MAJOR ARTICLE

Comparative Study of the Sensitivity of Different Diagnostic Methods for the Laboratory Diagnosis of Buruli Ulcer Disease

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Background. Several diagnostic laboratory methods are available for case confirmation of Buruli ulcer disease. This study assessed the sensitivity of various diagnostic tests in relation to clinical presentation of the disease, type of diagnostic specimen, and treatment history.

Methods. Swab samples, 3-mm punch biopsy tissue specimens, and surgically excised tissue specimens from 384 individuals with suspected Buruli ulcer disease were obtained at 9 different study sites in Ghana and were evaluated with dry reagent–based polymerase chain reaction (PCR), microscopic examination, culture, and histopathological analysis. The study subjects presented with nonulcerative and ulcerative lesions and were divided into 3 treatment groups: (1) previously untreated patients scheduled for antimycobacterial treatment, (2) patients treated with surgery alone, and (3) patients treated with surgery in combination with previous antimycobacterial treatment.

Results. Of 384 suspected cases of Buruli ulcer disease, 268 were confirmed by at least 1 positive test result. The overall sensitivity of PCR (85%) was significantly higher than that of microscopic examination (57%) and culture (51%). After data were stratified by treatment group, type of lesion, and diagnostic specimen type, analysis revealed that PCR of 3-mm punch biopsy tissue specimens (obtained from previously untreated nonulcerative lesions) and of swab samples (obtained from previously untreated ulcers) had the highest diagnostic sensitivity (94% and 90%, respectively). Although duration of the disease did not significantly influence the sensitivity of any test, previous antimycobacterial treatment was significantly associated with decreased sensitivity of PCR and culture.

Conclusions. Across all subgroups, PCR had the highest sensitivity. PCR assessment of 3-mm punch biopsy tissue specimens proved to be the best diagnostic tool for nonulcerative lesions, and PCR assessment of swab samples was the best diagnostic tool for ulcerative lesions. For monitoring of antimycobacterial treatment success within controlled trials, however, only culture is appropriate.

Buruli ulcer disease (BUD), which is caused by *Mycobacterium ulcerans,* affects the skin and subcutaneous adipose tissue. BUD occurs in >30 countries worldwide, with a focus and an increasing number of cases occurring in West Africa [1–3]. The disease initially presents as a painless nodule, papule, plaque, or edema and evolves into a painless ulcer with characteristically undermined edges. If untreated, scarring and contractures may cause serious functional disabilities [3, 4]. Previously, BUD was treated with wide surgical excision; in 2004, however, antimycobacterial treatment alone or in combination with surgery was introduced [3–8]. Currently available diagnostic laboratory tests include microscopic examination, culture, IS2404 PCR

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of swab samples and tissue specimens, and histopathological analysis.

According to various studies of the laboratory confirmation of clinically suspected cases of BUD, microscopic examination detects 29%–78% of BUD cases, and culture detects 34%–79%. Antimycobacterial treatment before specimen collection was shown to reduce culture positivity ratios (i.e., the number of positive test results divided by the number of cases of clinically suspected BUD) to <5%. Histopathological analysis confirmed >90% of clinically diagnosed BUD cases and >70% of clinically suspected BUD cases [8–15].

With an analytical specificity of 100% [16–19], a sensitivity of 79%–85% [10,12], and positivity ratios of 61%–72% [14, 15], IS2404 PCR is considered to be the most reliable technique for the detection of *M. ulcerans* in human diagnostic samples. Laboratory assessment of nonulcerative lesions is restricted to an analysis of tissue specimens, whereas diagnostic swab samples provide a valuable alternative for assessment of ulcerative lesions. According to recent data from Ghana, microscopic examination and/or PCR of swab samples allowed confirmation of up to 70% of suspected cases of BUD [14, 20].

In 2001, the World Health Organization recommended that 2 positive laboratory test results be obtained to confirm a positive diagnosis [21]. However, laboratory confirmation of suspected cases of BUD by 1 positive test result yields ~20% more confirmed cases than does confirmation by 2 positive test results. Because of the high positive predictive values of IS2404 PCR (100%) and microscopic examination (97%), 1 positive test result is considered to be sufficient for confirmation of a diagnosis of BUD [14, 20]. A positive IS2404 PCR result is also regarded as adequate evidence to commence antimycobacterial treatment [22].

In addition to swab samples, punch biopsy tissue specimens also allow the pretreatment laboratory confirmation of suspected BUD [3, 22]. Data on the diagnostic use of punch biopsy tissue specimens, however, are still scarce. Phillips et al. [23] reported sensitivities of 42% for microscopic examination, 49% for culture, 98% for IS2404 PCR, and 82% for histopathological analysis of 4-mm and 6-mm punch biopsy tissue specimens.

In the context of a research program funded by the European Commission on diagnosis and antimycobacterial treatment of BUD, various types of diagnostic specimens were obtained from patients who presented with different clinical forms of the disease and were grouped into 3 different treatment categories. The aim of this study was to determine the sensitivities of diagnostic laboratory methods for various types of specimens, depending on the type of lesions and prior treatment history.

PATIENTS, MATERIALS, AND METHODS

Study population and inclusion criteria. The study included individuals with clinical suspicion of BUD who had nonulcerative or ulcerative lesions and were seen from January 2006 through February 2008 at 9 different study sites in Ghana. The study subjects belonged to 1 of 3 different treatment groups: (1) the drug treatment group, which included patients who were scheduled for drug treatment, had received no antimycobacterial treatment before specimen collection, had a ⩽6-month duration of disease, had lesions ⩽10 cm in diameter, and were ⩾5 years of age; (2) the surgical treatment group, which included patients who were treated with surgical excision and had received no previous antimycobacterial treatment; and (3) the surgical treatment plus antimycobacterial treatment group, which included patients who were treated with surgical excision and had received at least 7 days of previous antimycobacterial treatment.

Standardized specimen collection. In the majority of cases, the diagnostic specimens were collected during the patients' initial presentation to the hospital. For a limited number of patients, additional follow-up samples were analyzed. Swab samples were taken by circling the entire undermined edges of ulcerative lesions. Three-millimeter punch biopsy tissue specimens and surgically excised tissue specimens with a maximum size of 10×10 mm were taken from the center of nonulcerative lesions or from undermined edges of ulcerative lesions, including necrotic tissue [20, 24].

The following sets of specimens were taken: for those in the drug treatment group with nonulcerative lesions, 3 punch biopsy specimens; for those in the drug treatment group with ulcerative lesions, 2 swab samples and 3 punch biopsy specimens; for those in the surgical treatment group and surgical treatment plus antimycobacterial treatment group with nonulcerative lesions, 3 surgically excised tissue specimens; and for those in the surgical treatment group and surgical treatment plus antimycobacterial treatment group with ulcerative lesions, 2 swab samples and 3 surgically excised tissue specimens obtained during the surgical procedure (figure 1). Standardized specimen collection bags, including containers with transport and storage media and data entry forms (BU01 and laboratory data entry form [3]) were provided to the study sites. PCR specimens were collected in 700 μL of cell lysis solution (Gentra Systems), culture specimens were collected in 5 mL of PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin), and histological specimens were collected in 5 mL of 10% buffered neutral formalin [14, 20].

Diagnostic methods and laboratories. Diagnostic specimens were processed at the Kumasi Centre for Collaborative Research in Tropical Medicine in Kumasi, Ghana, by dry reagent–based IS2404 PCR, microscopic examination, and culture with use of standardized procedures [14, 19–21]. Standard IS2404 PCR was performed and slides were reread for external quality assurance by the Department for Infectious Diseases and Tropical Medicine at the University of Munich (Munich,

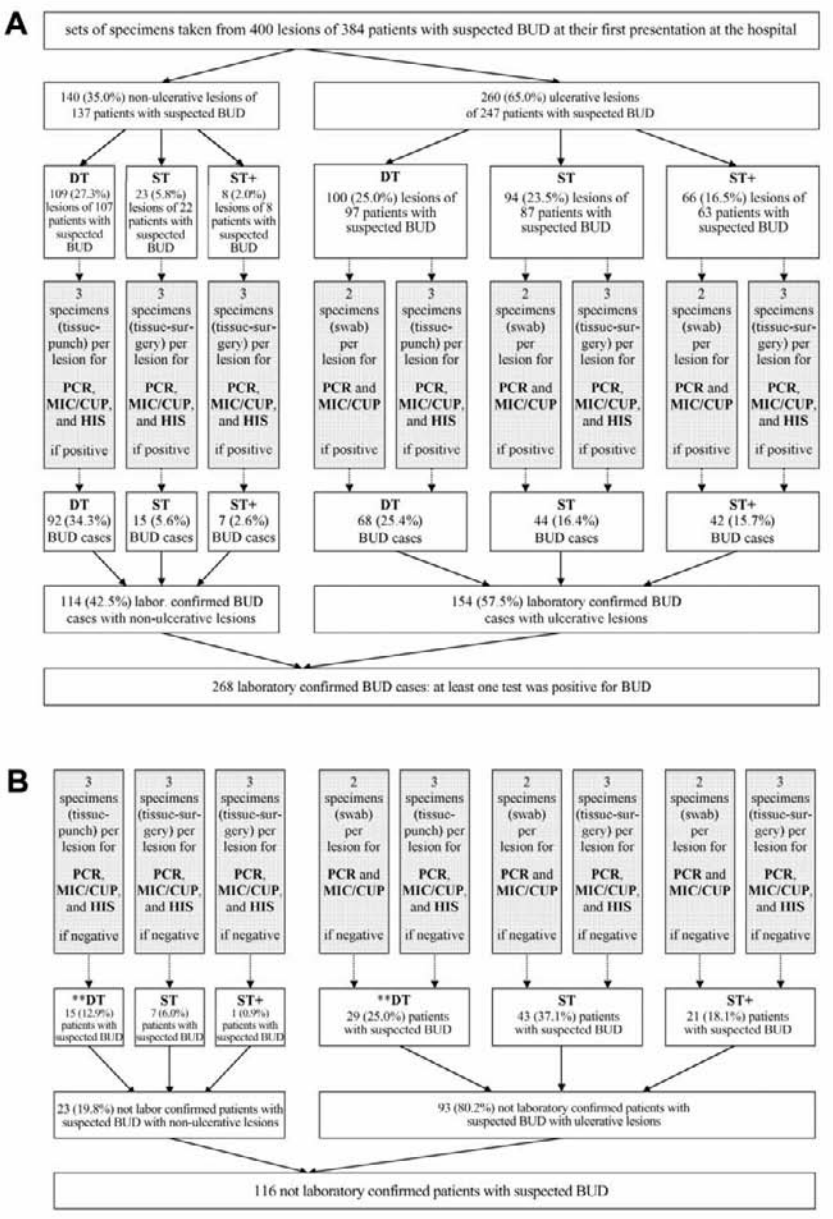

Figure 1. Flow chart of the study procedure. Specimens (swab samples [swab], punch biopsy tissue specimens [tissue-punch], and surgically excised tissue specimens [tissue-surgery]) obtained from 384 patients with suspected Buruli ulcer disease (BUD) from 3 different treatment groups (drug treatment [DT], surgical treatment without previous antimycobacterial treatment [ST], and surgical treatment with previous antimycobacterial treatment [ST+]) for BUD diagnostic testing with dry reagent–based IS2404 PCR (PCR), microscopy (MIC), culture with confirmatory IS2404 dry reagent–based PCR (CUP), and histopathologcial analysis (HIS). There were a total of 268 laboratory-confirmed cases of BUD *(A)* and 116 suspected cases of BUD without laboratory confirmation *(B)*. The statistics are based on the data obtained from each patient's first lesion (if there was >1 lesion per patient) and each patient's first visit at hospital (if there was >1 visit per patient). *Four of 16 patients with suspected BUD who had negative findings at initial presentation (3 in the ST group and 1 in the DT group) had BUD confirmed at follow-up visits. **Twenty-six of 44 patients in the DT group with suspected BUD that was not laboratory confirmed (15 patients with nonulcerative lesions and 29 patients with ulcerative lesions) received antimycobacterial treatment, because the clinical findings were suggestive of BUD. Three additional patients with suspected BUD in the DT group received treatment for onchocerciasis. The remaining 15 patients in the DT group with suspected BUD were lost to follow-up.

Germany), and histopathological examination was performed at the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany) and the Department of Pathology at the University of Munich [11, 17, 24].

In brief, DNA was prepared using the Puregene DNA isolation kit, with minor modifications [19]. For dry reagent–based PCR, the oligonucleotides MU5 and MU6 [17] were lyophilized in reaction tubes. PuReTaqTM Ready-To-GoTM PCR Beads (Amersham Biosciences) were added and dissolved in water before adding the template DNA. The standard PCR was performed according to the protocol described by Stinear et al. [17]. Both PCR assays included negative extraction and positive, negative, and inhibition controls.

Culture specimens were decontaminated by the Petroff method, inoculated on Loewenstein-Jensen media, and incubated at 32°C for 6 months. Microscopy smears were prepared from decontaminated material and were stained with the Ziehl-Neelsen technique [22].

Cultures with growth were subjected to Ziehl-Neelsen staining and a confirmatory IS2404 PCR. If a negative PCR result was obtained, sequence analysis of the *rpoB* gene (342 base pairs), *16S–23S*ribosomal RNA (rRNA) internal transcribed spacer gene (220 base pairs), *16S* rRNA gene (924 base pairs), and 65-kDa *HSP* gene (644 base pairs) was performed for strain identification [25–28].

Definition of sensitivity for each individual test. In this study, the sensitivity of a certain test was defined as the number of positive test results divided by the number of patients with at least 1 positive result of any diagnostic test [14, 20].

Statistical analysis. Approximative tests (χ^2 tests), exact tests (Fisher's exact tests), and Student's *t* tests as parametric test were conducted using Stata software, version 9.0 (Stata). Statistically significant differences were defined as *P* values <.05 or as nonoverlapping 95% CIs of proportions. Dependent variables were diagnostic test results. Type of lesion, duration of disease, duration of antimycobacterial treatment (if conducted before specimen collection), and type of diagnostic specimen were independent variables.

Ethical clearance and informed patient consent. Ethical clearance for the study was sought through the Committee of Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology, and the Komfo Anokye Teaching Hospital, Kumasi, Ghana. Informed patient consent was used for patients who received drug treatment and for patients who underwent surgical procedures.

RESULTS

Subjects. Four hundred sets of specimens from 384 individuals with suspected BUD (drug treatment group, 204 patients; surgical treatment group, 109 patients; and surgical treatment plus antimycobacterial treatment group, 71 patients) who presented with 140 nonulcerative (35%) and 260 ulcerative (65%) lesions were collected at the first presentation at the hospital. Sixteen patients had 2 lesions. A total of 43 sets of follow-up specimens were obtained from 30 study subjects; however, only data from the patients' initial specimen collection were considered for analysis. Forty-eight percent of the suspects were 5–14 years of age (age range, 1–95 years; mean age, 21 years; median age, 14 years); 40.9% of the patients were male (figure 1).

Laboratory-confirmed BUD cases. One hundred seventy-two (44%) of the BUD cases were confirmed by at least 2 positive laboratory test results; 268 (69.8%) were confirmed by at least 1 positive laboratory test result (figure 1). One hundred fourteen (42.5%) of the patients with BUD presented with nonulcerative lesions, and 154 (57.5%) presented with ulcerative lesions; 108 (40.3%) of the patients were male, and 150 (56.6%) of 265 patients (for 3 patients, age was unknown) were 5–14 years of age (range, 2–80 years; mean age, 18 years; median age, 12 years) (figure 2). In 231 (93.1%) of 248 patients, the lesions were located on the limbs or shoulders, with the right side being affected statistically significantly more often (in 138 [59.7%] of 231 patients; $P = .035$) than the left side (93 [40.3%] of 231 patients) (table 1). The lesions of 243 patients with confirmed cases with known lesion sizes were distributed according to World Health Organization categories [3], as follows: category I (a single lesion <5 cm in diameter), 108 patients (44.4%), including 61 in the drug treatment group, 23 in the surgical treatment group, and 24 in the surgical treatment plus antimycobacterial treatment group; category II (a single lesion 5–15 cm in diameter), 127 patients (52.3%), including 93 in the drug treatment group, 15 in the surgical treatment group, and 19 in the surgical treatment plus antimycobacterial treatment group; and category III (a single lesion >15 cm in diameter, multiple lesions, or osteomyelitis), 8 patients (3.3%), including 4 in the drug treatment group, 3 in the surgical treatment group, and 1 in the surgical treatment plus antimycobacterial treatment group.

Of 268 patients with BUD with at least 1 positive laboratory test result, 229 (85.4%) had a positive swab sample and/or tissue specimen with a positive PCR result, and 152 (56.7%) had a positive swab sample and/or a tissue specimen with positive microscopy findings. One hundred forty-nine (98.0%) of the 152 specimens with positive microscopy findings had those findings confirmed by at least 1 of the 3 other tests.

Of the 115 isolates (42.9%) obtained from swab sample and/or tissue specimen cultures with positive results, 108 isolates were confirmed by IS2404 PCR (positive predictive value, 93.9%). Among the remaining 7 isolates, sequence analysis identified 2 *M. ulcerans* strains. Two further strains were identified as *Mycobacterium mucogenicum* and *Mycobacterium phocaicum*, indicating a coinfection or superinfection in 2 indi-

viduals with confirmed BUD. For 3 isolates, sequencing did not provide definitive identification, probably because of contamination with closely related species that colonize the human skin.

Among the follow-up samples, sequencing identified 2 strains as *Mycobacterium gordonae* (cultured from an ulcer with a swab sample with positive IS2404 PCR) and *Mycobacterium szulgai* (isolated from an additional lesion at a different location on a patient whose initial lesion had a swab sample with positive IS2404 PCR results).

Histopathological examination confirmed results for 42 of 49 tissue specimens from individuals with otherwise laboratory-confirmed BUD (sensitivity, 85.7%) and for 17 (29.3%) of 58 specimens from individuals with suspected BUD who had negative microscopy findings and negative culture and PCR results (6 [10.3%] of these 58 specimens were obtained from lesions in the healing stages). In 4 (6.9%) of the 58 individuals with suspected BUD, histopathological features did not allow an unambiguous diagnosis, and histological findings in 37 (63.8%) were not suggestive of BUD (8 of these 37 patients received a diagnosis of onchocerciasis). Missing or poor-quality specimens did not allow histopathological analysis for the remaining 75 individuals with suspected BUD.

Of 30 individuals who were followed up over time, 16 received laboratory confirmation of BUD at their first presentation to the hospital. Of the remaining 14 subjects, 4 had BUD confirmed during subsequent follow-up visits. External quality assurance for microscopic examination and PCR showed >90% concordance of results (table 2).

Overall sensitivities of laboratory tests among all laboratory-confirmed BUD cases. The overall sensitivities were 85.4% (229 of 268 cases) for PCR, 56.7% (152 of 268 cases) for microscopic examination, and 48.0% (108 of 225 cases) for culture plus confirmatory IS2404 PCR. The sensitivity of PCR was significantly higher than that of microscopic examination and culture plus confirmatory IS2404 PCR ($P < .01$), with no statistically significant difference between microscopic exami-

Table 1. Location of nonulcerative and ulcerative lesions in 248 cases of laboratory-confirmed Buruli ulcer disease.

	No. (%) of lesions, by general location (n = 248)			
Specific location	Head and trunk	Right shoulder and limbs	Left shoulder and limbs	Total
Head, neck	6	…	…	6 (2.4)
Back	7	…	…	7 (2.8)
Abdomen	0	…	…	0 (0)
Buttock, hip	4	…	…	4 (1.6)
Shoulders	…	7	6	13 (5.2)
Arms				
Overall	…	65	39	104 (41.9)
Upper arm	…	24	17	41 (16.5)
Forearm	…	34	18	52 (21.0)
Wrist	…	3	1	4 (1.6)
Hand, dorsal	…	3	3	6 (2.4)
Hand, volar	…	1	0	1 (0.4)
Legs				
Overall	…	66	48	114 (46.0)
Thigh	…	20	13	33 (13.3)
Knee	…	9	7	16 (6.5)
Lower leg	…	26	18	44 (17.7)
Ankle	…	8	8	16 (6.5)
Foot, dorsal	…	3	2	5 (2.0)
Foot, plantar	…	0	0	0 (0)
Overall	17 (6.9)	138 (55.6)	93 (37.5)	248 (100)

NOTE. For 20 cases, the specific location of the lesions was not known.

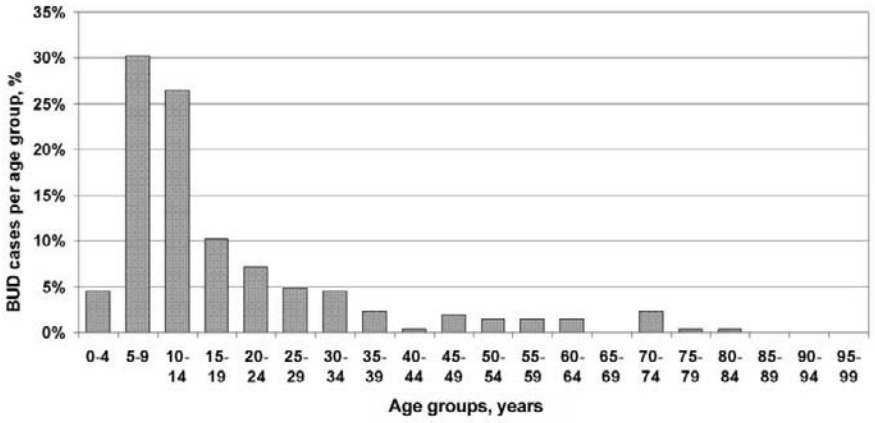

Figure 2. Age distribution of 268 patients with laboratory-confirmed Buruli ulcer disease (BUD). For 3 patients, age was unknown. Age range was 2–80 years, the mean age was 18 years, and the median age was 12 years. For the group of patients who received drug treatment, patients with suspected BUD who were <5 years of age were not included. For the surgical treatment without previous antimycobacterial treatment group and the surgical treatment with previous antimycobacterial treatment group, patients with suspected BUD were included regardless of age.

Table 2. External quality assurance for PCR and microscopic examination.

KCCR result, by method	DITM result			Concordant results	Concordance ratio, %[a]
	Positive	Negative	Total		
PCR[b]					
Positive	37	0[c]	37	37	…
Negative	7[c]	33	40	33	…
All	44	33	77	70	90.9
Microscopic examination					
Positive	56	5[d]	61	56	…
Negative	2[d]	33	35	33	…
All	58	38	96	89	92.7

NOTE. Data are no. of specimens, unless otherwise indicated. DITM, Department of Infectious Diseases and Tropical Medicine (Munich, Germany); KCCR, Kumasi Centre for Collaborative Research in Tropical Medicine (Kumasi, Ghana). Microscopic examination was performed for 96 slides at KCCR, and the slides were reexamined by staff at DITM.

[a] No. of specimens with concordant results divided by the total number of specimens tested with the same test at KCCR and DITM.

[b] PCR was performed with parallel testing of 77 DNA extracts. Dry reagent–based IS2404 PCR was performed at KCCR; standard PCR was performed at DITM.

[c] Dry reagent–based PCR performed at KCCR gave false-positive results for 0 (0%) of the specimens tested and false-negative results for 7 (9.1%) of the specimens tested.

[d] Microscopic examination performed at KCCR gave false-positive results for 5 (5.2%) of the specimens tested and false-negative results for 2 (2.1%) of the specimens tested.

nation and culture plus confirmatory IS2404 PCR ($P = .054$) (table 3).

Sensitivities of laboratory tests among confirmed BUD cases involving nonulcerative lesions. Among 114 confirmed nonulcerative BUD cases (drug treatment group, 92 cases; surgical treatment group, 15 cases; surgical treatment plus antimycobacterial treatment group, 7 cases), the sensitivities were 89.5% (102 of 114 specimens) for PCR, 57.0% (65 of 114 specimens) for microscopic examination, and 60.6% (57 of 94 specimens) for culture plus confirmatory IS2404 PCR analysis of tissue specimens. PCR was significantly more sensitive than microscopic examination and culture plus confirmatory IS2404 PCR ($P < .01$ for each). Stratified into treatment groups and specimens, the sensitivities for analysis of punch biopsy tissue specimens from the drug treatment group were 93.5% for PCR and 70.8% for culture plus confirmatory IS2404 PCR, which was statistically significantly higher than that for surgically excised tissue specimens in the surgical treatment group (table 3).

Sensitivities of laboratory tests among patients with confirmed BUD and ulcerative lesions. Among 154 laboratory-confirmed cases of ulcerative BUD (drug treatment group, 68 cases; surgical treatment group, 44 cases; surgical treatment plus antimycobacterial therapy group, 42 cases), the sensitivities were 69.8% (169 of 242 specimens) for PCR, 44.6% (108 of 242 specimens) for microscopic examination, and 25.0% (52 of 208 specimens) for culture plus confirmatory IS2404 PCR analysis of swab samples and tissue specimens. PCR was statistically significantly more sensitive than microscopic examination and culture plus confirmatory IS2404 PCR ($P < .01$ each). Stratified into treatment groups and specimens, the sensitivities for PCR analysis of swab specimens were 89.9% for the drug treatment group, 73.1% for the surgical treatment group, and 72.2% for the surgical treatment plus antimycobacterial therapy group. In all treatment groups, PCR sensitivity was greater for swab samples than it was for punch biopsy tissue specimens (67.8%) or surgically excised tissue specimens (surgical treatment group, 57.7%; surgical treatment plus antimycobacterial therapy group, 44.4%) (table 3).

Sensitivities of laboratory tests among patients with ulcerative lesions without previous antimycobacterial treatment depending on the duration of disease. According to duration of disease, 101 patients with laboratory-confirmed, previously untreated cases of BUD with ulcerative lesions were divided into 5 groups. In all groups, PCR of swab samples had a statistically significantly higher sensitivity (70%–91%) than did microscopic examination of swab samples (30%–67%) or culture plus confirmatory IS2404 PCR of swab samples (29%–59%). Despite a slight downward trend, no statistically significant association between test sensitivity and duration of disease for any test was found (figure 3).

Test sensitivity depending on duration of previous antimycobacterial treatment. In the 49 laboratory-confirmed cases of BUD in the surgical treatment plus antimycobacterial treatment group (7 nonulcerative case and 42 ulcerative cases), the test sensitivity was correlated with the duration of previous antimycobacterial treatment. Regardless of treatment duration, PCR was always the test with the highest sensitivity. The sensitivities of PCR and culture plus confirmatory IS2404 PCR were statistically significantly higher in the group of untreated patients than in the group of patients treated for >40 days (for

Table 3. Sensitivity of 3 different methods for the diagnosis of nonulcerative and ulcerative lesions of Buruli ulcer disease (BUD), by method of specimen collection and treatment group.

Type of lesion, treatment group (specimen type)	Sensitivity, % (no. of positive results/no. of specimens tested)			P[a]		
	Dry reagent–based IS2404 PCR	Microscopic examination	CUP	PCR vs. microscopic examination	PCR vs. CUP	Microscopic examination vs. CUP
Nonulcerative						
DT (punch biopsy tissue)	93.5 (86/92)[b]	57.6 (53/92)	70.8 (51/72)	<.01	<.01	.08
ST (surgically excised tissue)	66.7 (10/15)	40.0 (6/15)	40.0 (6/15)	.15	.15	1.00
P[c]	<.01	.21	.02			
ST+ (surgically excised tissue)	85.7 (6/7)[d]	85.7 (6/7)[e]	0 (0/7)	>.99	<.01	<.01
Overall	89.5 (102/114)[b]	57.0 (65/114)	60.6 (57/94)	<.01	<.01	.60
Ulcerative[f]						
DT (swab sample)	89.9 (53/59)[b]	67.8 (40/59)	57.4 (27/47)	<.01	<.01	.27
DT (punch biopsy tissue)	67.8 (40/59)[b]	33.9 (20/59)	23.4 (11/47)	<.01	<.01	.24
P[g]	<.01	<.01	<.01			
ST (swab sample)	73.1 (19/26)[b]	23.1 (6/26)	12.5 (3/24)	<.01	<.01	.34
ST (surgically excised tissue)	57.7 (15/26)[d]	38.5 (10/26)[e]	12.5 (3/24)	.17	<.01	.04
P[g]	.25	.24	>.99			
ST+ (swab sample)	72.2 (26/36)[d]	50.0 (18/36)[e]	3.0 (1/33)	.055	<.01	<.01
ST+ (surgically excised tissue)	44.4 (16/36)[d]	38.9 (14/36)	21.2 (7/33)	.63	.04	.11
P[g]	.02	.35	.03			
DT and ST (swab sample)	84.7 (72/85)[b]	54.1 (46/85)	42.3 (30/71)	<.01	<.01	.14
DT and ST (punch biopsy tissue and surgically excised tissue)	64.7 (55/85)[b]	35.3 (30/85)[e]	19.7 (14/71)	<.01	<.01	.03
P[h]	<.01	.01	<.01			
Overall[i]	69.8 (169/242)[b]	44.6 (108/242)[e]	25.0 (52/208)	<.01	<.01	<.01
All lesions[j]	85.4 (229/268)[b]	56.7 (152/268)	48.0 (108/225)	<.01	<.01	<.054

NOTE. Specimens were obtained for a total of 268 laboratory-confirmed cases of BUD. In 43 cases, however, no results for culture and confirmatory dry reagent–based IS2404 PCR were available. Therefore, the denominator for CUP is 225, not 268. CUP, culture and confirmatory PCR; DT, drug treatment with <7 days of previous antimycobacterial treatment; ST, surgical treatment with <7 days of previous antimycobacterial treatment; ST+, surgical treatment with ⩾7 days of previous antimycobacterial treatment.

[a] For comparison of sensitivity of tests performed on specimens taken from the same lesion.

[b] Sensitivity of PCR was statistically significantly ($P < .05$) higher than that of microscopic examination and CUP.

[c] P value comparing the sensitivity of PCR, microscopic examination, and CUP between punch biopsy tissue specimens and surgically excised tissue specimens obtained from patients with nonulcerative lesions.

[d] Sensitivity of PCR was not statistically significantly ($P > .05$) higher than that of microscopic examination and was statistically significantly ($P < .05$) higher than that of CUP.

[e] Sensitivity of microscopic examination was statistically significantly ($P < .05$) higher than that of CUP.

[f] Data for patients with BUD who had ulcerative lesions were included in the calculation for the swab samples if test results were obtained for both swab samples and tissue specimens taken from the same lesion.

[g] P value comparing the sensitivity of PCR, microscopic examination, and CUP between swab samples and tissue specimens obtained from the same ulcerative lesion.

[h] P value comparing the sensitivity of PCR, microscopic examination, and CUP between punch biopsy tissue specimens and surgically excised tissue specimens obtained from the same ulcerative lesion.

[i] Includes all results, regardless of whether 1 specimens (swab sample or tissue specimen) or 2 specimens (swab sample and tissue specimen) were obtained per lesion.

[j] Includes both nonulcerative and ulcerative lesions. For ulcerative lesions, only 1 result was included; if 2 results from the same ulcerative lesion (from a swab sample and a tissue specimen) were not concordant, then the lesion was considered to be positive if 1 of the 2 tests had a positive result.

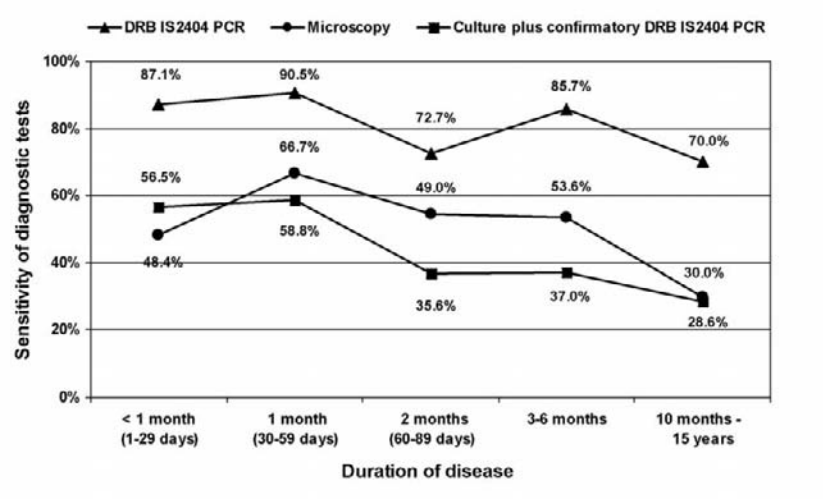

Figure 3. Sensitivity of diagnostic tests (dry reagent–based [DRB] IS2404 PCR, microscopic examination, and culture plus confirmatory DRB IS2404 PCR) by duration of disease among 112 patients with laboratory-confirmed Buruli ulcer disease and ulcerative lesions who had not received previous antibiotic treatment (68 swab specimens from patients in the drug treatment group and 44 swab specimens from patients in the surgical treatment without prior antimycobacterial therapy group). No patient reported a 7–9-month duration of disease.

PCR) or >20 days (for culture plus confirmatory IS2404 PCR). No statistically significant difference was found for microscopic examination (figure 4).

DISCUSSION

This is, to our knowledge, the largest study to date to evaluate the diagnostic yield of various diagnostic tests after the introduction of antimycobacterial therapy in a West African region in which BUD is highly endemic. Diagnostic tests proved to be reliable within a range of disease durations and across a range of clinical presentations and treatment groups. The results are relevant for case definition for drug trials. Furthermore, locally conducted laboratory confirmation may improve the reliability of epidemiological data.

The majority of recent data on the laboratory confirmation of clinically suspected BUD are from the era when surgical excision was considered to be the standard treatment, when surgically excised tissue specimens accounted for the majority of specimens analyzed. Following the introduction of antimycobacterial treatment, swab samples and punch biopsy tissue specimens, which provide the possibility of pretreatment diagnosis, have become increasingly important. Surgical excision and subsequent skin grafting, however, are still used to treat patients who experience treatment failure and have lesions that do not heal completely after antimycobacterial therapy. In these cases, surgically excised tissue specimens are available for laboratory analysis.

Comparable with previous data [20], laboratory confirmation by 1 positive test result gave 26% more confirmed cases than did confirmation by at least 2 positive test results. As in previous studies, histopathological examination identified an additional ~30% of cases, mainly in patients whose disease was in the paucibacillary stage [14, 19, 20]. However, histopathological features may not provide unambiguous identification, and the availability of the method is limited [11, 14, 19, 20]. Findings obtained with follow-up samples suggest retesting of patients who have typical clinical features but initial laboratory results that are negative.

The overall sensitivities of dry reagent–based PCR (85%), microscopic examination (57%), and culture plus confirmatory IS2404 PCR (48%), as determined in our study, are comparable with data published by other groups [8–15]. Independently of treatment group, type of lesion, or diagnostic specimen, the overall sensitivity of PCR was statistically significantly ($P <$ .01) higher than that of any other test, whereas there was no statistically significant difference between microscopic examination and culture plus confirmatory IS2404 PCR ($P = .054$).

The majority of mycobacterial isolates were confirmed to be *M. ulcerans* by IS2404 PCR or other methods. However, a few study patients, most of whom had received antimycobacterial treatment, harbored other mycobacteria. Because these strains were only isolated from these patients, laboratory contamination is unlikely. In the absence of other confirmatory tests or persistent mycobacterial growth after drug treatment, confirmation by molecular methods is important to identify coinfections or superinfections due to other mycobacteria.

Stratification by lesion type and treatment group gave the following major findings. The sensitivity (93.5%) of PCR performed on 3-mm punch biopsy tissue specimens obtained from previously untreated patients in the drug treatment group who

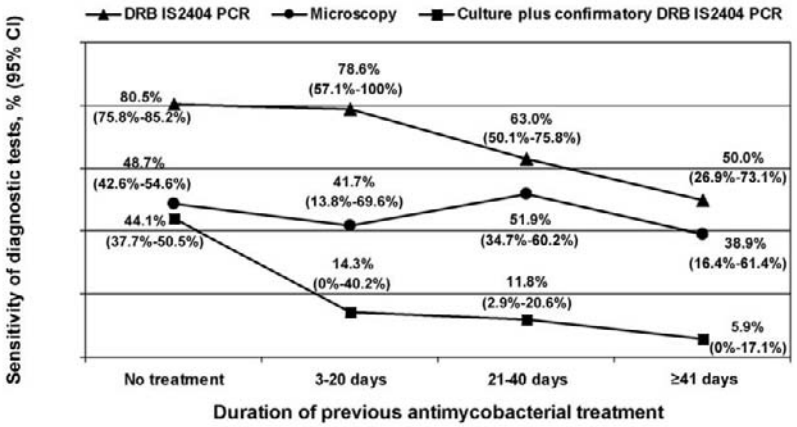

Figure 4. Sensitivity of diagnostic tests (dry reagent–based [DRB] IS2404 PCR, microscopy, and culture plus confirmatory DRB IS2404 PCR) among 49 patients with laboratory-confirmed cases of Buruli ulcer disease who received surgical treatment with previous antimycobacterial treatment (7 with preulcerative and 42 with ulcerative lesions), stratified by duration of previous antimycobacterial treatment (3–20 days, 9 patients; 21–40 days, 30 patients; ⩾40 days, 10 patients). A total of 219 patients received no treatment.

had nonulcerative lesions was significantly higher than the sensitivity of any other diagnostic test. These data are in line with the 98.3% sensitivity of PCR of 4-mm and 6-mm punch biopsy tissue specimens determined by Phillips et al. [23]. Culture plus confirmatory IS2404 PCR of punch biopsy tissue specimens also provided an excellent sensitivity of 70.8% among patients with BUD who had nonulcerative lesions. Therefore, 3-mm punch biopsy tissue specimens can be recommended for the pretreatment diagnosis of patients with nonulcerative lesions before initiation of antimycobacterial therapy. However, the small size of punch biopsy tissue specimens hampers histopathological analysis [29].

In previous studies in Ghana, PCR of swab samples confirmed 60%–70% of suspected cases of BUD with ulcerative lesions [14, 20]. The data on the sensitivity of PCR of swab samples obtained in this study also suggest considering PCR analysis of diagnostic swab samples to be the method of choice for cases that involve ulcerative lesions. Especially among the previously untreated patients in the drug treatment group with ulcerative early lesions, PCR of swab samples (sensitivity, 89.9%) proved to be superior to PCR analysis of 3-mm punch biopsy tissue specimens (sensitivity, 67.8%). In the surgical treatment group and the surgical treatment plus antimycobacterial treatment group, PCR of swab samples also had higher sensitivity than did analysis of tissue specimens. In accordance with previous observations, the lower sensitivity for tissue specimens obtained from patients who underwent surgery may be attributable to difficulties in determining the correct location for specimen collection once tissue is excised [14].

Despite a slight downward trend, no statistically significant association between duration of disease and diagnositic sensitivity was detected for swab specimens in this study. However, according to our own experience, in the course of the disease, the edges of ulcers often develop scarring, which can hinder the collection of swab samples. The duration of antimycobacterial treatment influenced the diagnostic sensitivities of PCR and culture plus confirmatory IS2404 PCR. Compared with their sensitivity in untreated patients, the sensitivities of both tests were statistically significantly lower after treatment. A statistically significant decrease in culture sensitivity was detected after 20 days of treatment. More than 40 days of treatment were required to produce the same effect for PCR. In pretreated patients, the sensitivity of PCR was still 50%, whereas the sensitivity of culture plus confirmatory IS2404 PCR decreased to 6%. In contrast with the rapid decrease in the viability of *M. ulcerans* in the first weeks after onset of treatment, PCR findings suggest extended persistence of *M. ulcerans* DNA in treated lesions.

This study describes the relative sensitivity of currently available diagnostic tests. Data on the specificity and the positive predictive values of these tests cannot be provided, because analysis of diagnostic samples from healthy individuals (including tissue specimens) would have been required to determine the number of false-positive test results. Assessment of positive and negative predictive values requires comparison with a reference test. Because of the limited availability of reference methods (e.g., histopathological examination as a reference test for PCR), the determination of positive and negative predictive values was not feasible for the entire range of tests used in this study.

According to the results of this study, IS2404 PCR was the test with the highest sensitivity overall and in all subgroups of

this study cohort; therefore, it is most suitable for the early diagnosis of all clinical forms of BUD. For monitoring of antimycobacterial treatment success within controlled trials, however, only culture seems to be the appropriate tool.

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