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Diagnostic Accuracy of Xpert MTB/RIF for Extrapulmonary Tuberculosis Specimens: Establishing a Laboratory Testing Algorithm for South Africa

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South Africa implemented Xpert MTB/RIF as the initial diagnostic test for pulmonary tuberculosis (TB). Xpert MTB/RIF's accuracy for diagnosing extrapulmonary tuberculosis (EPTB) was investigated. EPTB specimens ($n = 7,916$) from hospitalized patients received over a 6-month period at a high-throughput TB referral laboratory in Johannesburg were investigated. Large-volume specimens were centrifuged, tissue biopsy specimens homogenized, and all specimens checked for growth of contaminating bacteria on blood agar. Contaminated samples received NALC-NaOH (*N*-acetyl-L-cysteine–sodium hydroxide) decontamination prior to liquid culture. Residual specimens (volumes > 1 ml) after inoculation of culture ($n = 1,175$) were tested using the Xpert MTB/RIF sputum protocol. Using culture as the reference, Xpert MTB/RIF's overall sensitivity was 59% (95% confidence interval [95% CI], 53% to 65%) and specificity was 92% (CI, 90% to 94%), with the highest sensitivities of 91% (95% CI, 78% to 97%) for pus, 80% (95% CI, 56% to 94%) for lymph node aspirates, and 51% (95% CI, 44% to 58%) for fluids (ascitic, 59%; pleural, 47%). A difference in sensitivities was noticed between specimens classified as having a thick (87% [95% CI, 76% to 94%]) versus clear (watery) (48% [95% CI, 36% to 61%]) appearance. This was unchanged with traces of blood (52% [95% CI, 44% to 60%]) or precentrifugation (57% [95% CI, 28% to 82%]) among clear specimens. Xpert MTB/RIF generated an additional 124 specimen results that were contaminated by Mycobacterial Growth Indicator Tubes (MGIT; 10.5%) and diagnosed rifampin (RIF) resistance earlier (9.6% [25/260]). Xpert MTB/RIF's performance on EPTB specimens provides very promising results and should be considered for incorporation into national TB guidelines. Xpert MTB/RIF is less affected by contaminating bacteria and reduces laboratory labor and diagnostic delay compared to traditional methods.

Evidence from 138 studies published before 2008 suggested that nucleic acid amplification technologies (NAAT) could not replace conventional mycobacterial tests (microscopy, culture) for diagnosing pulmonary and, especially, extrapulmonary tuberculosis (EPTB) (1). Only a few years later, GeneXpert technology (2) has changed this paradigm, with a recent systematic review showing pooled sensitivity of 88% and pooled specificity of 98% (3) for diagnosis of pulmonary TB, but evidence (as of March 2012) for using Xpert MTB/RIF for diagnosing EPTB is still comparatively weak (4). Globally, there is still a dearth of studies involving the use of Xpert MTB/RIF in EPTB specimens, and few provide definitive answers. This is due mostly to the studies having small sample sizes across a range of various specimen types and differences in preprocessing methodologies and in input volumes and to studies having been conducted in different populations (adults, children, HIV infected). In one large published study, the overall sensitivity of Xpert MTB/RIF on tissue biopsy specimens/fine-needle aspirates (FNA), pleural fluid, gastric aspirates, pus, cerebrospinal fluid (CSF), urine, and peritoneal/synovial/pericardial fluid was reported as 81% (95% confidence interval [95% CI], 76% to 86%), with specificity of 99.8% (95% CI, 99% to 100%) (5). Other studies (3, 6–15) showed sensitivity ranges of 25% to 97% and specificity ranges of 89% to 100%.

In 2011, the prevalence of pulmonary TB in South Africa was 768/100,000 (95% CI, 399 to 1,250), with an incidence of 993/100,000 (95% CI, 819 to 1,180). There were 325,321 new pulmonary TB cases and 47,285 (15%) new EPTB cases reported, with the latter disease manifestation contributing to considerable morbidity, mortality, and diagnostic cost (16). Management of EPTB disease therefore needs to play a more prominent role in the National Tuberculosis Control Programmes, especially in high-HIV-burden settings (17). Xpert MTB/RIF has been nationally implemented in South Africa throughout smear microscopy laboratories as a replacement for smear microscopy for initial diagnosis of pulmonary TB. Three studies from the region on the performance of Xpert on EPTB specimens have to date focused only on pleural TB (8), TB lymphadenitis (18), and HIV-associated lymph node TB (LNTB) (19) in specific settings. The latter study performed Xpert MTB/RIF testing at the point of care at a large clinic, showing that all Xpert MTB/RIF-positive patients initiated treatment within 1 day and that Xpert MTB/RIF, with a sensitivity of 95%, could be endorsed as the initial diagnostic for HIV-associated LNTB (19). We there-

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fore investigated the use of Xpert MTB/RIF for diagnosing tuberculosis on all nonrespiratory specimens routinely received in a busy routine testing laboratory with the aim of establishing the most appropriate laboratory testing algorithm for South Africa with the current knowledge base and availability.

MATERIALS AND METHODS

Laboratory site and specimen receipt. The study was performed in the National Health Laboratory Service Mycobacteriology Referral Laboratory in Johannesburg, which provides a routine diagnostic service to public sector hospitals and clinics in 4 of 6 districts of the Gauteng province of South Africa. The laboratory processes on average 600 specimens daily for TB culture using Mycobacterial Growth Indicator Tubes (MGIT; Becton, Dickinson). On average, 80% of the specimens received in this laboratory are respiratory specimens, 10% blood specimens, and 10% nonrespiratory specimens. The majority of EPTB specimens are obtained from hospitalized patients. The EPTB specimens submitted for routine mycobacterial culture between 21 August 2012 and 21 February 2013 were evaluated in this study. This was purely a laboratory-based study, and no patient demographics were recorded other than those provided by the requesting clinician such as age, gender, and location. The HIV status was unknown as well as the results of any sputum or clinical follow-up testing. Ethics clearance for the use of residual specimens for laboratory evaluations was obtained through the University of the Witwatersrand Human Ethics Committee (approval no. M120875).

Laboratory processing of EPTB specimens. EPTB specimens from both children and adults were included in the study. Specimen contamination with bacteria was determined or excluded by plating all specimens onto a sheep blood agar plate and incubating at 37°C for 24 h. Nonsterile specimens underwent decontamination with NALC-NaOH (*N*-acetyl-L-cysteine–sodium hydroxide) to a final concentration of 1%, with the addition of phosphate buffer according to the manufacturer's instructions. Fluid specimens > 20 ml in volume were centrifuged before inoculation of 0.5 ml of the pellet into MGIT. Tissue and biopsy specimens were homogenized in 0.5 ml phosphate buffer before inoculation of 0.5 ml into MGIT culture media. If smear microscopy was requested, it was performed prior to liquid culture using concentrated auramine fluorescence microscopy. Growth of *Mycobacterium tuberculosis* complex was confirmed by Ziehl-Neelsen (ZN) staining. Definitive identification of the acid-fast bacilli (AFB) observed by ZN staining was performed using GenoType MTBDR*plus*v1 (Hain Lifescience, Nehren, Germany) (if drug susceptibility testing [DST] was requested by the clinician) or using a GenoType Mycobacterium CM line probe assay (Hain Lifescience) or the MPT64 antigen immunoassay (SD Bioline) if DST was not requested. If resistance was detected by the MTBDR*plus*v1 assay, further drug susceptibility testing by the MGIT proportion method (Becton, Dickinson) was performed. After routine processing and inoculation into MGIT, the residual specimen (1 ml unconcentrated) was tested by Xpert MTB/RIF per the manufacturer's instructions using sample reagent (SR). SR was added in a 2:1 ratio with 1 ml residual specimens that were not decontaminated. The residual volumes were documented as well as the macroscopic appearance of the specimen.

Data analysis. All data were entered into MS Excel and analyzed using STATA v.12. Liquid culture was considered the reference standard for sensitivity and specificity calculations. These calculations were performed on the total sample size as well as for individual specimen types. Confidence intervals (CI) at 95% were reported for the latter analyses, and overlapping CI data were regarded as showing no significant difference between the results determined for the corresponding sample types.

RESULTS

Description of routine EPTB specimens received and tested using Xpert MTB/RIF. A total of 7,916 EPTB specimens were received in the laboratory within the 6-month period and categorized as listed in column 2 of Table 1 according to what was inscribed by health care providers on the labels. Cerebrospinal fluid (CSF) accounted for 34% of samples, followed by 32% accounted for by fine-needle aspirates [FNA] (the majority of lymph nodes) and 25% accounted for by fluids (59% pleural, 17% ascitic, and 23% miscellaneous fluids). The remaining samples comprised pus (5%) and a miscellaneous 3% comprising bone, urine, dialysis fluid, skin scrapings, stool, tissue biopsy specimens, and catheter tips. This analysis focused on all specimen types except those identified as FNA, as this was previously reported from this region (19). The total number of specimens with sufficient residual specimen volume for Xpert MTB/RIF testing was 1,175 (as listed in column 3 of Table 1) and included 84 aspirates not labeled as FNA but rather as lymph node aspirates (LNA). The residual specimens were from patients ranging in age from <1 year to 96 years (median age of 39 years, with 43 samples from children <15 years of age); 55% were from males. Only 3% of CSF specimens had sufficient residual volume for Xpert testing.

TABLE 1 EPTB specimens received within 6 months and those with residual volumes for XpertMTB/RIF testing

Specimen type	Frequency received in 6 mos, *n* (%)	Frequency with residual vol tested by Xpert, *n* (%)
Cerebrospinal fluid	2,719 (34)	37 (3)
Fine-needle aspriate (mostly lymph node)	2,536 (32)	84 (7)[a]
Fluid (pleural, ascitic, other)	2,008 (25)	890 (76)
Pus	417 (5)	119 (10)
Tissue biopsy	184 (2)	31 (3)
Dialysis fluid or urine	35 (0.4)	10 (1)
Scrapings	7 (0.1)	0
Bone	5 (0.1)	4 (0.3)
Stool	3 (0.1)	0
Catheter tip	2 (0.3)	0
Total *n*	7,916	1,175

[a] Values represent only those labeled specifically as LNA (not as the majority labeled as FNA) where included in the analysis.

Of all the 1,175 specimens plated on sheep agar plates, 36 (3%) were shown to be contaminated with bacteria and followed NALC-NaOH processing. This was not specific to any one specimen type; however, 30.5% (11/36) were still contaminated on MGIT culture, and Xpert MTB/RIF testing was successful for all of these specimens. Overall, 10.5% (124/1,175) of MGIT cultures were contaminated, with the majority (58%, 72/124) being fluids that were thick (86%, 62/72). Of the MGIT-contaminated specimens, all (100%, 124/124) generated Xpert MTB/RIF results, of which 28% (35/124) were *M. tuberculosis* positive. Xpert MTB/RIF could not generate results in 0.5% (6/1,175) due to 5011 errors (signal loss due to loss of tube pressure).

Performance of Xpert MTB/RIF compared to MGIT culture. Table 2 describes the accuracy of Xpert MTB/RIF using mycobacterial culture as the reference standard for the various sample types and among the 1,175 specimens. *M. tuberculosis* positivity determined by Xpert MTB/RIF was 22% (260/1,175) compared to 23.5% (277/1,175) by MGIT culture. The sensitivity and specificity of Xpert MTB/RIF versus MGIT culture on 1,045 specimens by both methodologies were 59% (95% CI, 53% to 65%) and 92% (95% CI, 90% to 94%), respectively. Xpert MTB/RIF had the

TABLE 2 Xpert assay performance

Specimen category and type[a]	No. of specimens tested	No. (%) of specimens classified as:						No. of specimens with definitive results confirmed by reference culture	% Xpert sensitivity (95% CI)	% Xpert specificity (95% CI)
		M. tuberculosis positive		*M. tuberculosis* negative		Contaminated/error				
		MGIT culture	Xpert	MGIT culture	Xpert	MGIT culture	Xpert			
All	1,175	277 (23.5)	260 (22)	774 (65.9)	909 (77.4)	124 (10.5)	6 (0.5)	1,045	59 (53–65)	92 (90–94)
Pus	119	43 (36)	62 (52)	49 (41)	62 (52)	27 (23)		92	91 (78–97)	76 (60–85)
Aspirate	84	20 (23.8)	30 (46)	55 (65.5)	54 (64)	9 (10.7)		75	80 (56–94)	78 (65, 88)
Fluid	890	206 (23)	159 (18.3)	612 (69)	725 (81)	72 (8)	6 (0.7)	812	51 (44–58)	94 (92, 96)
Other										
CSF	37	4 (11)	3 (8)	32 (87)	34 (92)	1 (3)				
Tissue biopsy	31	3 (10)	5 (16)	19 (61)	26 (84)	9 (29)				
Urine	10	1		4	10	5				
Bone	4		1	3	3	1				
Fluids										
Plueral	528							482	47 (38–56)	94 (91–96)
Ascitic	153							139	59 (41–76)	97 (92–99)
Other	209							191	54 (39–69)	92 (87–96)

[a] Fluids considered as a group and fluid types examined individually reflect data from the same specimens.

highest sensitivity on pus (91% [95% CI, 78% to 97%]), followed by aspirates (80% [95% CI, 56% to 94%]) and fluids (51% [95% CI, 44% to 58%]), with nonoverlapping CIs between pus and fluids. The specificity values for Xpert compared to culture were lowest for pus (76% [95% CI, 60% to 85%]) and aspirates (78% [95% CI, 65% to 88%]). Among the fluids, Xpert MTB/RIF had a higher sensitivity for ascitic fluids (59% [95% CI, 39% to 69%]) than for pleural fluids (47% [95% CI, 38% to 56%]), but with overlapping CIs. Among the specimen types with sample numbers < 40, Xpert MTB/RIF detected one more *M. tuberculosis*-positive result for bone and two more positive results for tissue biopsy specimens than MGIT, whereas MGIT detected one more positive result on CSF and one more positive result on urine than Xpert MTB/RIF.

Fewer (10/132 [7.6%]) rifampin (RIF)-resistant cases were identified by traditional methods than by Xpert MTB/RIF, which provided an early diagnosis of RIF resistance (9.6%) in 25/260 cases (with 100% specificity) as detailed in Table 3. Traditional methods were hampered by contamination, PCR failure, or unclear banding patterns of MTBDR*plus*v1 or were not requested.

Table 4 further outlines Xpert MTB/RIF's performance based on specimen appearance (clear [and watery], bloody, thick, and traces of blood) and shows that 53% of Xpert MTB/RIF-tested specimens had some trace of blood. Overall, the sensitivity of Xpert (compared to MGIT) was highest (87% [95% CI, 76% to 94%]) among thick specimens and was significantly different from that determined among blood trace (52% [95% CI, 44% to 60%]) and clear (48% [95% CI, 36% to 61%]) specimens. There was no significant difference in sensitivity between specimens classified as clear (66% of which were fluids) and those classified as having a trace of blood. Among the clear fluid specimens, 57% (127/223) were pleural fluids. The Xpert MTB/RIF PCR was not inhibited, and no errors were reported for the 10 specimens described as bloody.

Specimens centrifuged before further processing (14.6%, 171/1,175) were mostly fluids (40%, 69/171) and pus (26%, 45/171), with Xpert MTB/RIF generating positive results in 32% (54/171) compared to 21% (36/171) in MGIT. The sensitivity of Xpert MTB/RIF compared to MGIT (excluding those contaminated on MGIT) in centrifuged samples ($n = 137$) was 75% (95% CI, 58% to 89%) compared to 57% (95% CI, 50% to 63%) for those not centrifuged ($n = 908$), but with overlapping CIs, showing no added benefit to fluids being centrifuged even if the specimen contained traces of blood.

DISCUSSION

The use of Xpert MTB/RIF for diagnosing pulmonary TB in South Africa has begun to result in a decline in the pulmonary TB positivity rate from ~16% at national implementation (March 2011) to ~13% after 2.4 million Xpert MTB/RIF tests (November 2013) at 100% program coverage (20). A number of factors, such as earlier diagnosis and treatment (17), treatment of microbiologically confirmed cases, and the expansion of ARV coverage, may be contributing to the decline. National policy does not yet include the use of Xpert MTB/RIF for diagnosing EPTB, largely for reasons of data insufficiency. Xpert MTB/RIF's introduction for pulmonary specimen testing has required changes in clinical and diagnostic algorithms and changes in the requisition of samples and their processing and the laboratory and clinic workflow, to name a few of the consequences (21). It has also exposed general weak-

TABLE 3 Xpert MTB/RIF results[a]

Xpert category and *M. tuberculosis* test-specific subcategory	No. of specimens				No. of specimens MGIT positive and tested by:	
	Tested	MGIT contaminated	MGIT negative	MGIT positive	MTBDR*plus*v1	MGIT DST
RIF resistant	25	2	6	17	17	11
MDR					4	
RIF monoresistant					5	1
INH/RIF sensitive					1	
No result					6	10
PCR fail					1	
RIF sensitive	231	32	56	143	143	47
INH/RIF sensitive					30	4
INH resistant/RIF indeterminate					1	
INH monoresistant					1	1
INH not tested/RIF resistant						1
No result					69	42
TUB negative					3	
Indeterminate					39	
Xpert negative	909	89	707	113	113	39
Indeterminate					22	
INH/RIF sensitive					22	12
INH sensitive/RIF indeterminate					1	
No result					63	27
TUB negative					5	
Total	1165					

[a] A total of 10 samples, including 6 that returned errors and 4 that were classified as rifampin indeterminate, were excluded from the results. INH, isoniazid; TUB, tuberculosis.

nesses in the general health care systems for TB, one being ongoing linkage to care postdiagnosis. In addition, the technology has challenged the processes used in certain culture laboratories and the need for further standardization of gold standard practices. This was also evidenced in this study, with the low detection of bacterial contamination in EPTB specimens on sheep blood agar plates (3.1%) and the NALC-NaOH decontamination processing unable to minimize MGIT contamination to a level below 10%. Xpert MTB/RIF, however, successfully generated results on all these specimens with less diagnostic delay and simultaneously reported RIF susceptibility results.

More than half of the specimens in this study were reported as containing traces of blood, but Xpert MTB/RIF's performance was no different for those specimens with a clear appearance (sensitivity, 52% [95% CI, 44% to 60%] versus 48% [95% CI, 36% to 61%], respectively). A protocol for Xpert MTB/RIF testing on blood samples (20 ml) has recently been developed and requires a special preprocessing buffer (22) to eliminate PCR inhibition; however, this does not appear to be required for the EPTB specimens routinely received in this laboratory. Among these clear specimens, pleural fluids performed most poorly (sensitivity, 47% [95% CI, 38% to 56%]). This may be due to specimen collection, storage, and preparation techniques (8) or to reduced numbers (below the 131 CFU/ml threshold) (23, 24) of *M. tuberculosis* in the specimen, a further dilution by SR buffer, or too harsh a treatment by SR buffer. The latter hypothesis may be the most realistic, since Xpert MTB/RIF's performance was significantly better (sensitivity, 87% [95% CI, 76% to 94%]) on fluid and pus specimens that had a thick appearance. These may be more similar in constitution to pulmonary specimens and therefore better suited to using SR buffer, which is designed for liquefaction. Elimination of SR from the Xpert MTB/RIF processing of clear specimens might improve the sensitivity; however, it would also prevent Xpert MTB/RIF processing being performed outside a laboratory environment (25). The study design, for reasons associated with current standards of care laboratory practice, where routine specimen testing was performed prior to Xpert MTB/RIF testing, dramatically limited the number of CSF specimens (also clear in appearance) for performance comparison; therefore, conclusions based on clear specimens in this study cannot be extrapolated to CSF. This might improve if Xpert MTB/RIF were to be performed as the first-line diagnostic on the received specimen but would require further research to optimize the Xpert MTB/RIF protocol for CSF and clear specimens if SR is eliminated. Fluid and pus specimens that were centrifuged were more likely (but not statistically significant) than uncentrifuged samples to give rise to a positive Xpert MTB/RIF result, in spite of the concentrated pellet being inoculated into MGIT and the residual supernatant being available for Xpert MTB/RIF testing. These specimens, however, were not paired, and centrifuged samples had a larger starting volume, but centrifugation would also be difficult to implement clinically in more-remote centers. The specificity of the Xpert MTB/RIF method was also lower among pus and aspirate specimens (76% and 78%, respectively), which may have been due to suboptimal (nonviable) growth of *M. tuberculosis* from these types of specimens in MGIT, whereas filtration, capture of whole bacteria, and amplification of *M. tuberculosis* DNA in the Xpert cartridge would be possible and could report a positive result. Importantly, Xpert

TABLE 4 Xpert MTB/RIF performance classified by specimen appearance, centrifuged specimens, and other[a]

Specimen category and group	No. (%) of specimens tested	No. of specimens classified by:						No. of specimens with definitive results confirmed by reference culture	% sensitivity (95% CI)	% specificity (95% CI)
		MGIT culture			Xpert MTB/RIF					
		Positive	Negative	Contaminated	Positive[b]	Negative	Error			
Appearance										
Clear (watery)	292 (25)	62	201	29	51	240	1	262	48 (36 to 61)	93 (89 to 96)
Bloody	10 (0.9)	0	9	1	1	9	0			
Thick	255 (22)	62	131	62	92	162	1	192	87 (76 to 94)	85 (77 to 90)
Trace of blood	618 (53)	153	433	32	116	498	4	582	52 (44 to 60)	93 (91 to 96)
Centrifuge treatment										
Not centrifuged	1,004 (850)	241	673	90	206	792	6	908	57 (50 to 63)	94 (91 to 95)
Centrifuged on receipt	171 (14.6)	36	101	34	54	117	0	137	75 (58 to 89)	81 (72 to 88)
Treatment with sheep blood agar followed by NALC-NaOH[c]										
Aspirate	8 (22)		5	3	1	7				
Fluid	12 (33)	2	8	2	2	10				
Pus	8 (22)	3	2	3	4	4				
Tissue	8 (22)		5	3		8				

[a] A total of 1,175 specimens were tested. A total of 124 samples (10.5% of 1,175 samples), including 9 aspirate samples, 1 bone sample, 1 CSF sample, 5 urine samples, 72 fluid samples, 27 pus samples, and 9 tissue biopsy samples, were classified as contaminated by MGIT culture testing. Xpert MTB/RIF classified 35 (28%) of these as positive and 89 (72%) of these as negative.

[b] Data include all RIF results (RIF sensitive, RIF resistant, and RIF indeterminate).

[c] A total of 36 samples were subjected to 24 h of bacterial growth on sheep blood agar followed by NALC-NaOH treatment.

MTB/RIF was positive in 62 specimens of the 774 that were negative by MGIT culture and positive in 35 of the 124 specimens that were contaminated on MGIT culture. The specificity of Xpert MTB/RIF on pulmonary specimens is excellent (99%) (2), and it is therefore likely that these positive Xpert MTB/RIF results represent true-positive cases. Using this assumption, overall, the number of cases for which Xpert MTB/RIF gave positive results was similar to that determined for MGIT culture (260 versus 277, respectively). Taking into account that Xpert MTB/RIF is less affected by contaminating bacteria, its use for diagnosing EPTB could significantly reduce labor in the laboratory and reduce the diagnostic delay. Sterility testing on blood agar plates followed by NALC-NaOH processing can be eliminated, and the need for MGIT culture and MPT64 antigen testing can be dramatically reduced, with a decrease in the turnaround time for patient result reporting. The number of requests for the MTBDR*plus* assay and phenotypic DST would also be reduced.

This report provides local data to support the introduction of TB screening of EPTB specimens with GeneXpert technology and similarly follows the pulmonary Xpert MTB/RIF algorithm with confirmation by DST and its use in the context of clinical suspicion. Without modification to the pulmonary Xpert MTB/RIF assay, clinicians practicing in the Johannesburg (Gauteng) region can expect the following sensitivities: 59% (95% CI, 53% to 65%) for all EPTB specimens; 91% (95% CI, 78% to 97%) for pus; 80% (95% CI, 56% to 94%) for aspirates (lymph node); 93% (95% CI, 88% to 97%) for HIV-associated FNA (19); and 51% (95% CI, 44% to 58%) for fluids (including pleural and ascitic fluids). It is acknowledged that the sample size for homogenized tissue biopsy specimens and bone in this study is low, but the sensitivity of Xpert MTB/RIF appears comparable to that of MGIT for these specimens.

These findings are not dissimilar from those of other studies from Spain (11), with 58% sensitivity (95% CI, 49% to 68%) for pleural fluid, lymph node, abscess aspirates, and tissues; from India (12), with 81% (95% CI, 76% to 85%) for tissue biopsy specimens, pus, and body fluids; and from Italy (5), with 81% (95% CI, 76% to 86%) for tissue biopsy specimens, FNA, pleural fluid, gastric aspirates, pus, CSF, urine, and peritoneal and synovial/pericardial fluids. Implementation will, however, require a full costing analysis and ongoing studies for specific tissue types such as CSF (the predominant EPTB specimen received) where volumes and specimen preparation procedures have not been defined.

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