non-specific changes	8	80%	39	66%	21	68%	68
Row %	12%		57%		31%		
Pathologic changes	2	20%	12	20%	10	32%	24
Row %	8%		50%		42%		
Totals	10		59		31		100

Then Lodox® scans and histology slides of the 100 cases reviewed by the forensic registrar were compared. The forensic registrar categorised 10 cases as minor changes histologically. Eight (8, 80%) of these cases were categorised as non-specific changes, and two (2, 20%) cases as pathologic on the Lodox® scans. There was 100% concordance in the interpretation of minor histologic changes and normal Lodox® scans. The forensic registrar categorised 59 cases as non-specific histologic changes. Eight (8, 14%) of these cases were categorised as normal Lodox® scans and twenty-one (12, 20%%) cases as pathologic changes on Lodox® scans. There was concordance in the thirty-nine (39, 66%) cases with non-specific histologic changes and minor Lodox® changes. The forensic registrar categorised 31 cases as pathologic on histologic examination. None of these cases were categorised as pathologic on Lodox® scan while twenty-one (21, 68%) were interpreted as demonstrating minor Lodox® changes. There was concordance in the ten (10, 32%) cases with pathologic changes. (*Table 12*).

The histograms below show the forensic registrar's Lodox® categorisation compared to the forensic registrar's interpretation of the histology slides (*Figure 17-19*).

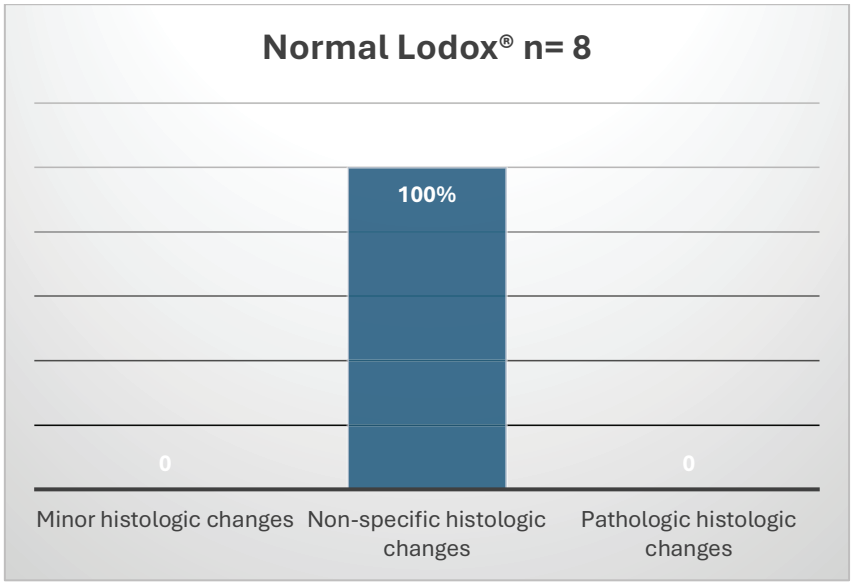


FIGURE 17 FORENSIC REGISTRAR NORMAL LODOX® VERSUS HISTOLOGIC INTERPRETATIONS. CHI-SQUARE (DF=1) =9.89, P=0.04 FISHER EXACT (R X C) P=0.15, KAPPA=0.01 (-0.15-0.17).

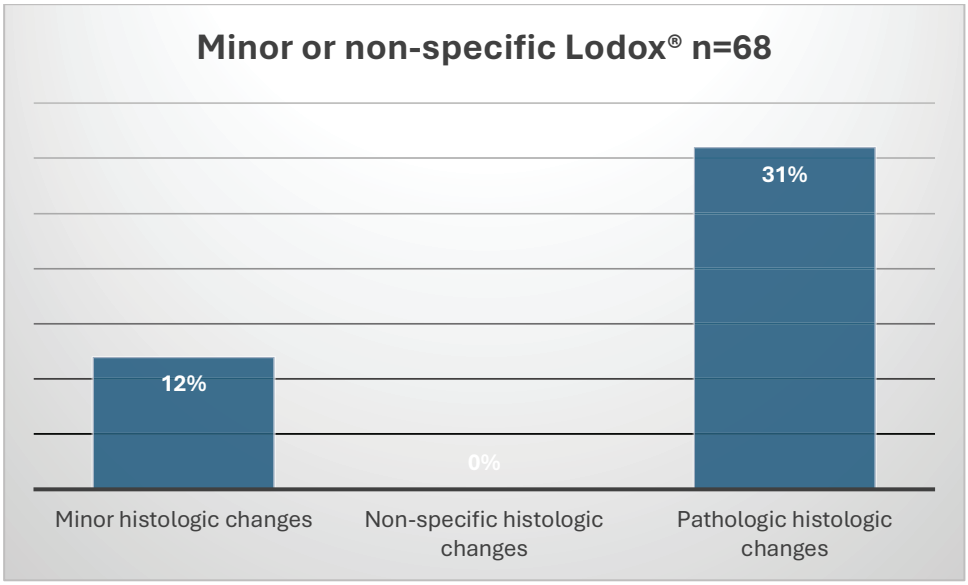


FIGURE 18 FORENSIC REGISTRAR MINOR TO NON-SPECIFIC LODOX® VERSUS HISTOLOGIC INTERPRETATIONS. CHI-SQUARE(DF=1) =9.89, P=0.04 FISHER EXACT (R X C) P=0.15, KAPPA=0.01 (-0.15-0.17).

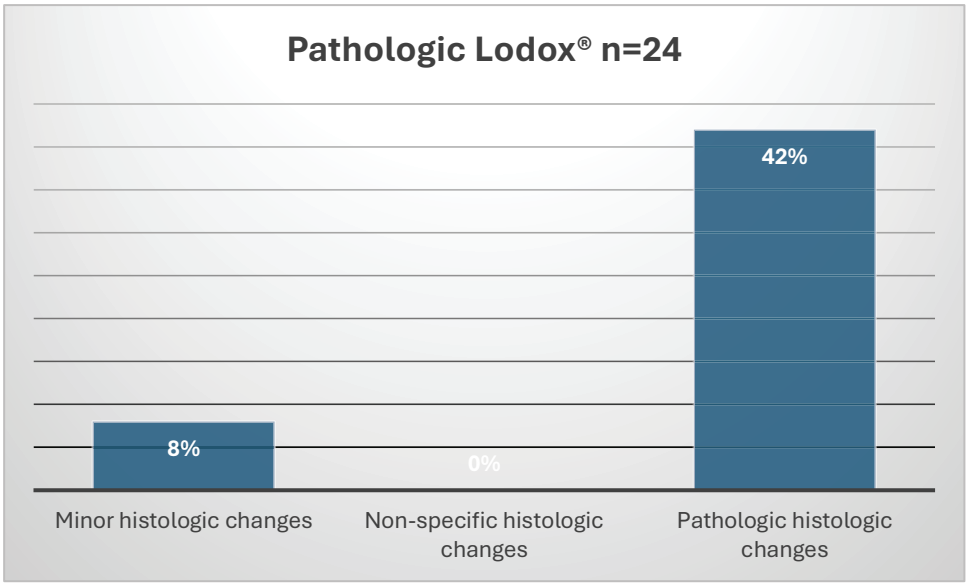


FIGURE 19 FORENSIC REGISTRAR PATHOLOGIC LODOX® VERSUS HISTOLOGIC INTERPRETATIONS. CHI-SQUARE(DF=1) =9.89, P=0.04 FISHER EXACT (R X C) P=0.15, KAPPA=0.01 (-0.15-0.17).

Inter-rater variability and correlation

The comparison between the histology and Lodox® categorisation and inter-rater agreement is provided in *Figure 20*.

	Weighted Kappa	Lower 95%	Upper 95%	n
Agreement between investigator Lodox® and histology categorisation	0,14	-0,02	0,29	100
Agreement between forensic registrar and radiologist Lodox® interpretation	0,27	-0,09	0,59	25
Agreement between investigator and moderator histology interpretation	0,29	0,03	0,49	25

FIGURE 20 AGREEMENT BETWEEN LODOX® AND HISTOLOGY RESULTS AND INTER-RATER AGREEMENT

	Pearson	Pearson p-value	Spearman	Spearman p-value	n
Correlation between investigator Lodox® and histology categorisation	0,14	0,16	0,15	0,13	100
Correlation between investigator and moderator Lodox® interpretation	0,35	0,09	0,31	0,13	25
Correlation between investigator and moderator histology interpretation	0,4	0,05	0,42	0,04	25

FIGURE 21 CORRELATION BETWEEN LODOX® AND HISTOLOGY AND INTER-RATER CORRELATION

When comparing the forensic registrar and radiologist Lodox® categorisations, the weighted-κ is 0.27, which signifies fair inter-rater agreement; however, this is less accurate, due to the wide 95%CI [-0.09, 0.59]. Correlation between the forensic registrar and radiologist Lodox® categorisations is moderate, according to the Pearson and Spearman coefficients. These results are considered statistically insignificant because the p-values for the Pearson and Spearman tests, which are 0.09 and 0.13 respectively, are greater than 0.05.

Histology categorisation by the forensic registrar and anatomic pathologist shows a fair level of inter-rater agreement. The weighted-κ is 0.29 with a 95%CI of 0.03, 0.49 (*Figure 20*).

A negligible relationship between Lodox® and histology categorisation is indicated by a Pearson coefficient of 0.14 and Spearman coefficient of 0.15. These results are considered statistically insignificant because the p-values for the Pearson and Spearman tests, which are 0.16 and 0.13 respectively, are greater than 0.05 (*Figure 21*).

Pathologic versus non-pathologic results

TABLE 13 PATHOLOGIC VERSUS NON-PATHOLOGIC LODOX® AND HISTOLOGY RESULTS

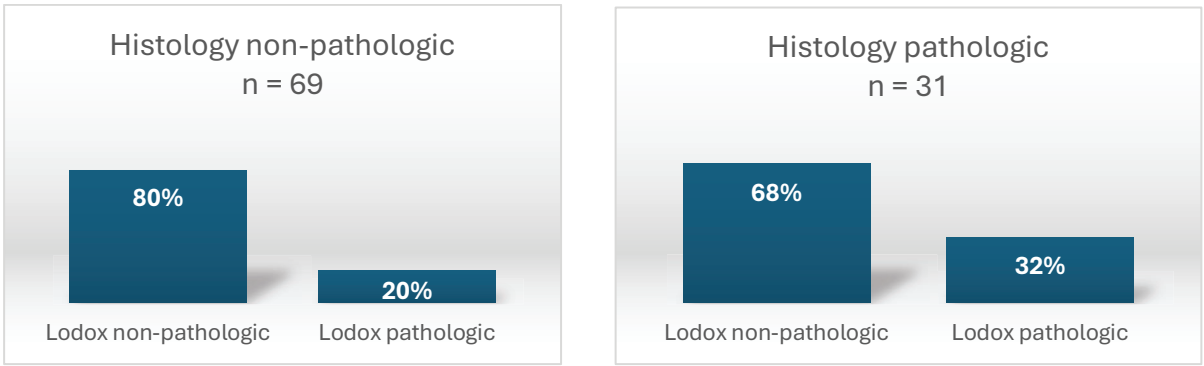
	Chi-square(df=1) =1.62, p=0.20 Fisher Exact p=0.21					
	Pathologic histology		**Non-pathologic histology**	**Column %**	**Row Totals**	
Pathologic Lodox®	10 True negative	32%	14 False positive	20%	24	42% (22%, 63%) (Positive predictive value)
Row %	42%		58%			
Non-pathologic Lodox®	21 False negative	68%	55 True positive	80%	76	72% (61%, 82%) (Negative predictive value)
Row %	28%		72%			
Column Totals	31		69		100	
	32% (17%, 51%) (sensitivity)		80% (68%, 88%) (specificity)			

After combining the normal/minor and non-specific categories of both Lodox® and histology, a 2x2 contingency table was created, comparing these non-pathologic results to the frankly pathologic results (*Table 13*). Of the 24 cases with pathologic Lodox® scans 10 (42%) were histologically confirmed to be pathologic (true positive result). The remaining 14 cases (58%) were categorised as pathologic on Lodox® but non-pathologic on histology, representing false positive results. Of the 76 cases with non-pathologic Lodox® scans, 55 (80%) were histologically confirmed to be non-pathologic (true negative result). The remaining twenty-one cases (67.74%) were categorised as non-pathologic on Lodox® but pathologic on histology, representing false negative Lodox® results.

The post-mortem Lodox® scan demonstrated a low sensitivity of 32.3% (16.7%, 51.4%) to diagnose pneumonia, while its specificity was high at 79.7% (68.3%, 88.4%). Similarly, the positive predictive value (PPV) was 41.7% (22.1%, 51.4%) while the negative predictive value (NPV) of the Lodox® to diagnose pneumonia was 72.4% (60.9%, 82.0%).

The histograms below show pathologic and non-pathologic histology results versus pathologic and non-pathologic Lodox® results.

FIGURE 22 HISTOGRAM OF NON-PATHOLOGIC VERSUS PATHOLOGIC CATEGORISATION OF LODOX® AND HISTOLOGY.



The sample size of this study is too small to draw the conclusion that the Lodox® diagnosis of pneumonia is sufficient to diagnose pneumonia post-mortem, as the Fisher's exact p-value for the aforementioned test is 0.21. Once more, since the p-value determined using the Fisher exact test is 0.21, no inferences can be made based on the calculated sensitivity, specificity, NPV, or PPV.

Chapter 4: Discussion

This chapter discusses the demographic features of the study participants and the interpretations of their Lodox® scans and histology slides. Perspectives of the radiologist, anatomical pathologist, and forensic registrar are presented. The study aims and objectives are assessed in light of the data, and the study's limitations are examined.

Demographics

SUIDs selected for this study were drawn from the SRM-serviced West Metropole of the City of Cape Town. Healthfield, Martin, and Ramesar (2020) reported that between 2013 and 2017, male infants made up 51.71% of SUID admissions to SRM, while female infants accounted for 48.29% of the total. A similar gender distribution was noted in this study. This is consistent with male gender being a recognised risk factor for SUID, specifically SIDS. Moreover, male infants made up a larger percentage of the study's infants aged 1 to 11 months compared to female infants; while females accounted for a higher proportion of sudden deaths in infants under the age of one month (*Figure 5*).

Most sudden infant deaths occur between 2 and 4 months, although sudden death can occur at any age (Sens, Hughes, 2021). According to Heathfield, Martin, and Ramesar, 73.56% of infant deaths occurred before 4 months of age (2020). Similarly, in this study infants younger than 6 months (n = 72, 72%) accounted for the majority of infant deaths. Although differing slightly, the age ranges used in the studies were comparable and the selected study participants were representative of the larger study population.

Notably, in the event of premature birth, the gestational age was not used to calculate a 'corrected' infant age. This impacted the number of infants in each age category. More importantly, it would have affected radiologic and histologic interpretation because immature lungs may appear artefactually pathological without knowledge of the developmental stage.

Lodox® discussion

When Lodox® scans were initially reviewed for inclusion, a sizable portion of them (approximately 40%) revealed thoracic rotation and were deemed ineligible for inclusion in the study. This suggests that the positioning of the deceased should be done with more care, as this has a significant impact on the interpretation of the scan results. While forensic

pathology officers handle the body's placement on the Lodox® table, ensuring its correctness remains the ultimate responsibility of the forensic medical practitioner. The radiologist made the comment that none of the moderated scans were non-diagnostic, indicating that the quality of the Lodox® images is acceptable for viewing soft tissues such as the lungs.

It is important to note that radiological image quality does not necessarily correspond to correct interpretation of those images. The Lodox® scans were interpreted using the WHO framework, which offers a standardized approach to radiologic review. Although the framework is simple to use, it assumes that the user is properly trained in the interpretation of conventional radiology scans. As previously stated, post-mortem radiology is not a specific area of training for forensic medical practitioners. Instead, experience is gained in daily mortuary practice and based on interpretation of living patients' x-rays, as taught during medical school. The radiologist, who has considerably greater experience with post-mortem images, categorised more Lodox® scans as normal than the forensic registrar. It is possible that insufficient post-mortem radiology training impacted the non-specialist's interpretation of Lodox® scans. This was confirmed during the consensus meeting which took place after the forensic registrar and radiologist gave their initial, separate interpretations of the scans. After discussion, it became clear that the forensic registrar had overclassified normal post-mortem changes as pathologic.

A particular challenge experienced by the forensic registrar was assessment of interstitial infiltrates, which appear as patchy and linear infiltrates in a lacey pattern. This is line with the observations of Voigt et al., who found that an interstitial pattern and peribronchial thickening had the lowest levels of interrater agreement (2021). This was similar to the Daya 2009 study, where diffuse interstitial lung disease was the area where specialists disagreed the most and diagnostic limitations with a high false positive rate were observed. Daya also pointed out that experience and training should be considered in further studies when interpreting conventional radiology, including Lodox® scan interpretations, as they are subjective.

The forensic registrar noted that it seemed the Lodox® images were simpler to interpret with each scan that was assessed. This might reflect the Baader-Meinhof phenomenon. This phenomenon happens when something one has recently learnt seems to appear "everywhere" shortly after one is made aware of it. On the other hand, had the results been misinterpreted at the outset, this perception might have remained throughout the investigation. Even so, the likelihood of the phenomenon occurring was lowered by using the WHO standardization tool as a guideline. Alternatively, it might just be evidence that,

with additional training and experience, the registrar has improved their ability to interpret Lodox® scans.

Despite some inherent problems, the guideline allowed for the most repeatable results. Crucially, if the guideline is to be utilized for additional research and the provision of forensic services, post-mortem artefacts must be considered and integrated into it. For example, pathologic changes should only be diagnosed when the Lodox® scan clearly shows pathology, such as pleural effusion and consolidation, rather than subtle changes which may be artefactual. Furthermore, in order to reduce radiologic post-mortem artefacts, Lodox® scans ought to be carried out as soon as possible after death.

Minimizing post-mortem interval and improved training notwithstanding, it is clear that viewing a Lodox® scan in isolation is not ideal. A thorough history affects interpretation, for instance knowledge of the post-mortem interval and gestational age. In addition, ancillary investigations such as a respiratory viral panel should be used conjunction with a Lodox® scan. For example, some respiratory pathogens such as respiratory syncytial virus are known to be virulent and can be fatal. As well as being essential for diagnosis, the pathologic process of pneumonia can be better understood through microscopic examination, which can assist in interpreting results from the Lodox® scan.

Histology discussion

Tissue processing and staining quality are critical in order to ensure diagnostic-quality slides for microscopic examination. In this study, the quality of the slides that were supplied for case inclusion was acceptable. The ability of the pathologist to identify patterns in the lung tissue and decipher their morphology will also depend on their training and experience. The forensic registrar had completed the anatomical pathology rotation and had an additional eight months of previous experience in anatomical pathology, in contrast to other registrars at the same level. One year of focused anatomical histopathology training is the typical amount of training received during the fellowship training in forensic pathology in South Africa, along with the experience gathered from reviewing post-mortem cases over time. During the study, the forensic registrar noted that histological identification of pneumonic processes was improved with the experience gained from conducting this research and using the SIDS consensus tool.

The purpose of the consensus tool in this study was to standardize lung slide interpretation because histologic interpretation, like Lodox® interpretation, can be subjective. One of the issues raised was that the tool was designed primarily to rule out SIDS, rather than to diagnose pneumonia. While specific numbers of alveolar neutrophils correspond to each category (SIDS, borderline SIDS, and explained infant death), it makes no mention of the quantity of other inflammatory cells, such as lymphocytes, required for categorisation. This is a concern since viral and fungal infections tend to be associated with lymphocytes and/or eosinophils.

Another issue was that some findings, such as pulmonary haemorrhage, were not accounted for by the histologic tool. Pulmonary haemorrhage may occur in viral infection (Weisenberg, 2024), as an agonal event, in trauma or due to decomposition. However, these cases—which may very well have been pathologic—were classified as having non-specific changes.

In the circumstances described above, respiratory virus investigation would be beneficial. While immunohistochemistry is an option, its cost is prohibitive in the local context. Furthermore, understanding the case history is essential for interpreting histology, which was not possible in this study.

The moderating anatomical pathologist commented that in general, three-tier classification systems are suboptimal since cases tend to be assigned to the middle category if the observer is unsure. It is preferred that two-tier systems are developed, with more stringent criteria that ensure reproducible, consistent inter-rater categorization. The results of this study show that the anatomical pathologist classified substantially fewer cases as non-specific compared to non-existent /minor changes. This was because post-mortem artefacts were interpreted as minor changes by the anatomical pathologist, while the forensic registrar classified these cases as non-specific. The non-specific category is equivocal due to the subjective nature of histologic interpretation and some degree of overlap between non-specific and pathologic changes. The use of two categories, pathologic and non-pathologic, would have reduced the amount of uncertainty regarding non-specific findings. Furthermore, the two-category approach would have simplified the statistical analysis and produced potentially significant conclusions even with a sample size smaller than the calculated 1400. If the non-existent/minor changes and non-specific categories were combined, formulation of cause of death would also not significantly change. Thus, the two-category approach is recommended for future studies.

Accuracy of non-specialist forensic registrar Lodox® and histology interpretation

To address objective 7 regarding the accuracy of non-specialist interpretation, the moderators' interpretations of the 25 moderated cases were recognized as the accurate diagnosis. The variability in both Lodox® and histology interpretation was confirmed in this study. Interestingly, Lodox® interpretation appeared to differ more than histologic interpretation between the forensic registrar and the respective moderators. The p-values yielded by the Fisher exact (0.01), and chi-squared (0.04) tests indicate a *statistically significant* strongly positive association between the anatomical pathologist and the forensic registrar interpretations. A strongly positive correlation was observed between the histology raters' results. Both the Pearson and Spearman test p-values (0.05 and 0.04, respectively) are less than 0.05, indicating statistical significance for these results; whereas the correlation between the radiologist and forensic registrar showed a statistically insignificant moderate association. Although the correlation and agreement results between the radiologist and forensic registrar were not statistically significant, they suggest that the less experienced non-specialist requires more training in post-mortem radiologic interpretation. Increasing the number of moderated scans and histology in future studies would also assist in more significant conclusions regarding inter-rater variability.

The moderators did not interpret the Lodox® and histology of the same 25 cases because of the blinding process. Moderation of the same cases would potentially produce more reliable conclusions about the validity of Lodox® as a substitute for histology.

Validity of post-mortem Lodox® as substitute for histologic diagnosis of pneumonia

The degree of agreement between the Lodox® scan and the current best standard for diagnosing pneumonia in cases of sudden infant death, histologic examination, must be considered in order to practice evidence-based forensic pathology.

When evaluating the dependability of a screening or diagnostic test, various statistical measures are used. In this study, the sensitivity quantifies how well Lodox® identifies histologically-confirmed cases of pneumonia. The low sensitivity of 32.3% implies that it is usually not possible to diagnose pneumonia based on Lodox® images. In line with this, only 10 out of 24 Lodox® scans that were initially classified as pathologic were later confirmed to

be cases of pneumonia on histology, resulting in a low positive predictive value of 41.7%. The corresponding 58% false positive rate is concerning. These are scans which were interpreted as pathologic but do not correspond to pathologic histological changes. These cases would not proceed to internal review and additional investigations under some FMPs' current practices. The consequences of misinterpreting the post-mortem Lodox® as pneumonia could prevent the identification of unnatural causes of death that might not be apparent on history, Lodox®, and external examination. Misdiagnosis can also result in low-quality cause-of-death data, which can jeopardize public health initiatives that are based on these statistics.

On the other hand, both the high specificity (80%) and the high negative predictive value (74%) are reassuring. Thus, it is reasonable to assume that a non-pathologic Lodox® scan is indeed non-pathologic.

Given the small sample size in this study (n:100), it was expected that the agreement between Lodox® and histology would not be statistically significant (p-value greater than 0.05). Nonetheless, the findings imply that a Lodox® scan diagnosis of pneumonia does not always correspond to the histologic diagnosis. It can be deduced that some of the diagnoses made during the period Healthfield, Martin, and Ramesar (2020) researched at Salt River Mortuary were inaccurate. In their study, 64% of SUIDs were classified as dying of natural causes after clinical review, Lodox®, and external examination alone. The 2020 study includes all causes of natural death, respiratory and non-respiratory; however, from 2013 to 2017, over half of all SUID cases were diagnosed as lower respiratory tract infections.

The Lodox® scans that the forensic registrar classified as normal did not exhibit pathologic changes on histology; instead, they were all classified as non-specific changes. Since no pathology was found in this group, additional forensic investigation of these cases would have been necessary. A comparison between the diagnoses obtained during this study and the actual diagnoses made by the assigned FMPs in these cases would be illuminating and would be an interesting avenue of future investigation.

Lodox® scans revealed non-specific or minor changes in almost 70% of cases in this study. Histologic results across all three categories demonstrate the importance of histology within this grouping. This indicates that the post-mortem process should continue despite non-specific findings on Lodox® and that these cases should be thoroughly investigated as the cause may be natural or unnatural.

Slight agreement and a negligible correlation between the Lodox® and histology interpretations, albeit not statistically significant, suggest that the Lodox® scan should not be used as a substitute for the histologic diagnosis of pneumonia due to its unreliability. These findings expand on previously published research by De Lange et al. (2007), which demonstrated poor radio-pathologic correlation for soft tissue diagnosis (lung tissue) in the paediatric post-mortem population. The outcomes are also consistent with those of the adult population as reported by Quarrie (2015), who observed a negligible correlation between Lodox® and histology interpretations. Both this study and the earlier ones show that further investigation with larger sample sizes is needed to definitively support or refute the correlation between the two modalities.

Limitations

The study design and time constraints influenced the methodological decisions. A prospective study instead of a retrospective study would enable more accurate standardization of histology and Lodox® scan interpretation with development of better guidelines for interpretation of post-mortem cases.

The limited sample size and lack of statistically significant findings have an impact on the data's reliability. A single investigator (forensic registrar) may not have accurately reflected the range of experience that forensic medical practitioners possess in one department. It would be beneficial for future research to involve multiple investigators with varying degrees of experience.

Conclusion

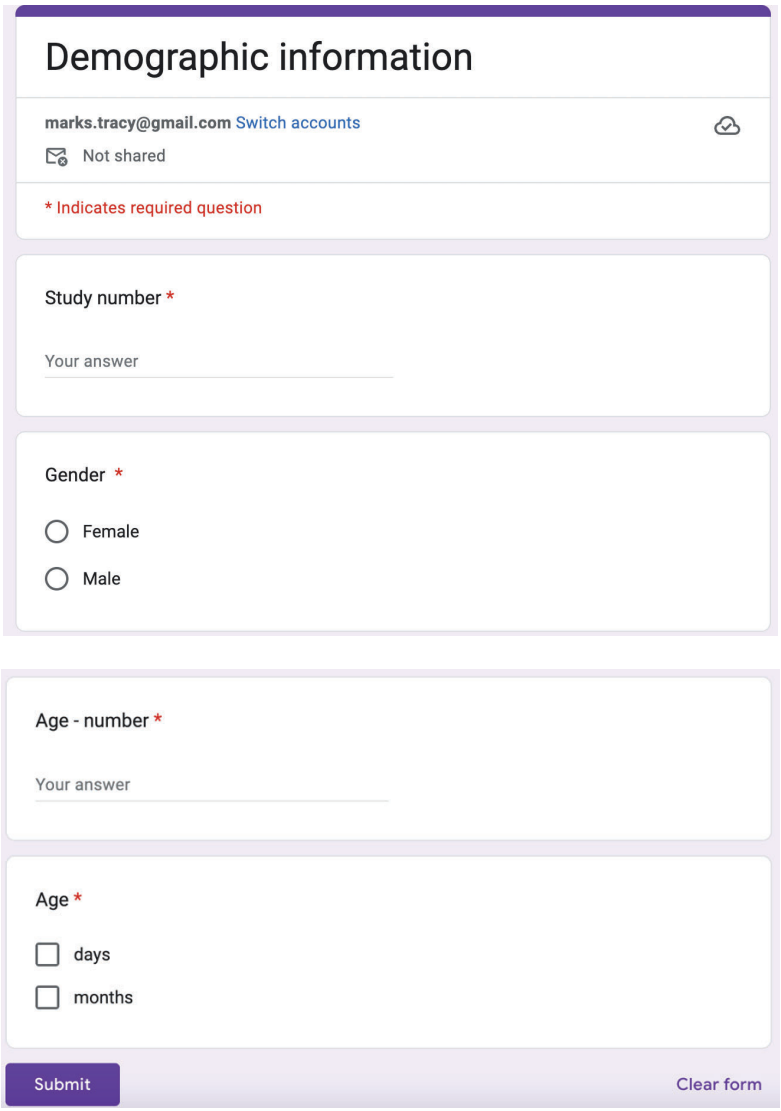
This retrospective cross-sectional study aimed to evaluate whether interpretation of a post-mortem Lodox® scan is sufficient to diagnose pneumonia in alleged sudden infant deaths, without the need for confirmatory histology. The potential utilisation of the Lodox® scan as a screening tool would make a vast difference in forensic practice in lower income countries, where caseloads are high and access to sophisticated imaging is limited. Unfortunately, the small sample size did not yield enough information to conclude that a statistically significant relationship exists between Lodox® and histology categorisation. Nonetheless, results suggest that the diagnosis of pneumonia on Lodox® may be unreliable, resulting in failure to identify both natural and unnatural causes of death that may not be apparent on history, imaging, and external examination alone. One of the consequences of inaccurate cause of death data is that public health interventions would be compromised.

It is recommended that lung histology should be performed until such time as there is definitive evidence from future studies supporting the use of Lodox® as a substitute for the histologic diagnosis of pneumonia in sudden infant death. This research reveals that forensic histology interpretation is accurate, more so than Lodox® diagnosis. The implementation of post-mortem radiology training programs for FMPs is therefore suggested. Future studies should involve detailed two-category interpretation tools for both post-mortem radiology and histology. Larger sample sizes, involvement of more forensic practitioners at varying levels of experience, and more moderation of results are required.

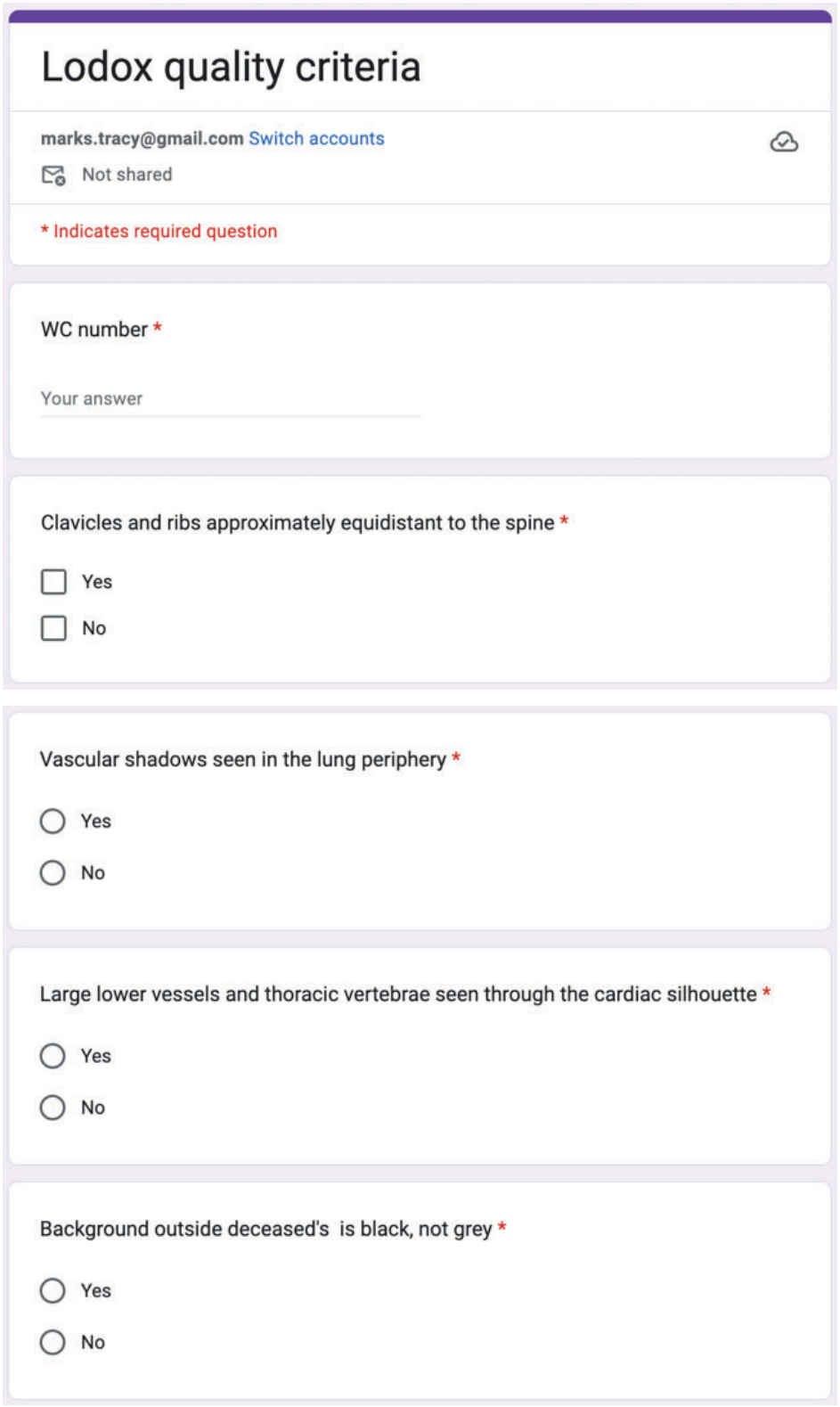
In closing, it is important for forensic professionals to adapt their practice according to the latest developments in medicolegal death investigation and to strive for the most objective, accurate results.

Appendices

Appendix 1 Demographic information



Appendix 2 Lodox slide quality



Lodox quality criteria

marks.tracy@gmail.com Switch accounts

Not shared

* Indicates required question

WC number *

Your answer

Clavicles and ribs approximately equidistant to the spine *

- [ ] Yes
- [ ] No

Vascular shadows seen in the lung periphery *

- ◯ Yes
- ◯ No

Large lower vessels and thoracic vertebrae seen through the cardiac silhouette *

- ◯ Yes
- ◯ No

Background outside deceased's is black, not grey *

- ◯ Yes
- ◯ No

Bones and tissues easily distinguishable from soft tissue *

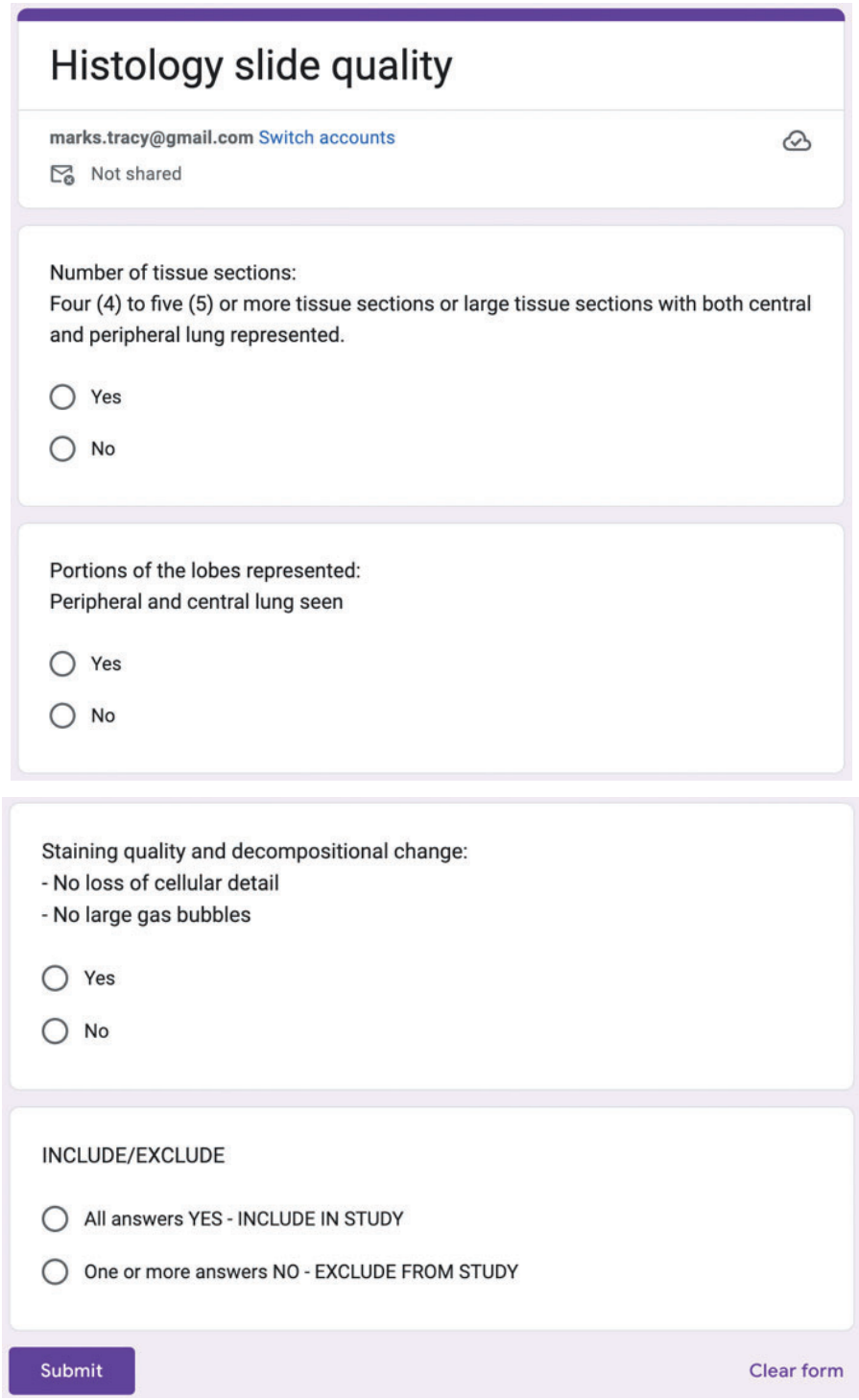
- ◯ Yes
- ◯ No

Include/Exclude *

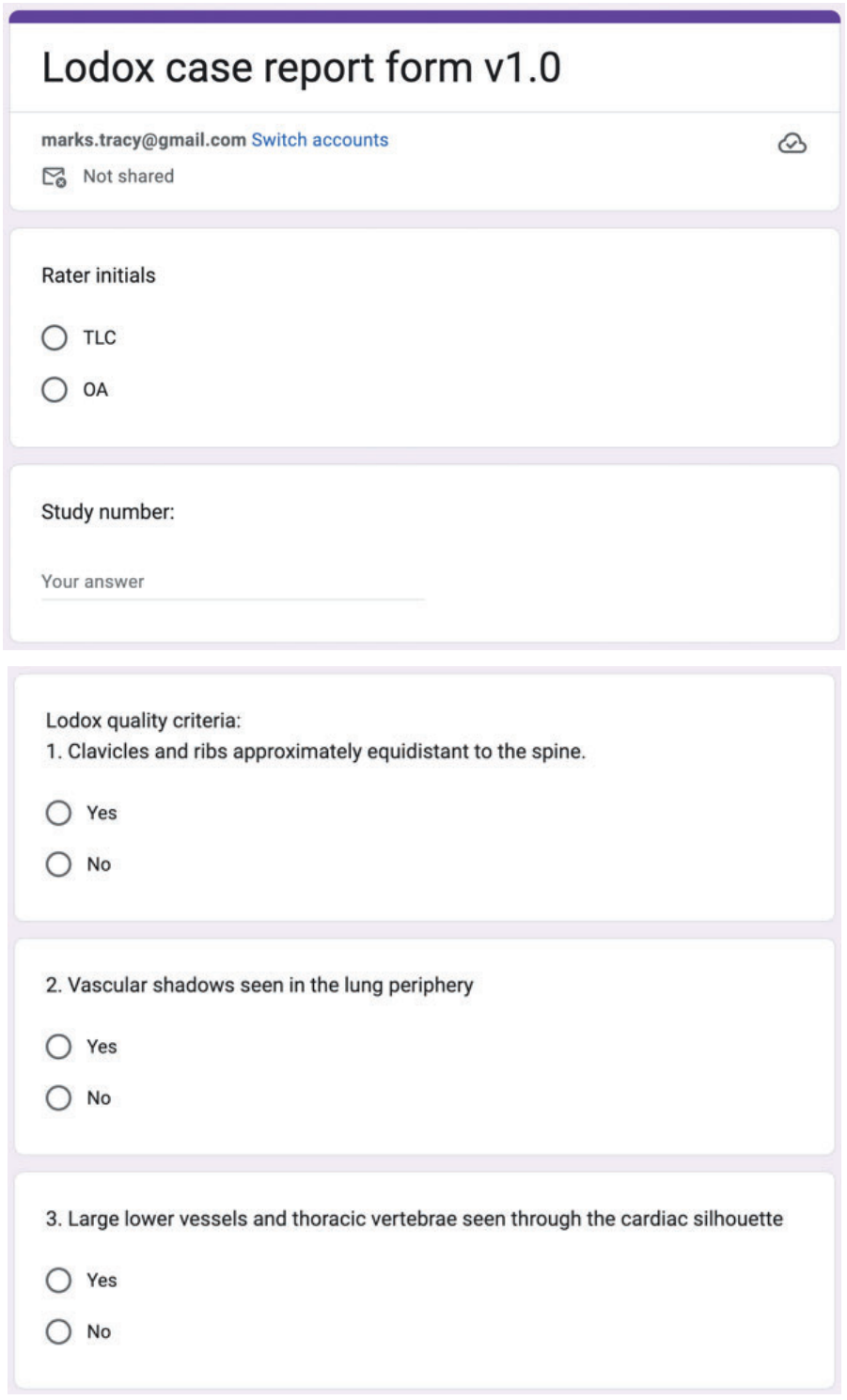
- ◯ All answers YES - CHECK HISTO SLIDES FOR INCLUSION
- ◯ One or more answers NO - EXCLUDE from study

Submit Clear form

Appendix 3 Histology slide quality



Appendix 4 Lodox case report form



Lodox case report form v1.0

marks.tracy@gmail.com Switch accounts

Not shared

Rater initials

- TLC
- OA

Study number:

Your answer

Lodox quality criteria:
1. Clavicles and ribs approximately equidistant to the spine.

- Yes
- No

2. Vascular shadows seen in the lung periphery

- Yes
- No

3. Large lower vessels and thoracic vertebrae seen through the cardiac silhouette

- Yes
- No

4. Background outside deceased's silhouette is black, not grey.

- ◯ Yes
- ◯ No

5. Bones and tissues easily distinguishable from soft tissue.

- ◯ Yes
- ◯ No

- ◯ All above answers YES - PROCEED TO INTERPRETATION
- ◯ One or more answers NO - EXCLUDE FROM STUDY

Rater interpretation - Free text

Your answer

Lodox characterisation

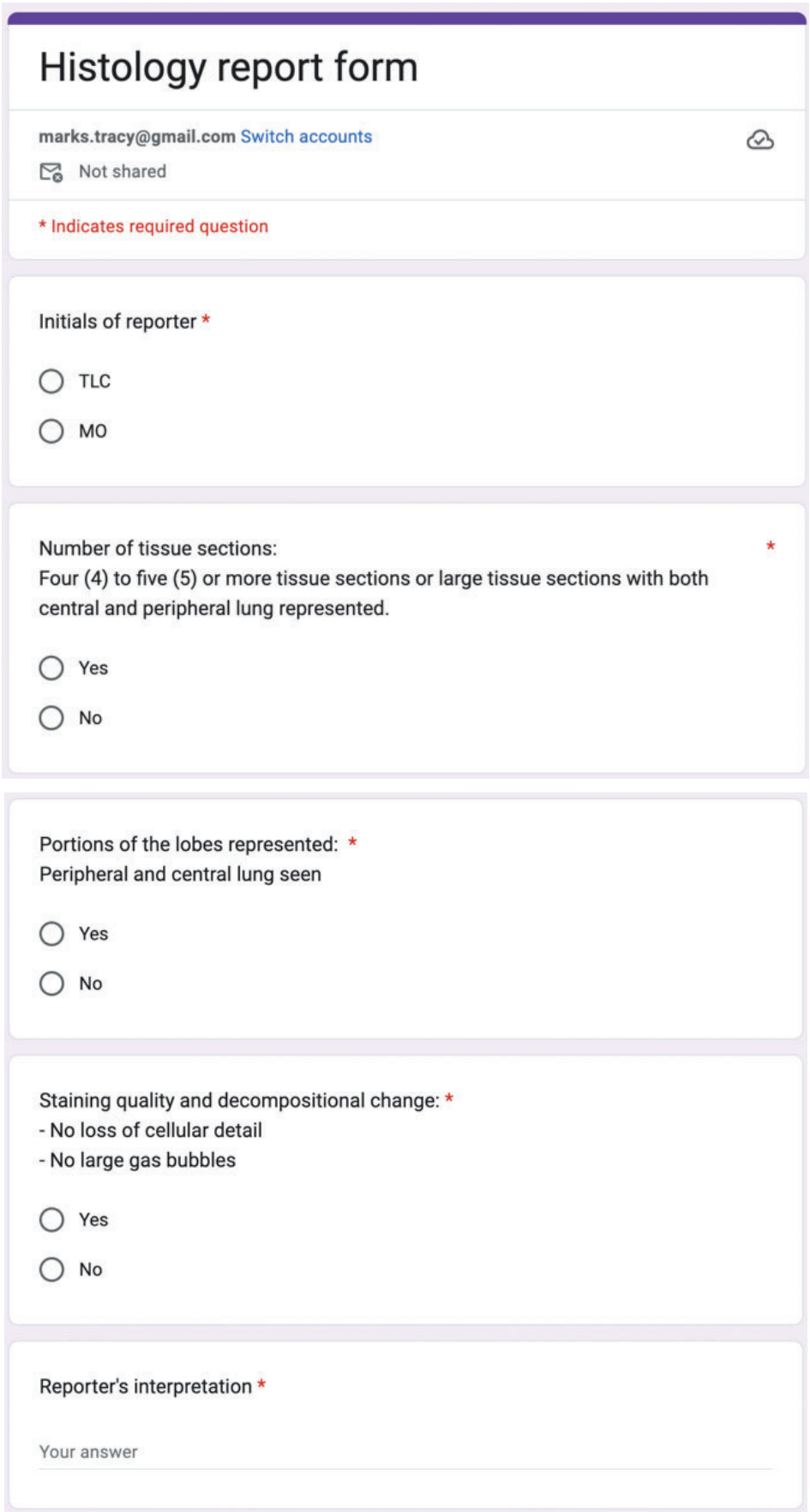
- ◯ Normal scan: No infiltrates
- ◯ Minor/Non-specific changes: Lung inflation normal to increased, minor patchy infiltrates not of sufficient magnitude to constitute primary endpoint consolidation/small areas of atelectasis
- ◯ Pathologic changes: Presence of consolidation, infiltrates, or effusion, linear and patchy (interstitial infiltrate) in a lacey pattern involving both lungs, featuring peribronchial thickening and multiple areas of atelectasis.

Additional comments

Your answer

Submit Clear form

Appendix 5 Histology Report Form



Histology report form

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* Indicates required question

Initials of reporter *

- TLC
- MO

Number of tissue sections: *
Four (4) to five (5) or more tissue sections or large tissue sections with both central and peripheral lung represented.

- Yes
- No

Portions of the lobes represented: *
Peripheral and central lung seen

- Yes
- No

Staining quality and decompositional change: *
- No loss of cellular detail
- No large gas bubbles

- Yes
- No

Reporter's interpretation *

Your answer

Portions of the lobes represented: *
Peripheral and central lung seen

- ◯ Yes
- ◯ No

Staining quality and decompositional change: *
- No loss of cellular detail
- No large gas bubbles

- ◯ Yes
- ◯ No

Reporter's interpretation *

Your answer

Histology categorisation *

Choose

Additional Comments

Your answer

Submit Clear form

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