Comparison of Minimally Invasive Tissue Sampling With Conventional Autopsy to Detect Pulmonary Pathology Among Respiratory Deaths in a Resource-Limited Setting

Drucilla J. Roberts, MD,[1] Henry N. Njuguna, MBChB, MPH,[2] Barry Fields, PhD,[3] Corinne L. Fligner, MD,[6] Sherif R. Zaki, MD,[4] M. Kelly Keating, PhD,[4] Emily Rogena, MMed,[7] Edwin Walong, MMed,[7] Andrew K. Gachii, MMed,[8] Elizabeth Maleche-Obimbo, MMed, FPulm,[7] Grace Irimu, MMed, PhD,[7] John Mathaiya, MMed,[7] Noelle Orata, MMed,[7] Rosemarie Lopokoiyit, MMed,[7] Jackson Michuki, MMed, MPH,[7] Gideon O. Emukule, PhD,[2] Clayton O. Onyango, PhD,[2] Stella Gikunju,[9] Collins Owuor, MSc,[9] Peter K. Muturi, MPH,[10] Milka Bunei,[10] Marc-Alain Widdowson, VetMB, MSc,[2,3] Joshua A. Mott, PhD,[5] and Sandra S. Chaves, MD, MSc[2,5]

From the [1]Massachusetts General Hospital, Boston; [2]Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya; [3]Division of Global Health Protection, [4]Division of High-Consequence Pathogens and Pathology, Infectious Diseases Pathology Branch, and [5]Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA; [6]University of Washington, Seattle; [7]University of Nairobi, Kenya; [8]Kenyatta National Hospital, Nairobi, Kenya; [9]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya; [10]IHRC, Nairobi, Kenya.

Key Words: Conventional autopsy; Minimally invasive tissue sampling technique; Postmortem investigation; Pediatric mortality; Pulmonary pathology; Respiratory illness death; Resource limited; TaqMan Array Card

Am J Clin Pathol 2019;XX:1–13

DOI: 10.1093/AJCP/AQZ016

ABSTRACT

Objectives: *We compared minimally invasive tissue sampling (MITS) with conventional autopsy (CA) in detection of respiratory pathology/pathogens among Kenyan children younger than 5 years who were hospitalized with respiratory disease and died during hospitalization.*

Methods: *Pulmonary MITS guided by anatomic landmarks was followed by CA. Lung tissues were triaged for histology and molecular testing using TaqMan Array Cards (TACs). MITS and CA results were compared for adequacy and concordance.*

Results: *Adequate pulmonary tissue was obtained by MITS from 54 (84%) of 64 respiratory deaths. Comparing MITS to CA, full histologic diagnostic concordance was present in 23 (36%) cases and partial concordance in 19 (30%), an overall 66% concordance rate. Pathogen detection using TACs had full concordance in 27 (42%) and partial concordance in 24 (38%) cases investigated, an overall 80% concordance rate.*

Conclusions: *MITS is a viable alternative to CA in respiratory deaths in resource-limited settings, especially if combined with ancillary tests to optimize diagnostic accuracy.*

Accurate data on cause of death (CoD) are critical to formulating cost-effective public health policies and include death certificates, physician documentation, autopsy, and epidemiologic surveys. Death certificates are commonly used to estimate mortality rates and assess disease-specific burden in countries where they are available. However, these data can be inaccurate because listed causes are often provided by physicians untrained in determining CoD and are rarely supported by pathologic or other medical diagnostic data.[1,2] These inaccuracies are exacerbated in resource-limited settings.[3,4]

The conventional autopsy (CA) is the gold-standard procedure for the determination of CoD. However, it is a resource-intensive and invasive procedure requiring experienced personnel and extensive laboratory support. In most resource-limited settings, CA determination of CoD is often restricted to forensic external and limited internal gross examination in deaths due to injury or unnatural causes. Natural or "medical" deaths are rarely investigated for several reasons, including family hesitancy, limited resources at family or health facility level, religious prohibitions, or concerns about appearance of the body after CA.[5]

These challenges have led to the development of new tools for determination of CoD. These include minimally invasive tissue sampling (MITS), which aims to obtain diagnostic samples of tissues postmortem by various biopsy techniques without major incisions or organ removal[6,7] and can potentially be performed at less cost and by nonphysicians in lower resourced settings.[8-10]

Despite these advantages, the MITS technique introduces the possibility of sampling errors, especially for identification of nondiffuse disease pathology as seen with localized pneumonia. There are no universally accepted MITS procedures as there are for the CA. Most studies employ needle biopsies of a limited number of accessible organs and tissues, either directed by imaging techniques (directed MITS) or guided only by visual inspection and palpation (blind MITS), the latter a more feasible approach in resource-limited settings. However, only a few studies comparing MITS technique with the CA as gold standard exist, and they do not focus on deaths associated with infectious diseases and/or have small sample sizes.[9,11-17]

Our study aimed to compare the specimen adequacy and histologic and laboratory findings on lung tissue collected using blind-needle biopsy MITS with those from CA in a pediatric respiratory disease mortality study undertaken at a large public hospital in Kenya, assessing the contribution of molecular microbiologic testing.

Materials and Methods

This substudy was part of the Pediatric Respiratory Etiology and Surveillance Study (PRESS) that investigated causes and etiologies of death among children hospitalized with respiratory disease at Kenyatta National Hospital (KNH), Kenya's leading national referral hospital and the teaching hospital of the University of Nairobi (UoN).[18] Briefly, the PRESS study identified children aged 1 to 59 months who were hospitalized in pediatric medical wards with respiratory illness (defined as either cough or difficulty in breathing regardless of other existing conditions or if diagnosed as having respiratory illness by the attending physician) and followed them over the course of their hospitalization. If death occurred, the family received extensive grief counseling, and written informed consent for autopsy was sought from adult caretakers.

For consented deaths, the bodies had to be refrigerated at 4°C to 8°C in an on-site morgue and anthropometric measurements, including weight, height, and mid–upper arm circumference, gathered before the postmortem examinations. (Details of these procedures are reported elsewhere.[18]) Bodies were excluded from postmortem examination study if the autopsy interval was more than 5 days after death, the body was not refrigerated within 8 hours of death, caretakers did not provide written consent, or the child was not hospitalized with respiratory illness (per above case definition).

The MITS and CA were performed within 5 days of death by experienced and trained local anatomic pathologists and trained ancillary personnel. The procedures for the MITS and CA were designed by two authors (C.L.F. and D.J.R.) in conjunction with pathologists at the KNH/UoN (E.R., A.K.G., and E.W.) and the CDC (S.R.Z. and M.K.K.), all of whom underwent a 5-day training on the procedure to ensure standardization and competency.

Specimen Collection

Biopsy specimens were obtained from two sites in each lung via an anterior approach using anatomical landmarks and a single 11-gauge biopsy needle (ACE-111152, ACECUT automatic biopsy system 11GX115mm; TSK Laboratory Europe B.V.). Before needle insertion, the skin was wiped with alcohol swabs until the swabs were clean, and the skin was left to air dry. The first site in each lung was the apical pulmonary region, targeted via a supraclavicular placement of the needle in a midclavicular location and directed inferiorly. The second site targeted the middle lobe by inserting the needle at the fourth intercostal space at the midclavicular line. Three core biopsy specimens were obtained from each site on both sides of the chest, targeting both lungs.

The MITS procedure was followed by a specialized CA optimized for obtaining biopsy specimens for molecular microbiologic studies using sterile techniques as previously described.[18] Briefly, CA included detailed lung examination and routine collection of tissue sections from each lobe. Additional sections were collected based on gross pathologic abnormalities. At least five sections of lung parenchyma (one section from each lobe) were obtained during each CA.

All lung tissue specimens from both the MITS and CA were triaged for histology and molecular studies **Figure 1**. The tissue for histology was immediately placed in 10% neutral formalin, fixed for 4 to 24 hours, and then stored in 70% ethanol. The specimens for molecular study were placed in phosphate-buffered solution and transported to the Kenya Medical Research Institute (KEMRI) and the Centers for Disease Control and Prevention (CDC) laboratory in Nairobi for testing.

Laboratory Procedures

Histology

The pulmonary biopsy specimens were formalin fixed, routinely processed, and paraffin embedded, and

DOI: 10.1093/AJCP/AQZ016

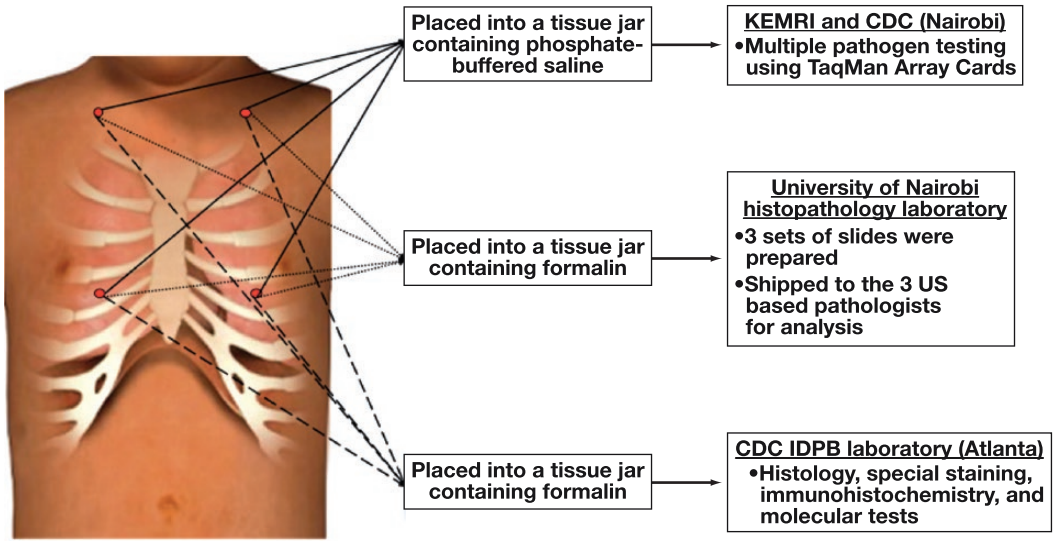

Figure 1 Diagram illustrating the sites for lung tissue specimen collection using minimally invasive tissue sampling techniques, sample handling and transport, and test and laboratory location. CDC, Centers for Disease Control and Prevention; IDPB, Infectious Diseases Pathology Branch; KEMRI, Kenya Medical Research Institute.

H&E-stained slides were prepared. The sequentially cut and stained slides were made available to four sources: KNH/UoN, Massachusetts General Hospital Pathology, University of Washington Anatomic Pathology, and the US CDC, Infectious Diseases Pathology Branch. In this study, only lung histology was evaluated as described below; diagnoses from the other institutions and results from the main PRESS study in determining CoD are reported separately.[18]

Two pathologists (C.L.F. and D.J.R.) independently reviewed the H&E-stained lung histology slides from the MITS and CA samples from each death unlinked from the case (therefore unpaired) and without knowledge of the source or any pathologic, histologic, or molecular microbiology findings. The slides were reviewed independently by each pathologist.

An important aspect to determining the utility of blind MITS is the adequacy of the procedure in obtaining specimens. Correct tissue targeting is thus a significant issue that must be evaluated and involves two separable variables. First, the tissue targeted must actually be sampled in a volume adequate to make a diagnosis, which we term *targeting for adequacy*. Second, the tissue sampled must contain the pathologically diagnostic findings, which we term *targeting for specificity*.

After independent pathology review, a consensus histologic diagnosis was reached between the two pathologists for both the MITS and CA specimens. Histologic diagnoses were categorized by primary pathology and secondary pathology as follows: bronchopneumonia, granulomatous inflammation, diffuse alveolar damage, interstitial pneumonitis, aspiration pneumonia, specific pathogen, normal, or other (eg, pulmonary edema, hemorrhage, atelectasis). The primary pathology was considered the most significant histologic finding, most likely responsible for the respiratory symptoms/signs, and the secondary pathology was considered contributory but not the most significant pathology. If more than one histologic diagnosis was deemed pathologically relevant, a combination of diagnoses was considered as the primary pathology.

To control for bias related to "visual memory" (ie, bias involving a pathologist's recollection when sequentially reviewing both MITS and CA specimens from the same case), in the diagnostic interpretation of the MITS, a subset of the MITS samples was reviewed by one pathologist (C.L.F.) without review of the corresponding CA histology.

The consensus MITS histologic diagnoses were compared with the corresponding histologic diagnoses from the CA samples from the same case to determine concordance. Full concordance was present if the primary diagnosis was the same in both sampling techniques. Partial concordance in diagnosis was present if any of the diagnoses were concordant, even if the primary diagnoses in CA and MITS were not concordant.

Molecular Studies

Lung tissues and lung core needle biopsy specimens were stored in phosphate-buffered saline (PBS) and transported to the laboratory for storage at –80°C. Each of the tissue specimens was thawed and placed into

DOI: 10.1093/AJCP/AQZ016

a sterile 2-mL microcentrifuge tube containing 200 µL PBS. The tissue specimens were lysed for 2 minutes at maximum oscillation frequency using the Tissue Lyser LT (Qiagen) following the addition of the 5-mm steel lysis beads to each of the tubes according to manufacturer's instructions. After lysis, total nucleic acid extraction was done using the MagNA Pure 96 instrument and the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics), following the manufacturer's instructions. Then, 46 µL nucleic acid extract was used to load TaqMan Array Cards (TACs) as described below.

We used a polymerase chain reaction (PCR)–based respiratory TAC to test for 21 pathogens: 13 bacteria (*Bordetella pertussis*, *Chlamydia pneumoniae*, *Haemophilus influenzae* all types [pan], *Haemophilus influenzae* type b, *Klebsiella pneumoniae*, *Legionella* spp, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* [group A streptococci], and *Mycobacterium tuberculosis*), one parasite (*Pneumocystis jirovecii*), and 15 viral pathogens (adenoviruses; coronaviruses 1, 2, 3, 4; enteroviruses; human metapneumovirus; influenza A and B viruses; parainfluenza 1, 2, 3, 4; respiratory syncytial virus; and rhinoviruses). TAC is a 384-well microfluidic card with eight ports containing 48 connected wells[19] (Supplementary Figure; all supplemental materials can be found at *American Journal of Clinical Pathology* online). This design allows the simultaneous testing of 384 individual quantitative PCRs for both RNA and DNA targets. Primers and probe for each assay were preloaded and dried onto the designated wells. Each card was used to run six samples, one negative control, and one positive control. The card has three control assays, the internal positive control and two specimen quality control assays; glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH); and ribonuclease P gene (RNP3). Each of the eight lanes on the card was loaded with 50 µL 2× AgPath-ID (Thermo Fisher Scientific), 4 µL 25× enzyme mix, and 46 µL of individual total nucleic acid prior to running on an Applied Biosystems ViiA7 Real-Time PCR system (Life Technologies). Thermal cycling conditions included 45°C for 10 minutes for reverse transcription, 94°C for 10 minutes for polymerase activation, and 45 cycles of 94°C for 30 seconds (denaturation) and 60°C for 60 seconds (annealing and extension).

Real-time fluorescence data were examined manually for each TAC to confirm amplification of the positive controls and to detect anomalous baseline fluorescence. A cutoff cycle threshold (Ct) of less than 35 for positivity was set for all assays. Previous studies have shown that Ct values less than 35 can be supported by sequencing and other data.[19,20]

Specific pathogens identified by TAC using MITS and CA samples were then compared for concordance. Specimens were scored as fully concordant if the same pathogens were detected in both tissue types, partially concordant if at least one pathogen was detected in both tissue types, and discordant if the pathogens detected differed in both tissue types.

Human Immunodeficiency Virus Testing

Human immunodeficiency virus (HIV) antigen testing was done at the Kenya AIDS Vaccine Initiative laboratory on dry blood spots collected during autopsy. The Roche Amplicor HIV DNA PCR kit was used, with some modifications. Briefly, a clean handheld punch (one-fourth inch) was used to punch a disk (6 mm^2) from the dry blood spots into a 2-mL screw-cap tube, and 1 mL Roche Specimen Wash Buffer was added. DNA extraction, PCR amplification, and analysis by enzyme-linked immunosorbent assay were done per the manufacturer's recommendations. Specimens were considered unequivocally positive if they had an OD of 0.8 and negative if they had an OD less than 0.2 using an A450 filter. Specimens that had ODs higher than 0.2 but less than 0.8 were considered indeterminate and retested.

Statistical Analysis

We used proportions to describe clinical and demographic characteristics of study patients and the distribution of histologic findings (full or partial concordance or discordant findings) on tissues collected through CA and MITS techniques. We also compared TAC results on pathogen detection in CA- and MITS-collected tissue for each patient. We used logistic regression to determine demographic, clinical, and postmortem characteristics associated with concordance in histology findings and concordance in pathogen detection by TAC.

We described the number of pathogen codetections in MITS- and CA-collected tissue for each patient. Codetections were categorized by pathogen groups (ie, bacteria and viruses). We also described the weighted distribution of viral and bacterial pathogens by time of death to time of autopsy, termed *postmortem interval* (range, 0-5 days). To determine the weighted distribution by time from death to autopsy for each day, we determined the fraction of autopsies done and multiplied this fraction by the number of pathogens detected in autopsies done within that respective time interval after death. We used scatterplots to map the distribution of these pathogens by number of days from death to autopsy. We used linear regression to determine the line of best fit for these scatterplots for viral and bacterial pathogens detected in

DOI: 10.1093/AJCP/AQZ016

CA- and MITS-collected tissue and determined the gradient of each slope.

Statistical analyses were performed using Stata version 13.1 (StataCorp). *P* values less than .05 were considered statistically significant.

Ethical Considerations

The study protocol and procedures were reviewed and approved by the institutional review board at KEMRI (SSC 2692). We obtained ethical approval reliance from the institutional review board at UoN/KNH and CDC (6599). The study was registered by the KNH Research & Programs Department (registration PEADS/0014/2014). Written informed consent was sought from the child's parent/guardian in either English or Swahili as appropriate.

Results

Characteristics of Patients

From August 2014 to December 2015, we performed autopsy examination on 64 young children; 31 (48%) were male, and the median age was 7 months (interquartile range [IQR], 4-12 months). Overall, 40 (63%) children had moderate to severe malnutrition, and 15 (23%) were dehydrated. The median number of days from hospitalization to death was 2 (IQR, 1-4.5 days). All autopsies were completed within 5 days of death (one on day 0, eight on day 1, 18 on day 2, 17 on day 3, 13 on day 4, and seven on day 5), with a median of 3 days and IQR of 2 to 4 days **Table 1**.

Histology Findings

No tissues collected by MITS and CA showed any significant histologic autolysis, suggesting that the postmortem interval of up to 5 days with refrigeration had no major effect on histologic quality. Targeting for adequacy rate was 84%, with 54 of 64 MITS-collected tissues showing adequate (ie, quantity) pulmonary tissue for diagnosis **Table 2**. Of the 10 inadequate MITS samples, most were due to insufficient lung tissue for diagnosis (eight patients) and only rarely due to no pulmonary tissue obtained (two patients). The inadequate cases did not cluster around patient ages and did not correlate with experience of the pathologist doing the procedure. Twenty-eight samples included tissues other than lung obtained in the slides using MITS. These nonpulmonary tissues inadvertently biopsied included heart, liver, lymph node, adipose tissue, thymus, fibrous tissue, and skeletal muscle. The presence of nonpulmonary tissues correlated with inadequate biopsy specimens. In one case, the liver tissue present in the MITS right lung biopsy specimen revealed a new pathologic diagnosis: giant cell hepatitis (case 35).

The most common histopathologic diagnoses were bronchopneumonia, interstitial pneumonitis, and diffuse alveolar damage in both the CA and the MITS samples. Full diagnostic concordance between MITS and CA samples was seen in 23 (36%) cases. Partial concordance was seen in 19 (30%) cases. The combined full and partial concordance rate was 66% (42 of 64 cases), and the discordance rate for histologic diagnosis was 17% (11 cases) (Table 2). Discrepancies were most often due to pathology not present in the MITS but evident in the CA and did not correlate with age, sex, or nutritional status of the child or pulmonary diagnostic category identified at CA **Table 3**. The 24-sample subset of MITS biopsy specimens in which one pathologist (C.L.F.) did not review the corresponding CA specimens revealed no increase in the discrepancy rate, suggesting an absence of observer bias based on visual memory.

Molecular Findings

TAC results in CA- and MITS-collected tissues were fully concordant among 28 (44%) cases and partially concordant in 24 (36%) cases. This resulted in a full or partial concordance of 52 (80%) of 64 cases. Only 13 (20%)

Table 1

Characteristics of Autopsied Children Hospitalized With Respiratory Illness at Kenyatta National Hospital, 2014-2015 (n = 64)[a]

Characteristic	Value
Age, median (IQR), mo	7 (4-12)
Sex	
Male	31 (48)
Female	33 (52)
Nutrition status	
Wasting[b]	
Absent/mild (–2 < *z* score < +2)	24 (37)
Moderate (–3 < *z* score < –2)	14 (22)
Severe (*z* score <–3)	26 (41)
Child HIV status	
Positive	5(8)
Negative	59 (92)
Mother HIV status	
Positive	3 (5)
Negative	26 (40)
Unknown	35 (55)
Days from onset of illness to hospitalization, median (IQR)	10 (6-21)
Days from hospitalization to death, median (IQR)	2 (1-4.5)
Days from death to autopsy, median (IQR)	3 (2-4)

HIV, human immunodeficiency virus; IQR, interquartile range.
[a]Values are presented as number (%) unless otherwise indicated.
[b]Wasting: weight-for-height. A deficit (*z* score below –2) reflects moderate malnutrition, and a *z* score below –3 reflects severe malnutrition.

DOI: 10.1093/AJCP/AQZ016

Table 2

Findings and Concordance on Histology Diagnosis on Lung Tissue Specimen Collected From Children Dying of Respiratory Illness Using Conventional Autopsy (CA) to Minimally Invasive Tissue Sampling (MITS)

Case No.	Time From Death to Autopsy, d	Histology Diagnosis Based on Conventional Autopsy	Histology Diagnosis Based on Minimally Invasive Tissue Sampling	Concordance[a]
1	4	Bronchopneumonia	Bronchopneumonia	Full
2	3	Interstitial pneumonitis	Inadequate	Inadequate tissue
3	1	Aspiration	Aspiration	Full
4	1	Interstitial pneumonitis	Interstitial pneumonitis	Full
5	4	Interstitial pneumonitis, bacteria	Normal	None
6	5	Bronchopneumonia	Bronchopneumonia, interstitial pneumonitis	Full
7	2	Possible PJP, interstitial pneumonitis	Other (edema, intra-alveolar squamous cells), bacteria, possible PJP	None
8	2	Other (patchy chronic inflammation and increased intra-alveolar macrophages)	Normal	Full
9	3	DAD, possible PJP, interstitial pneumonitis	Other (atelectasis)	None
10	3	Bronchopneumonia, DAD	DAD	Partial
11	3	Bronchopneumonia, bacteria	Other (atelectasis)	None
12	2	DAD	Inadequate	Inadequate tissue
13	4	Bronchopneumonia, DAD, bacteria	Interstitial pneumonitis, bronchopneumonia	Partial
14	5	Other (hemorrhage, congestion, atelectasis)	Other (atelectasis)	Full
15	2	Interstitial pneumonitis, bronchopneumonia, bacteria	Other (edema, intra-alveolar squamous cells), bacteria	None
16	5	DAD, possible PJP, possible viral, bronchopneumonia	DAD, possible viral infection	Partial
17	4	DAD, possible PJP, CMV	DAD, possible PJP, CMV	Full
18	2	DAD, bronchopneumonia, interstitial pneumonitis	Normal	None
19	3	DAD, possible PJP	DAD, possible PJP	Full
20	4	Adenovirus, fungus, granulomatous inflammation	Fungus, granulomatous inflammation	Partial
21	2	DAD, bronchopneumonia, possible PJP	Inadequate	Inadequate tissue
22	3	DAD, possible viral, possible PJP, fungus	DAD, possible viral, fungus	Full
23	2	Interstitial pneumonitis	Inadequate	Inadequate tissue
24	3	Interstitial pneumonitis, bronchopneumonia, aspiration, bacteria	Interstitial pneumonitis, aspiration, bacteria	Partial
25	3	Other (atelectasis)	Other (atelectasis)	Full
26	1	Patchy bronchopneumonia, other	Inadequate	Inadequate tissue
27	5	Bronchopneumonia, DAD, other (atypical hematopoietic cells), interstitial pneumonitis	Interstitial pneumonitis, other (atypical hematopoietic cells)	Partial
28	4	Interstitial pneumonitis	Possible viral, interstitial pneumonitis	Full
29	1	DAD, bronchopneumonia	DAD	Partial
30	4	Other (congestion), bacteria	Other (congestion)	Partial
31	2	Bronchopneumonia	Bronchopneumonia	Full
32	4	Interstitial pneumonitis, bronchopneumonia	Interstitial pneumonitis	Partial
33	1	Interstitial pneumonitis	Normal	None
34	4	DAD, interstitial pneumonitis, possible viral pneumonia and PJP	Bronchopneumonia, possible PJP, possible viral	Partial
35	4	Bronchopneumonia	Bronchopneumonia	Full
36	2	DAD, bronchopneumonia, possible viral	Bronchopneumonia	Partial
37	3	Interstitial pneumonitis, bronchopneumonia, other (edema)	Interstitial pneumonitis	Partial
38	5	Interstitial pneumonitis, other (thrombus in large vessel)	Inadequate	Inadequate tissue
39	5	Other (atelectasis)	Inadequate	Inadequate tissue
40	3	DAD, interstitial pneumonitis, bronchopneumonia, aspiration	Bronchopneumonia, aspiration	Partial
41	4	Interstitial pneumonitis, other (peribronchiolar chronic inflammation)	Possible PJP, interstitial pneumonitis	Full
42	1	Interstitial pneumonitis, other (hemorrhage)	Interstitial pneumonitis	Full
43	3	Interstitial pneumonitis, bronchopneumonia, possible PJP or CMV	Interstitial pneumonitis, bronchopneumonia, possible PJP	Full

(continued)

DOI: 10.1093/AJCP/AQZ016

Table 2 (cont)

Case No.	Time From Death to Autopsy, d	Histology Diagnosis Based on Conventional Autopsy	Histology Diagnosis Based on Minimally Invasive Tissue Sampling	Concordance[a]
44	1	Possible viral	Normal	None
45	2	DAD, bronchopneumonia, possible measles, other (increased intra-alveolar macrophages)	Bronchopneumonia	Partial
46	4	Other (mucus plugs, edema), bacteria	Normal	Partial
47	2	DAD, bronchopneumonia, possible viral, other (edema)	DAD, possible PJP, interstitial pneumonitis	Partial
48	0	Possible PJP, interstitial pneumonitis, bronchopneumonia, possible virus, other (mucus plugs), bacteria	Interstitial pneumonitis, possible PJP	Partial
49	2	Other (congestion, hemorrhage, atelectasis, edema), interstitial pneumonitis	Normal	None
50	3	Bronchopneumonia, granulomatous inflammation, possible RSV, arteritis	Bronchopneumonia, granulomatous inflammation	Full
51	3	Bronchopneumonia, DAD, bacteria	Bronchopneumonia	Partial
52	2	Bronchopneumonia, possible aspiration, bacteria	Bronchopneumonia, possible aspiration	Full
53	1	Interstitial pneumonitis	Interstitial pneumonitis	Full
54	5	Other (atelectasis, congestion, increased intra-alveolar macrophages)	Normal	Full
55	4	Interstitial pneumonitis	Inadequate	Inadequate tissue
56	2	Focal mild bronchopneumonia, focal hemorrhage	Inadequate	Inadequate tissue
57	3	Granulomatous inflammation, possible TB	Inadequate	Inadequate tissue
58	3	Bronchopneumonia, DAD	Bronchopneumonia	Partial
59	2	Other (atelectasis increased intra-alveolar macrophages)	Other (atelectasis increased intra-alveolar macrophages)	Full
60	2	Bronchopneumonia, viral inclusions, aspiration with granulomas	Bronchopneumonia, possible viral	Full
61	3	DAD, other (edema, increased alveolar macrophages)	Other (atelectasis)	None
62	2	Bacteria	Bacteria	Full
63	3	Bronchopneumonia	Inadequate	Inadequate tissue
64	2	Other (atelectasis)	Interstitial pneumonitis	None

CMV, cytomegalovirus; DAD, diffuse alveolar damage; PJP, *Pneumocystis jiroveci* pneumonia; RSV, respiratory syncytial virus; TB, tuberculosis.
[a]Concordance defined as follows: Full, primary diagnosis was same in both MITS- and CA-collected tissues; Partial, primary diagnosis not the same in MITS- and CA-collected tissues but the other diagnoses were the same; None, diagnoses were not the same in either MITS- or CA-collected tissues; and Inadequate tissue, tissue not adequate for histologic analysis. Concordance summary: Full, n = 23 (36%); Partial, n = 19 (30%); None, n = 11 (17%); Inadequate tissue, n = 11 (17%); Concordant + partially concordant, n = 42 (66%).

cases were fully discordant **Table 4**. There was a significant association between severely malnourished bodies and concordance on TAC results (odds ratio, 12.5; 95% confidence interval, 1.43-109.64), although no other factors were statistically significantly associated with concordance (Table 3).

Detection and codetection of one (n = 28 [44%]) or two (n = 8 [13%]) bacterial pathogens were higher in MITS-collected tissue compared with CA-collected tissue (n = 21 [33%] and n = 6 [9%], respectively). There were fewer CA-collected tissues positive for bacteria (n = 35 [55%]) compared with MITS-collected tissue (n = 42 [66%]). There was a similar distribution of specimens positive for viral agents in MITS- and CA-collected tissues, n = 22 (34%) and n = 23 (37.5%), respectively (Table 4).

The most commonly detected bacterial pathogens were *K pneumoniae*, both in MITS-collected (n = 24 [38%]) and CA-collected (n = 19 [30%]) tissues. Considering MITS- and CA-associated results respectively, the other frequently detected bacterial pathogens were *H influenzae* (n = 10 [16%] and n = 12 [19%]) and *S aureus* (n = 9 [14%] and n = 8 [13%]), and viruses were respiratory syncytial virus (n = 8 [13%] and n = 8 [13%]), parainfluenza type 3 (n = 5 [8%] and n = 8 [13%]), and adenovirus (n = 7 [11%] and n = 4 [6%]). *P jiroveci* was detected in 14 (22%) MITS-collected tissue and 12 (19%) CA-collected tissue. Some bacteria considered to be frank pathogens were detected in a few cases, including *B pertussis* (n = 1), *C pneumoniae* (n = 1), *M tuberculosis* (n = 2), and *M pneumoniae* (n = 2) **Figure 2**.

DOI: 10.1093/AJCP/AQZ016

Table 3

Factors Associated With Concordance on Histology and TAC Test Results on Lung Tissues Collected Using CA and MITS Techniques From Children Dying of Respiratory Illness at Kenyatta National Hospital, Nairobi, Kenya[a]

	Histology Results Comparing CA to MITS			TAC Results comparing CA to MITS		
Characteristic	Concordance on Histology Findings (n = 43[b]), No. (%)	OR (95% CI)	*P* Value	Concordance on TAC results (n = 51[c]), No. (%)	OR (95% CI)	*P* Value
Age category, mo						
1-5	18 (86)	Reference		18 (75)	Reference	
6-11	12 (67)	0.33 (0.70-1.60)	.17	19 (83)	1.32	.71
12-59	13 (93)	2.17 (0.20-23.25)	.52	14 (82)	1.3	.75
Sex						
Male	19 (76)	Reference		24 (77)	Reference	
Female	24 (86)	1.89 (0.47-7.69)	.37	27 (82)	1.31 (0.39-4.45)	.66
Postmortem body features						
Dehydration						
No	33 (80)	Reference		38 (76)	Reference	
Yes	10 (83)	1.21 (0.22-6.66)	.77	13 (93)	4.11 (0.48-34.72)	.22
Lung congestion						
No	13 (85)	Reference		18 (90)	Reference	
Yes	30 (87)	0.58 (0.11-3.10)	.66	33 (75)	0.33 (0.07-1.67)	.11
Overt lung pathology						
No	10 (83)	Reference		12 (80)	Reference	
Yes	33 (80)	0.83 (0.15-4.53)	.97	39 (80)	0.98 (0.23-4.13)	.69
Pleural fluid						
No	31 (84)	Reference		37 (84)	Reference	
Yes	12 (75)	0.58 (0.14-2.43)	.51	14 (70)	0.44 (0.12-1.54)	.15
Nutritional status (wasting)						
Adequate (–2 < *z* score < +2)	18 (42)	Reference		16 (67)	Reference	
Moderate (–3 < *z* score < 2)	9 (21)	0.75 (0.11-5.32)	.77	10 (71)	1.25 (0.30-5.26)	.76
Severe (*z* score < –3)	16 (37)	0.53 (0.20-2.59)	.44	25 (96)	12.5 (1.43-109.64)	**.02**
HIV status						
Negative	38 (79)	—		47 (80)	Reference	
Positive	5 (100)	—		4 (80)	1.02 (0.10-10.00)	
Tissue adequacy						
Adequate	—	—		42 (79)	Reference	
Not adequate	—	—		9 (82)	1.18 (0.22-6.26)	.85

CA, conventional autopsy; CI, confidence interval; HIV, human immunodeficiency virus; MITS, minimally invasive tissue sampling; OR, odds ratio; TAC, TaqMan Array Card.

[a]Bold number represents statistical significance using the Wald test. HIV status was not considered as a covariate in the histology concordance model, as all five HIV-positive patients had concordant results.

[b]Total number with partial or full concordance on histology findings (see Table 2).

[c]Total number with partial or full concordance on TAC results (see Table 4).

Detection of bacteria but not viruses increased as the postmortem interval increased for both MITS- and CA-collected tissues **Figure 3**.

Discussion

In our study, we found an 84% targeting for adequacy rate and a 66% targeting for specificity rate. We found that the histologic diagnostic concordance (combining full and partial concordance) based on lung samples collected using MITS compared with CA was 66%. The concordance of the molecular studies based on MITS tissues compared with those from CA was 80%. Our study found that the histologic concordance between MITS and CA was not affected by age, sex, or nutritional status of the child. Nonetheless, malnutrition was associated with a higher concordance rate among pathogens detected by TAC. Tissue obtained by MITS can be used for special studies in resource-limited settings, especially when adding ancillary tests (such as microbiologic studies), which may improve the accuracy of the diagnoses.

Our study was limited to respiratory pathologies, and we did not intend to determine CoD, which limits direct comparison to other studies. Causes of discordance of CoD analysis in other studies included sampling protocol. For example, in the Huston et al study,[21] coronary

DOI: 10.1093/AJCP/AQZ016

Table 4

Concordance on TaqMan Array Card Results of Pathogens Tested in Lung Tissue Specimen Collected Using CA and MITS in Children Hospitalized With Respiratory Illness at Kenyatta National Hospital, Nairobi, Kenya, 2014-2015

Case No.	CA	MITS	Concordance[a]
1	*Klebsiella pneumoniae* (20.52) *Haemophilus influenzae*—all types (29.63) *Staphylococcus aureus* (32.88) *Streptococcus pneumoniae* (26.43/26.55) *Pneumocystis jirovecii* (34.04)	*K pneumoniae* (23.45) *H influenzae*—all types (27.75) *S aureus* (32.02) *S pneumoniae* (27.18/27.82)	Partial
2	Parainfluenza virus type 1 (32.2) *H influenzae*—all types (25.32)	Parainfluenza virus type 1 (34.69) *H influenzae*—all types (28.05) *K pneumoniae* (33.97)	Partial
3	Negative	Negative	Full
4	*K pneumoniae* (22.79)	*K pneumoniae* (23.38) Adenovirus (31.12/32.80)	Partial
5	*H influenzae*—all types (33.88) *S aureus* (30.62) *Moraxella catarrhalis* 31.79/33.73) *P jirovecii* (27.21) Human metapneumovirus (31.99/31.63)	*H influenzae*—all types (32.32) *S aureus* (32.72) *M catarrhalis* (34.41/Indeterminate) *P jirovecii* (29.02) Human metapneumovirus (31.99/31.63) *K pneumoniae* (33.3)	Partial
6	*K pneumoniae* (29.58) *H influenzae*—all types (29.17) *M catarrhalis* (28.05/29.37)	*K pneumoniae* (29.23) *H influenzae*—all types (30.41) *M catarrhalis* (30.49/30.26)	Full
7	*K pneumoniae* (19.11)	Negative	None
8	Negative	*K pneumoniae* (27.22)	None
9	*K pneumoniae* (31.85)	*K pneumoniae* (26.12)	Full
10	Negative	*K pneumoniae* (29.4)	None
11	*H influenzae*—all types (30.8)	*K pneumoniae* (32.23)	None
12	Negative	Negative	Full
13	*K pneumoniae* (28.71)	*K pneumoniae* (26.14)	Full
14	Negative	Negative	Full
15	Human metapneumovirus (29.80/29.99) *K pneumoniae* (30.42)	Human metapneumovirus (30.25/30.61) *K pneumoniae* (32.04)	Full
16	*P jirovecii* (30.75)	*P jirovecii* (29.81)	Full
17	*P jirovecii* (21.07) Respiratory syncytial virus (30.71/31.30)	*P jirovecii* (19.65) Respiratory syncytial virus (30.37/32.44) *S aureus* (34.37)	Partial
18	Negative	*P jirovecii* (32.4)	None
19	*P jirovecii* (18.19) *Bordetella pertussis* (33.19/32.76)	*P jirovecii* (20.62) *B pertussis* (34.87/35.18)	Full
20	Adenovirus (19.89/20.28) *Pseudomonas aeruginosa* (30.62)	Adenovirus (19.14/18.65) *P aeruginosa* (27) *P jirovecii* (33.72)	Partial
21	Human metapneumovirus (22.71/22.94) *P aeruginosa* (32.7)	Human metapneumovirus (23.67/23.29) *S aureus* (33.16) *P aeruginosa* (29.52)	Partial
22	Adenovirus (23.88/24.84) Human metapneumovirus (30.81/30.62)	Adenovirus (25.74/25.61) Human metapneumovirus (26.42/27.04) *P jirovecii* (29.03) *S aureus* (32.58)	Partial
23	Negative	Negative	Full
24	Parainfluenza virus type 3 (22.57)	Parainfluenza virus type 3 (24.47)	Full
25	*H influenzae*—all types (32.98)	Negative	None
26	*S aureus* (29.24) Parainfluenza virus type 3 (33.86)	*S aureus* (32.94) *P aeruginosa* (31.25)	Partial
27	Respiratory syncytial virus (22.40/22.38) *P aeruginosa* (30.41) *S aureus* (30.19)	Respiratory syncytial virus (19.48/19.72) *P aeruginosa* (27.52) *S aureus* (31.56)	Full
28	Respiratory syncytial virus (23.39/23.39) *P aeruginosa* (34.69) *P jirovecii* (29.44) *S aureus* (34.58)	Respiratory syncytial virus (26.90/25.08) *P jirovecii* (31.56) *S aureus* (34.87)	Partial

(continued)

DOI: 10.1093/AJCP/AQZ016

Table 4 (cont)

Case No.	CA	MITS	Concordance[a]
29	Respiratory syncytial virus (16.66/16.99) *K pneumoniae* (19.86)	Respiratory syncytial virus (18.57/18.57) *K pneumoniae* (34.26)	Full
30	*K pneumoniae* (31.18) Respiratory syncytial virus (34.81/Indeterminate)	*K pneumoniae* (28.88) Respiratory syncytial virus (34.15/33.55)	Partial
31	Negative	Negative	Full
32	*M catarrhalis* (29.92/Indeterminate) *S pneumoniae* (31.39/31.91) *S aureus* (34.65) *K pneumoniae* (32.32) *P aeruginosa* (31.95)	 *S pneumoniae* (34.18/34.50) *K pneumoniae* (18.68) *P aeruginosa* (29.83)	Partial
33	*P jirovecii* (29.99)	*P jirovecii* (29.52)	Full
34	*P jirovecii* (25.49)	*P jirovecii* (20.2)	Full
35	*K pneumoniae* (30.43)	*K pneumoniae* (25.51) *P jirovecii* (32.75)	Partial
36	Negative	*K pneumoniae* (26.85)	None
37	*H influenzae*—all types (26.65) *M catarrhalis* (31.75/30.52) *K pneumoniae* (23.74)	*H influenzae*—all types (26.75) *M catarrhalis* (30.66/30.60) *K pneumoniae* (25.81)	Full
38	*K pneumoniae* (30.12) *S aureus* (31.49)	*K pneumoniae* (29.38)	Partial
39	Negative	*K pneumoniae* (30.25)	None
40	*S pneumoniae* (32.24/31.52) *P jirovecii* (39.68)	*S pneumoniae* (33.15/33.55)	Full
41	Respiratory syncytial virus (24.31/24.90)	Respiratory syncytial virus (24.94/24.91) CP (33.05/34.32)	Partial
42	Negative	Negative	Full
43	*K pneumoniae* (28.89) *P jirovecii* (23.43)	*K pneumoniae* (26.22) *P jirovecii* (22.21)	Full
44	Respiratory syncytial virus (31.15/32.03)	Respiratory syncytial virus (34.26/Indeterminate)	None
45	Respiratory syncytial virus (15.17/15.53) Parainfluenza virus type 3 (29.93)	Respiratory syncytial virus (11.72/12.77) Parainfluenza virus type 3 (33.19)	Full
46	Negative	Negative	Full
47	Influenza virus type A (16.19/15.85) Parainfluenza virus type 3 (33.46)	Influenza virus type A(18.49/18.10)	Partial
48	HCV4 (25.17) *H influenzae*—all types (23.49) Parainfluenza virus type 3 (26.29) *P jirovecii* (30.85)	HCV4(27.07) *H influenzae*—all types (25.75) Parainfluenza virus type 3 (26.91) *P jirovecii* (28.99)	Full
49	Parainfluenza virus type 3 (34.85)	Negative	None
50	*K pneumoniae* (29.64) *Mycobacterium tuberculosis* (31.14/29.54) *P jirovecii* (33.85)	*K pneumoniae* (32.35) *M tuberculosis* (29.85/29.73)	Partial
51	Negative	*Mycoplasma pneumoniae* (31.61/31.19)	None
52	*M catarrhalis* (30.16/30.97) *H influenzae*—all types (32.86) Adenovirus (35.37/34.73) *K. pneumoniae* (31.99)	*M catarrhalis* (26.14/26.11) *H influenzae*—all types (28.51) Adenovirus (35.65/33.25)	Partial
53	*K pneumoniae* (34.06)	*K pneumoniae* (31.05)	Full
54	Rhinovirus (31.37)	 *S aureus* (31.03)	None
55	*S pneumoniae* (31.41/31.46) *H influenzae*—all types (29.73) *M pneumoniae* (29.82/29.86) Rhinovirus (29.58) *S aureus* (31.32)	*S pneumoniae* (33.62/34.12) *H influenzae*—all types (32.5) *M pneumoniae* (32.07/32.36)	Partial
56	Negative	Negative	Full
57	*M tuberculosis* (31.07/30.94) *K pneumoniae* (28.03)	*M tuberculosis* (32.49/Indeterminate) Adenovirus (35.42/33.78)	None
58	*S pneumoniae* (33.84/34.87) *M catarrhalis* (32.45/32.02) *H influenzae*—all types (30.96)	 *M catarrhalis* (32.44/32.74) *H influenzae*—all types (31.89)	Partial

(continued)

DOI: 10.1093/AJCP/AQZ016

Table 4 (cont)

Case No.	CA	MITS	Concordance[a]
59	Negative	Negative	Full
60	Adenovirus (21.29/20.52) Parainfluenza virus type 3 (31.27)	Adenovirus (20.81/21.66) Parainfluenza virus type 3 (31.53)	Partial
61	*P jirovecii* (23.61)	*P jirovecii* (28.33)	Full
62	*K pneumoniae* (24.01)	*K pneumoniae* (23.85)	Partial
63	Parainfluenza virus type 3 (18.95) *K pneumoniae* (28.97)	Parainfluenza virus type 3 (18.5) *K pneumoniae* (28.7)	Full
64	*H influenzae*—all types (33.55)	*H influenzae*—all types (30.63) Adenovirus (33.69/34.25)	Partial

CA, conventional autopsy; MITS, minimally invasive tissue sampling.
[a]Concordance defined as follows: Full, identical pathogens detected in both tissue types (CA and MITS); Partial, at least one identical pathogen detected in both tissue types; None, different pathogens detected in both tissue types. Concordance summary: Full, n = 28 (44%); Partial, n = 23 (36%); and None, n = 13 (20%).

artery disease or bony metastasis could not be diagnosed by the MITS as biopsy specimens of these tissues were not included in their protocol. We believe that determination of CoD using MITS techniques alone can be limited, as not all tissues/organs are examined and affected areas could be missed, which is not the case when using CA. Focused autopsy questions may be more amenable to the MITS technique, unless multiple specimens collected from various organs can be done using this technique.

To our knowledge, only three publications have compared blind MITS with CA in terms of targeting for specificity. Garg et al[12] studied cause of death in 25 neonates in India, with a median age between 3.5 and 4 months. This India-based study found full concordance in 56% and partial concordance in an additional 12% (68%), with a 32% discordance rate. This compares well with our results of a 66% concordance rate (full and partial). Unlike the Garg et al[12] study, which targeted the lungs from the upper portion of the midaxillary line, we used the supraclavicular, aiming the needle inferiorly, and midclavicular line, aiming dorsally, and combined these specimens. Another blind MITS study with CA specificity validation is the study by Huston et al[21] of 20 adult deaths that reported a 60% to 67% confirmation of the MITS-determined CoD by the CA. A higher confirmation rate (75%) was described for major diagnoses.

The third study comparing blind MITS to CA was the largest and most recently published.[22,23] In this Uganda-based study, Cox et al[22] enrolled 96 HIV-positive adult patients for blind MITS followed by a CA. They found diagnostic concordance rates of only 46%, citing targeting as the likely reason for discordance. The addition of an ultrasound-directed MITS improved the diagnostic concordance only modestly, from 46% to 52%. Other directed MITS studies have found a significantly higher accuracy rate/concordance rate. In a study using a "keyhole" technique (magnetic resonance imaging and endoscopic examination), the adequacy rate was 90%.[24] In a study of a directed approach using ultrasonography to identify organ and pathology location, accuracy was only 67% for kidneys but 100% for lung.[16]

With imaging or endoscopic targeting for adequacy, published diagnostic accuracy rates range from 77%[8] to greater than 90%.[24,25] A recent publication in children, neonates, and stillbirths in a resource-limited setting had accuracy rates greater than 80%.[15] Although improved targeting accuracy increases the diagnostic accuracy of the procedure, the use of the directed MITS in resource-limited regions may be impractical for large deployment. The paucity of centers with mobile imaging technologies (eg, ultrasonography) or access to computed tomography, magnetic resonance imaging, or endoscopy obviates use of these techniques. Without them, the accuracy may fall to approximately 60% to 70%. These low accuracy rates need to be considered when interpreting data obtained by MITS procedures. Methods to improve accuracy that are affordable and available in the field and in resource-limited regions are needed.[6]

TAC detected a number of potential pathogens in the lung tissues, and overall, there was general concordance between the CA and MITS specimens for molecular microbiology. More bacterial agents were detected than viral agents, and many of these bacteria could represent contamination of the tissue or bacterial overgrowth following death, which is a limitation of all autopsy microbiologic studies, but in our study, this was more pronounced with MITS. The most frequently detected bacteria were organisms that could represent flora normally present in the upper respiratory tract. Conversely, agents like *M tuberculosis* likely reflect current or past infection. Viral agents detected from MITS and CA samples showed more consistency. However, it is unclear (based on TAC findings) whether detected viruses could be associated with respiratory illness etiology without thorough investigation of the histopathology of affected tissue and the clinical and other systems' pathologic context. In some cases, TAC results were important to confirm or refute the suspected organism identified by MITS histology alone. For example, in case 16, the MITS histology suggested a viral pathology,

DOI: 10.1093/AJCP/AQZ016

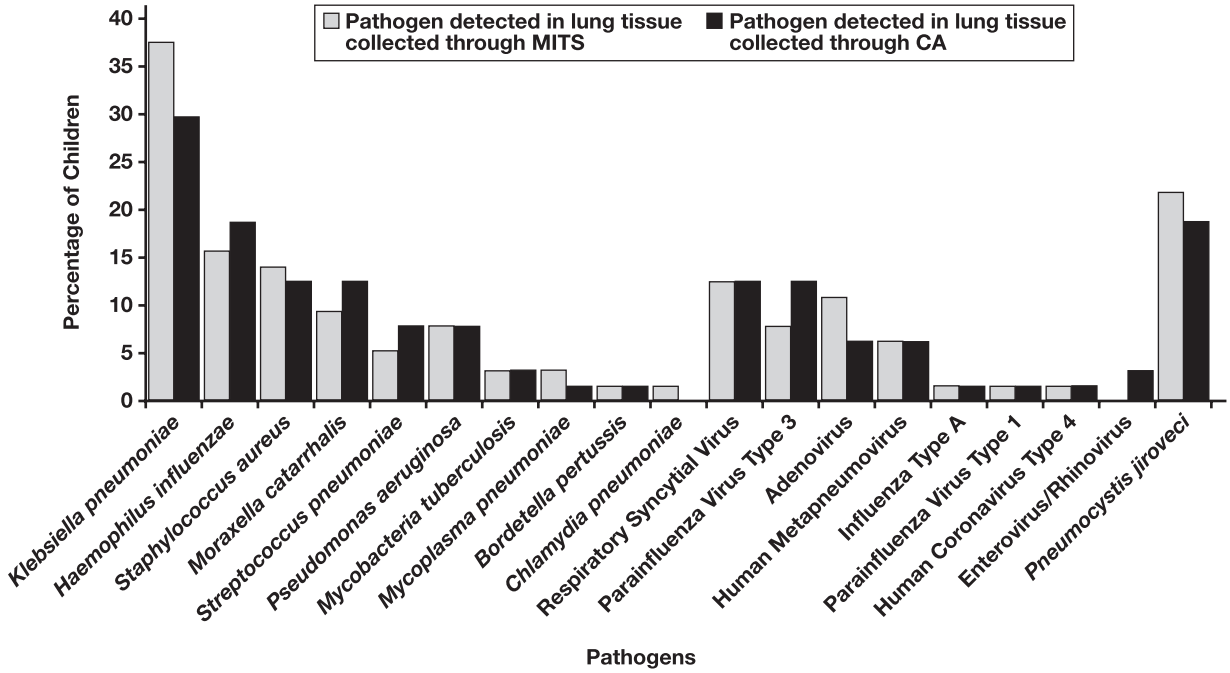

Figure 2 Distribution of pathogens detected using TaqMan Array Card in tissues collected through conventional autopsy (CA) and minimally invasive tissue sampling techniques (MITS) in children younger than 5 years hospitalized with respiratory illness in Kenya from 2014 to 2015.

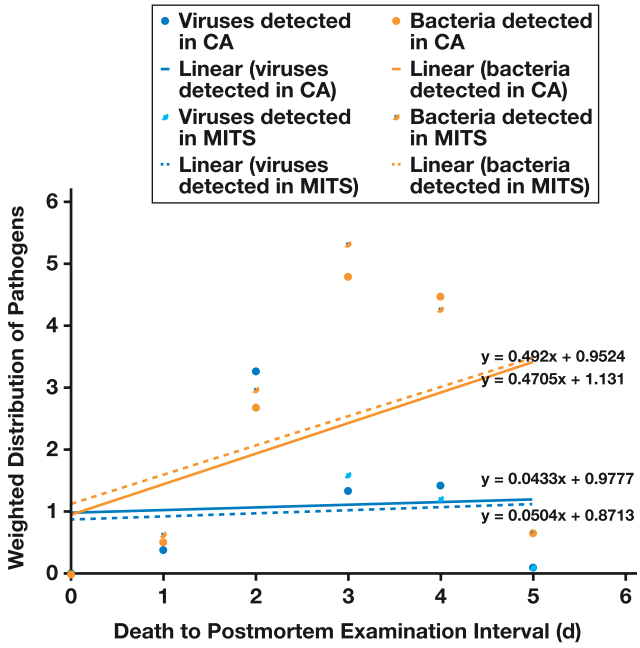

Figure 3 Distribution of viral and bacterial pathogens detected using TaqMan Array Card (TAC) weighted by number of autopsies done over the 5 days from death to postmortem examination. CA, conventional autopsy; MITS, minimally invasive tissue sampling.

but TAC confirmed *P jiroveci* pneumonia (PJP). In case 17, MITS histology was suspicious for a potential role of cytomegalovirus (CMV) or PJP, and TAC confirmed PJP, although CMV could not be diagnosed by TAC since it was not one of the targets on the card. We see utility in the combined diagnostic potential of histology by MITS and special studies using molecular diagnostic tools like TAC, although interpretation of a definitive diagnosis in infectious respiratory pathologies is challenging.

Another important factor of MITS that should be discussed is the infrastructure necessary to do this work. We found that in our study setting, the morgue team, the pathologists, and the laboratory technicians were all able to conduct study procedures after training. The MITS was not excessively burdensome, taking on average less than 1 hour to complete. The histology laboratory produced well-processed, good-quality H&E-stained slides from the tissues they received. Tissues for molecular studies were properly handled, and reliable results were obtained. However, even in the setting of a MITS procedure instead of a CA, reliable results require adequate laboratory facilities, durable and disposable equipment, reagents, and trained personnel necessary for postmortem examination and the requisite tissue processing. This infrastructure may be available only in certain locations in resource-limited countries.

DOI: 10.1093/AJCP/AQZ016

The strengths of our study include the relatively large number of pediatric lung tissues studied. Targeting biopsy specimens by imaging was not possible by the design of this study but would be useful to improve accuracy, as it can ensure adequate biopsy material obtained from organs with focal pathologies.

In conclusion, we found that a blind approach to a MITS designed to investigate respiratory pathologies in low-resource settings achieved concordance with the CA in 66% of cases. The biopsy tissue via MITS used for molecular and microbiologic studies[14,17,26] could improve the clinical utility and diagnostic accuracy of the procedure, especially when considering targeted organs. The utility of the blind MITS for molecular microbiologic studies was confirmed in our study with an important caveat that tissue adequacy and specificity should be kept in mind when interpreting results.

Corresponding author: Drucilla J. Roberts, MD; djroberts@mgh.harvard.edu.

Acknowledgments: We thank the families of all the deceased children involved in this study. We acknowledge the many who supported this work clinically (nurses and house staff) and technically (mortuary attendants) and the surveillance officers involved in data collection. We also thank the histology staff of the University of Nairobi and the CDC, as their work was critical to the success of the project.

This work was supported by the US Centers for Disease Control and Prevention (cooperative agreement GH002133). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

1. Nashelsky MB, Lawrence CH. Accuracy of cause of death determination without forensic autopsy examination. *Am J Forensic Med Pathol.* 2003;24:313-319.
2. Turner EL, Metcalfe C, Donovan JL, et al. Contemporary accuracy of death certificates for coding prostate cancer as a cause of death: is reliance on death certification good enough? A comparison with blinded review by an independent cause of death evaluation committee. *Br J Cancer.* 2016;115:90-94.
3. Akakpo PK, Awuku YA, Derkyi-Kwarteng L, et al. Review of errors in the issue of medical certificates of cause of death in a tertiary hospital in Ghana. *Ghana Med J.* 2017;51:30-35.
4. Hazard RH, Chowdhury HR, Adair T, et al. The quality of medical death certification of cause of death in hospitals in rural Bangladesh: impact of introducing the international form of medical certificate of cause of death. *BMC Health Serv Res.* 2017;17:688.
5. Oluwasola OA, Fawole OI, Otegbayo AJ, et al. The autopsy: knowledge, attitude, and perceptions of doctors and relatives of the deceased. *Arch Pathol Lab Med.* 2009;133:78-82.
6. Bassat Q, Ordi J, Vila J, et al. Development of a post-mortem procedure to reduce the uncertainty regarding causes of death in developing countries. *Lancet Glob Health.* 2013;1:e125-e126.
7. Kean S. Cause of death. *Science.* 2015;347:1410-1413.
8. Weustink AC, Hunink MG, van Dijke CF, et al. Minimally invasive autopsy: an alternative to conventional autopsy? *Radiology.* 2009;250:897-904.
9. Bassat Q, Castillo P, Martínez MJ, et al. Validity of a minimally invasive autopsy tool for cause of death determination in pediatric deaths in Mozambique: an observational study. *PLoS Med.* 2017;14:e1002317.
10. Ben-Sasi K, Chitty LS, Franck LS, et al. Acceptability of a minimally invasive perinatal/paediatric autopsy: healthcare professionals' views and implications for practice. *Prenat Diagn.* 2013;33:307-312.
11. Denzer UW, von Renteln D, Lübke A, et al. Minimally invasive autopsy by using postmortem endoluminal and transluminal endoscopy and EUS. *Gastrointest Endosc.* 2013;78:774-780.
12. Garg S, Punia RP, Basu S, et al. Comparison of needle autopsy with conventional autopsy in neonates. *Fetal Pediatr Pathol.* 2009;28:139-150.
13. Rüegger CM, Bartsch C, Martinez RM, et al. Minimally invasive, imaging guided virtual autopsy compared to conventional autopsy in foetal, newborn and infant cases: study protocol for the paediatric virtual autopsy trial. *BMC Pediatr.* 2014;14:15.
14. van der Linden A, Blokker BM, Kap M, et al. Post-mortem tissue biopsies obtained at minimally invasive autopsy: an RNA-quality analysis. *PLoS One.* 2014;9:e115675.
15. Menendez C, Castillo P, Martínez MJ, et al. Validity of a minimally invasive autopsy for cause of death determination in stillborn babies and neonates in Mozambique: an observational study. *PLoS Med.* 2017;14:e1002318.
16. Castillo P, Ussene E, Ismail MR, et al. Pathological methods applied to the investigation of causes of death in developing countries: minimally invasive autopsy approach. *PLoS One.* 2015;10:e0132057.
17. Castillo P, Martínez MJ, Ussene E, et al. Validity of a minimally invasive autopsy for cause of death determination in adults in Mozambique: an observational study. *PLoS Med.* 2016;13:e1002171.
18. Njuguna HN, Zaki SR, Roberts DJ, et al. Determining the cause of death among children hospitalized with respiratory illness in Kenya: protocol for Pediatric Respiratory Etiology Surveillance Study (PRESS). *JMIR Res Protoc.* 2019;8:e10854.
19. Liu J, Ochieng C, Wiersma S, et al. Development of a TaqMan array card for acute-febrile-illness outbreak investigation and surveillance of emerging pathogens, including Ebola virus. *J Clin Microbiol.* 2016;54:49-58.
20. Kodani M, Yang G, Conklin LM, et al. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. *J Clin Microbiol.* 2011;49:2175-2182.
21. Huston BM, Malouf NN, Azar HA. Percutaneous needle autopsy sampling. *Mod Pathol.* 1996;9:1101-1107.
22. Cox JA, Lukande RL, Kalungi S, et al. Needle autopsy to establish the cause of death in HIV-infected hospitalized adults in Uganda: a comparison to complete autopsy. *J Acquir Immune Defic Syndr.* 2014;67:169-176.

DOI: 10.1093/AJCP/AQZ016

23. Cox JA, Lukande RL, Kalungi S, et al. Practice of percutaneous needle autopsy: a descriptive study reporting experiences from Uganda. *BMC Clin Pathol.* 2014;14:44.
24. Sebire NJ, Weber MA, Thayyil S, et al. Minimally invasive perinatal autopsies using magnetic resonance imaging and endoscopic postmortem examination ("keyhole autopsy"): feasibility and initial experience. *J Matern Fetal Neonatal Med.* 2012;25:513-518.
25. Catheline JM, Turner R, Guettier C, et al. Autopsy can be performed laparoscopically. *Surg Endosc.* 1999;13:1163-1164.
26. Martínez MJ, Massora S, Mandomando I, et al. Infectious cause of death determination using minimally invasive autopsies in developing countries. *Diagn Microbiol Infect Dis.* 2016;84:80-86.

DOI: 10.1093/AJCP/AQZ016