MAJOR ARTICLE

Evaluation of Laboratory Methods for Diagnosis of Varicella

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Background. The incidence of varicella disease is declining as a result of vaccination, making clinical diagnosis more challenging, particularly for vaccine-modified cases. We conducted a comprehensive evaluation of laboratory tests and specimen types to assess diagnostic performance and determine what role testing can play after skin lesions have resolved.

Methods. We enrolled patients with suspected varicella disease in 2 communities. Enrollees were visited at the time of rash onset and 2 weeks later. Multiple skin lesion, oral, urine, and blood or serum specimens were requested at each visit and tested for varicella zoster virus (VZV) immunoglobulin (Ig) G, IgM, and IgA antibody by enzyme-linked immunoassay; for VZV antigen by direct fluorescent antibody; and/or for VZV DNA by polymerase chain reaction (PCR). Clinical certainty of the diagnosis of varicella disease was scored. PCR results from first-visit vesicles or scab specimens served as the gold standard in assessing test performance.

Results. Of 93 enrollees, 53 were confirmed to have varicella disease. Among 20 unmodified cases, PCR testing was 95%–100% sensitive for macular and/or papular lesions and for oral specimens collected at the first visit; most specimens from the second visit yielded negative results. Among 27 vaccine-modified cases, macular and/or papular lesions collected at the first visit were also 100% sensitive; yields from other specimens were poorer, and few specimens from the second visit tested positive. Clinical diagnosis was 100% and 85% sensitive for diagnosing unmodified and vaccine-modified varicella cases, respectively.

Conclusions. PCR testing of skin lesion specimens remains convenient and accurate for diagnosing varicella disease in vaccinated and unvaccinated persons. PCR of oral specimens can sometimes aid in diagnosis of varicella disease, even after rash resolves.

Since 1995, when varicella vaccine was licensed, the incidence of varicella disease has decreased by >80% [1, 2]. Before vaccine licensure, varicella disease was ubiquitous among children and had a characteristic clinical presentation that allowed for a clinical diagnosis [1, 3]. However, the decrease in the rate of disease has likely been accompanied by a decrease in the positive predictive value of the clinical diagnosis, especially by new physicians with limited experience with varicella. In addition, varicella disease in previously vaccinated persons is relatively common and often highly modified, with lesions that are fewer in number, more transient, and often macular and/or papular rather than vesicular. Laboratory testing is therefore increasingly important for diagnosis of varicella disease and for case confirmation by public health authorities. There is also an increasing need for alternative specimen types for diagnosis of varicella disease, especially during outbreak investigations, which often commence after rashes have resolved.

We evaluated various laboratory tests and specimen types to assess their performance in diagnosis of varicella disease and to see whether they can serve as adjuncts to polymerase chain reaction (PCR) testing of skin lesions for varicella zoster virus (VZV) DNA, which is currently regarded as the most sensitive and

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specific method. We also explored whether diagnostic performance varied in persons with a history of varicella disease or vaccination.

METHODS

Study enrollment. During the period from January 2005 through June 2006, we enrolled persons of any age in Philadelphia, Pennsylvania, and New Haven County, Connecticut, who were suspected by physicians and school nurses as having varicella disease. Both sites have had extensive experience with varicella surveillance and have participated in population-based studies of varicella disease and vaccine effectiveness [4–9]. This study was approved by the institutional review boards of the Centers for Disease Control and Prevention (CDC) and both study sites. Enrollees or their parents or guardians provided informed consent.

Data and specimen collection. Study participants were visited twice by a physician or study nurse experienced with diagnosis of varicella disease and specimen collection. The first visit occurred as soon as possible after rash onset; the second visit occurred ~2 weeks later. Standard questionnaires were used for enrollee interviews. During the first visit, the study nurses or physicians were asked to rate the clinical certainty of their diagnosis of varicella disease on a 5-point scale (1, unlikely; 5, very likely).

During both visits, we obtained skin lesion, buccal and throat swab, oral fluid, urine, and blood specimens. Using established methods, we obtained samples from each skin lesion type (vesicles, macular and/or papular lesions, and scabs) present [10, 11]. With the exception of urine and blood samples, we collected duplicate specimens whenever possible.

Samples from vesicles, macular and/or papular lesions, and scabs were collected with a slide and/or polyester swab. All samples from lesions were placed in a sealed container and maintained at ambient temperatures.

Buccal and throat swab samples were collected by holding 2 swabs together and gently swabbing the buccal mucosa and the oropharynx (including tonsillar pillars), respectively, for several seconds. Oral fluid was collected by using 2 OraSure sponges (OraSure Technologies) together and gently swabbing the cheeks and gums for several seconds. The sponges were then left in the mouth for an additional minute to absorb oral fluid.

Urine was collected in a standard urinalysis cup. Blood samples were obtained by venipuncture and/or finger-stick, depending on the enrollee's consent. Blood samples obtained via finger-stick were blotted onto 2 filter paper circles (10 mm in diameter).

Laboratory methods. Specimens were tested at the National VZV Laboratory, CDC (Atlanta, GA), using multiple methods.

Serum samples were tested for VZV immunoglobulin (Ig) G using an in-house whole-cell enzyme-linked immunoassay (ELISA); samples that yielded negative or equivocal results were retested using the more sensitive glycoprotein ELISA, as previously described [10]. In-house capture ELISAs were used to detect VZV IgM and IgA antibody [10]. IgG titration was done for patients with both acute- and convalescent-phase venipuncture blood samples, as previously described [10]; a $\geqslant$4-fold increase in titer was considered diagnostic of disease infection. For acute-phase serum samples with detectable VZV IgG, avidity was tested, as previously described, to provide supporting information regarding history of varicella disease [11, 12].

Direct fluorescent antibody (DFA) testing requires intact infected cells and, thus, was performed only on vesicular samples (Chemicon; Chemicon International). Results were interpreted on the basis of the manufacturer's recommendations. Slides with $\geqslant$20 cells were classified as adequate.

DNA samples for PCR testing were extracted and purified using an automated platform (Magna Pure; Roche Diagnostics) [13–15] and genotyped as wild-type or Oka strain, as described elsewhere [10, 11]. Because VZV is highly cell associated, oral and urine specimens were prepared to concentrate cellular material. Oral fluid specimens were prepared for DNA extraction by soaking collection sponges overnight in phosphate-buffered saline, centrifuging at 400*g* for 10 min, and adding 200 μL of reconstituted oral fluid to 300 μL of Magna Pure lysing buffer. Urine samples were prepared for DNA extraction by centrifuging for 10 min at 400*g,* aspirating 200 μL from the tube bottom, and mixing with 300 μL of Magna Pure lysing buffer. Actin was used as a PCR control; specimens with undetectable actin DNA were considered to be inadequate. Real-time Fröster resonance energy transfer PCR protocols targeting 4 vaccine-associated DNA polymorphisms in opening reading frames 38 (69349), 54 (95241), and 62 (106262, 107252) were done as described elsewhere [13–15].

Classification of cases. PCR results from a vesicle or scab specimen collected at the first visit was defined as our gold standard used to rule-in or rule-out a diagnosis of varicella disease. Enrollees with a vesicle or scab specimen with a positive PCR result were classified as confirmed varicella case patients; enrollees with a specimen with a negative result were considered non–case patients. If vesicle or scab specimens were not available or if results were discordant, varicella case status was defined as indeterminate, and the participants were excluded from analysis. Case patients were categorized on the basis of prior varicella disease and on vaccination history. Cases in patients with no varicella vaccination or disease history were termed "unmodified varicella cases," and cases in those with prior varicella vaccination were termed "vaccine-modified cases." Prior varicella disease and vaccination were validated by medical chart review. If the reported disease history was inconsistent with serologic results, a hierarchical approach was used to clas-

sify prior varicella disease status, with IgG and/or avidity test results as the diagnostic reference standard.

Analysis of sensitivity and specificity. We defined sensitivity for each diagnostic test as the percentage of confirmed varicella case patients (ie, on the basis of our gold standard) who tested positive by the diagnostic assay. Specificity was defined as the percentage of non–case patients (also on the basis of our gold standard) who tested negative by the diagnostic assay. We used results from the first adequate specimen of each type collected to calculate sensitivity and specificity. We calculated 95% exact binomial confidence intervals. SAS software, version 9.1 (SAS Institute) [16], was used to analyze all data.

RESULTS

Demographic data and clinical and epidemiologic results. Of 299 patients with suspected varicella disease, 93 (66 from New Haven and 27 from Philadelphia) agreed to participate and were eligible for the study. Ages ranged from 0 to 48 years (median, 10 years), and most patients were white and non-Hispanic (Table 1). Because of differences in case ascertainment by study site, the proportion of suspected varicella disease cases that were vaccine modified varied by age and race. Of note, no vaccinated case patients had received >1 dose of varicella vaccine. Vesicle or scab lesions sampled from 53 of 93 enrollees tested positive for VZV DNA, including specimens obtained from 20 of 27 enrollees who had no history of varicella disease or vaccination, 3 of 11 who had a history of varicella, 27 of 50 who had been vaccinated, and 3 of 5 whose disease (4 patients) or vaccination (1 patient) status was unknown (Figure 1). Vesicle and scab PCR results for remaining enrollees were either negative (15 of 93) or indeterminate (unavailable or discordant; 25 of 93).

Clinical characteristics of the 53 case patients and 15 non–case patients are shown in Table 2. Vaccine-modified cases-tients with vaccine-modified disease had fever, ⩾50 lesions, or vesicles.

Diagnostic performance. Data regarding specimen and test performance are shown in Tables 3–5. Only 3 confirmed case patients had a history of varicella disease (performance was not assessable because of the small sample size).

Among 20 case patients classified as having unmodified varicella disease (ie, they had no history of varicella disease or vaccination), PCR testing of macular and/or papular lesions, cheek and throat swab samples, and oral fluid samples collected during the first visit was 95%–100% sensitive (Table 3). Just 4 of 15 specimens were deemed adequate for DFA testing; 3 of these 4 tested positive. High clinical suspicion of varicella disease by clinicians was 100% sensitive. During the second visit, oral specimens were collected from almost all case patients with unmodified varicella disease, and scab, urine, and blood samples were obtained from approximately one-half of them. None of these participants had vesicles, and only 1 had a macular and/or papular lesion at this visit. PCR testing of scab specimens was 90% sensitive; it was considerably less sensitive for oral, urine, and blood specimens, although some samples had positive test results up to 20 days after rash onset.

During the first visit, PCR testing of macular and/or papular lesion, throat swab, and oral fluid samples obtained from 27 enrollees with confirmed vaccine-modified varicella disease was 70%–100% sensitive (Table 4). Sensitivity did not change by interval between rash onset and specimen collection during the 5 days after rash onset (data not shown). Sensitivity of PCR testing of macular and/or papular lesions also did not vary by lesion number among case patients with <50 lesions (data not shown). There were only 3 patients with vaccine-modified disease with whole-blood specimens. High clinical suspicion of varicella disease was 85% sensitive. At the second visit, we collected oral and urine specimens from almost all enrollees in this group and scab specimens from 14 (52%) of them. Only 1 case patient had a vesicle; it tested negative. PCR testing of scab specimens was 69% sensitive. Sensitivity was low for other specimens, although some samples had positive results up to 17 days after rash onset.

Varicella disease was excluded for 15 patients. We were able to use their results to determine that the diagnostic tests were specific (Table 5). Low clinical suspicion of varicella by clinicians was 70% specific.

We did not have sufficient number of participants to assess sensitivity of either clinical or laboratory diagnosis by day number following rash onset. For all enrollees tested positive by PCR, VZV was wild-type.

Discordance in gold standard results. We established PCR results from a vesicle or scab specimen collected at the first visit as our gold standard which to classify varicella case-status. To support this choice, we attempted to collect at least 2 vesicle or scab specimens at the first visit to see whether they yielded concordant results.

At the first visit, we obtained 97 specimens (2–8 per patient) from 20 enrollees with unmodified varicella disease; PCR results for all 97 specimens were concordant-positive. We obtained 13 specimens (2–6 per patient) from 4 enrollees with prior varicella disease; PCR results for specimens from 3 of 4 case patients were concordant-positive. The patient with disconcordant results had 2 vesicles or scabs with negative PCR results and 1 scab with positive PCR results. We obtained 106 specimens (1–6 per patient) from the 29 enrollees with vaccine-modified varicella disease; PCR results from 27 of 29 were concordant-positive. The 2 patients with disconcordant results each had 3–5 vesicles or scabs test positive by PCR and 1 vesicle test negative by PCR. Finally, we obtained 38 specimens (1–5 per patient) from 15 confirmed non–case patients; all PCR results were

Table 1. Demographic Characteristics and Clinical Diagnosis, by Vaccination and Disease History, in Patients with Suspected Cases of Varicella Disease

Variable, group	No. (%) of enrollees			
	Unvaccinated, no history (unmodified varicella) (n = 27)	Unvaccinated, prior history (n = 11)	Vaccinated, no history (vaccine-modified varicella) (n = 50)	Unknown vaccination or disease history (n = 5)
Study site				
Philadelphia, PA	14 (52)	7 (64)	3 (6)	3 (60)
New Haven County, CT	13 (48)	4 (36)	47 (94)	2 (40)
Age group, years				
0–4	9 (33)	0 (0)	8 (16)	0 (0)
5–19	11 (41)	8 (73)	42 (84)	4 (80)
⩾20	7 (26)	3 (27)	0 (0)	1 (20)
Sex				
Male	18 (67)	4 (36)	27 (54)	5 (100)
Female	9 (33)	7 (64)	23 (46)	0 (0)
Ethnicity				
Hispanic	7 (26)	0 (0)	5 (10)	1 (20)
Non-Hispanic	20 (74)	11 (100)	45 (90)	4 (80)
Race				
Black	12 (44)	4 (36)	3 (6)	1 (20)
White	14 (52)	7 (64)	46 (92)	4 (80)
Other	1 (4)	0 (0)	1 (2)	0 (0)
Chickenpox likely diagnosis[a]				
Yes	22 (88)	9 (90)	38 (83)	4 (80)
No	3 (12)	1 (10)	8 (17)	1 (20)
Unknown	2	1	4	0
Certainty of diagnosis when chickenpox was likely diagnosis				
Uncertain (rated 1–3)[b]	3 (14)[c,d]	5 (56)[c,e]	8 (21)[c,f]	3 (75)[c,g]
Highly certain (rated 4–5)[b]	19 (86)[c,d]	4 (44)[c,e]	30 (79)[c,f]	1 (25)[c,g]

[a] Percentage of enrollees excludes enrollees categorized as "unknown" and calculated only among those with known information.
[b] At the first patient visit, diagnosing study nurse or physician rated the clinical certainty of their diagnosis of varicella disease on a 5-point scale, with 1 being unlikely and 5 being very likely.
[c] No. (%) of suspect case-patients among those the physician or study nurse thought varicella was the most likely diagnosis.
[d] Data are for 22 patients.
[e] Data are for 9 patients.
[f] Data are for 38 patients.
[g] Data are for 4 patients.

negative concordant. In total, our gold standard provided discordant results for 3 (4%) of 68 suspect VZV cases.

DISCUSSION

Varicella vaccination rates have reached high levels in the United States [1]. The resulting decrease in the incidence of varicella disease, coupled with its modification among vaccinated individuals, is likely to lead to increasing uncertainty in the clinical diagnosis of varicella and greater reliance on the laboratory testing. To our knowledge, this is the first study to systematically evaluate a wide range of laboratory procedures for their ability to diagnose varicella in both vaccinated and unvaccinated people. We tested specimens collected at the time of rash onset to inform clinicians seeing patients with acute illness, and again 2 weeks after rash onset, to assist public health workers who are often only able to investigate sporadic or outbreak-associated cases after resolution of the illness. We attempted to determine whether alternatives to PCR testing exist when skin lesions are no longer available for sampling.

PCR testing of vesicles or scabs sampled during early illness provides sensitive and specific evidence of varicella [17–22], and previous studies have revealed it to be considerably more sensitive than shell viral culture or standard culture [18, 19, 23]. This test served as the gold standard for our study. We found that almost all PCR results were concordant, providing reassuring evidence about the performance of our gold stan-

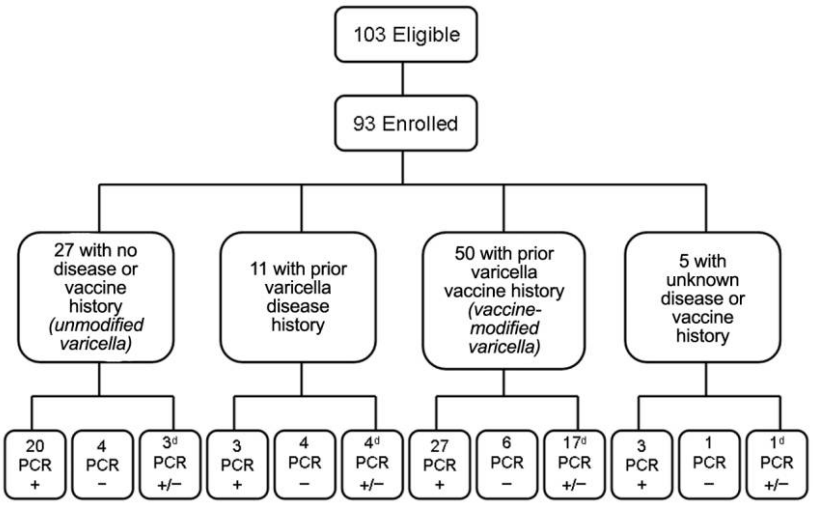

Figure 1. Study enrollment flowchart—final enrollee varicella classification (positive, negative, or indeterminate) as determined by polymerase chain reaction (PCR) testing. +, positive; -, negative; +/-, indeterminate. [a]PCR result from a vesicle or scab specimen collected at the first visit was defined as the gold standard and used to classify the disease status of suspect case-patients. [b]Of 93 study participants, 12 had equivocal whole-cell enzyme-linked immunoassay (ELISA) immunoglobulin (Ig) G results from either the first or second visit. Of the 12 equivocal results, 3 were reclassified as IgG positive, 5 were re-classified as IgG negative, and the remaining 4 could not be classified on the basis of glycoprotein ELISA (gpELISA) results. Among the laboratory-confirmed unvaccinated case patients, there was 1 who was reclassified as having a negative IgG as determined on the basis of the gpELISA result and 1 who could not be reclassified. There were 2 laboratory-confirmed vaccinated case patients with equivocal whole-cell IgG ELISA results, which were re-tested with gpELISA: one was reclassified as having a positive IgG, and the other's results remained equivocal after gpELISA testing. Of the 93 participants, 20 had negative whole-cell ELISA IgG results for specimens obtained during either the first or second visit; all remained IgG negative after gpELISA testing. [c]There were 14 unvaccinated enrollees with both disease history information and avidity test results, which were concordant with the exception of a 1-month old infant, who had reported no prior varicella disease history, but had a high varicella zoster virus (VZV) IgG avidity result. The high avidity in this case is likely from maternal antibodies and therefore this enrollee was classified as unvaccinated with no prior disease history. There were 3 vaccinated enrollees with both disease history information and avidity test results. All had no prior varicella disease history, but had high VZV IgG avidity results; their high avidity is likely due to their varicella vaccination. [d]Varicella status for enrollees categorized as PCR +/− was defined as indeterminate because they were either missing a vesicle or scab sample at the first visit or they had disconcordant vesicle or scab PCR results in the first visit. All but 3 cases were classified as indeterminate because the patients were missing a vesicle and scab specimen at the first visit; the 3 exceptions included 1 unvaccinated patient with a prior disease history and 2 vaccinated patients.

dard. We found that PCR testing of macular and/or papular lesions collected soon after rash onset also yielded sensitive results. These findings are particularly important, because vaccine-modified varicella disease often manifests with macular and/or papular lesions only [3, 24]. Sensitivity did not vary by number of total lesions during rash, suggesting that any available lesions should be adequate. PCR testing of oral specimens was also sensitive, particularly among case patients with unmodified varicella disease. Both skin lesions and oral specimens yielded specific results as well. All tests were less useful 2 weeks after rash onset; skin lesions were not readily available once the illness resolved, and test results for oral specimens were mostly negative.

Other tests were less valuable for diagnosing varicella [10, 17, 25]. DFA requires properly collected specimens and yielded mostly indeterminate results, even in the hands of experienced staff. IgM was insensitive, although one study found IgM to be 75% sensitive among vaccine-modified cases [10]. Although the reason for this discrepancy is unknown, it may be due to the timing of specimen collection; 78% of vaccine-modified cases in our study were sampled 0–3 days after rash onset, whereas 77% of cases in Weinmann et al [10] were sampled at 1–7 days. When detectable, VZV-specific IgM antibody is an indication of recent exposure to the virus, but it may not discriminate between primary infection, reinfection, or reactivation [10, 17]. Documentation of increases in IgG antibody titers requires collection of 2 specimens, making it unsuitable for diagnosing varicella disease during the acute illness. Furthermore, increases in IgG titers may be difficult to demonstrate in people with preexisting titers due to vaccination or previous infection. During primary infection, IgG responses may not be detectable at the acute time-point and are probably still developing 2 weeks after rash onset [26]; end point titration did not improve our test sensitivity. Urine yielded insensitive results. Although VZV in urine or oral fluid specimens should all be cell-associated, false-negative results could have been possible if some VZV occurred in a cell-free state.

Oral specimens and urine samples do not appear to offer clear advantages for diagnosing varicella disease, particularly because skin lesions can be sampled readily and with minimal

Table 2. Clinical Characteristics of Case Patients and Non–Case Patients

Variable, group	No. (%) of enrollees				
	Case patients				
	Unvaccinated, no history (unmodified varicella) (n = 20)	Unvaccinated, prior history (n = 3)	Vaccinated, no history (vaccine-modified varicella) (n = 27)	Unknown vaccination or disease history (n = 3)	Non-case patients (n = 15)
Fever (temperature, ⩾37.25°C [⩾99°F])[a,b]					
Yes	13 (65)	1 (33)	11 (42)	1 (33)	1 (7)
No	7 (35)	2 (67)	15 (58)	2 (67)	13 (93)
Unknown	0	0	1	0	1
Itchy rash[b]					
Yes	16 (89)	3 (100)	24 (92)	3 (100)	8 (53)
No	2 (11)	0 (0)	2 (8)	0 (0)	7 (47)
Unknown	2	0	1	0	0
No. of lesions					
<50	2 (10)	1 (33)	13 (48)	0 (0)	8 (53)
⩾50	18 (90)	2 (67)	14 (52)	3 (100)	7 (47)
Type of lesions[c]					
Macules	7 (35)	1 (33)	22 (81)	1 (33)	10 (67)
Papules	11 (55)	1 (33)	19 (70)	1 (33)	10 (67)
Pustules	9 (45)	0 (0)	4 (15)	0 (0)	3 (20)
Scabs	14 (70)	3 (100)	24 (89)	2 (67)	7 (47)
Vesicles	15 (75)	1 (33)	11 (41)	3 (100)	4 (27)
Chickenpox likely diagnosis[b]					
Yes	19 (100)	2 (67)	26 (100)	3 (100)	9 (90)
No	0 (0)	1 (33)	0 (0)	0 (0)	1 (10)
Unknown	1	0	1	0	5
Certainty of diagnosis when chickenpox likely diagnosis					
Uncertain (rated 1–3)[d]	0 (0)[e]	0 (0)[e]	4 (15)[e]	0 (0)[e]	6 (67)[e]
Highly certain (rated 4–5)[d]	19 (100)[e]	2 (100)[e]	22 (85)[e]	3 (100)[e]	3 (33)[e]

[a] Measured tactilely or by thermometer.
[b] Percentage of enrollees excludes enrollees categorized as unknown and calculated only among those with known information.
[c] Represents whether type of lesion was present during rash. Categories are not mutually exclusive, so cases may have involved multiple types of lesions.
[d] At the first patient visit, diagnosing study nurse or physician rated the clinical certainty of their diagnosis of varicella disease on a 5-point scale, with 1 being unlikely and 5 being very likely.
[e] No. (%) of enrollees calculated among those where the physician or study nurse thought varicella disease was the most likely diagnosis.

invasiveness. However, in select circumstances, such unconventional specimens can serve as useful alternatives, because they are easy to obtain and can sometimes yield positive results after skin lesions have resolved. This might be particularly relevant in the setting of outbreak investigations in which many children need to be evaluated after their rashes have cleared. Better laboratory tools are still needed to fill this public health need.

Among our study participants with unmodified or vaccine-modified varicella disease, clinical diagnosis was 100% and 85% sensitive, respectively. We were able to rule out cases of suspected varicella disease through laboratory testing, allowing us to determine that the specificity of clinical diagnosis was 70%. Our results suggest that clinical diagnosis of varicella can be accurate, even among vaccinated persons, particularly when the clinician is confident about the diagnosis. Scabs, recognized exposure to varicella or herpes zoster disease, and school attendance have been found to support the diagnosis of vaccine-modified varicella disease [27].

Eleven study participants reported prior episodes of varicella disease, but only 3 had evidence of prior disease. Second episodes of varicella disease have been reported in the literature [28–31], including one study suggesting that the proportion of such cases is increasing [31], perhaps in association with reduced VZV-specific immunity as exposures to varicella decreases or with careful case seeking for vaccine-modified disease. Avidity testing may provide a useful tool to monitor this phenomenon more comprehensively.

Table 3. Sensitivity of Clinical Diagnosis and Laboratory Testing among 20 Persons with Unmodified Varicella Disease

Visit no., diagnostic test	No. of specimens collected	No. of specimens found to be adequate	No. of VZV- positive specimens	Sensitivity, %[a] (95% CI)
Visit 1[b]				
Clinical diagnosis (rated 4–5)[c]	19	NA	19	100 (82–100)
Vesicle swab DFA	15	4	3	75 (19–99)
Vesicle slide DFA	0	0	0	—
Macular and/or papular swab PCR	15	15	15	100 (78–100)
Macular and/or papular slide PCR	7	7	7	100 (59–100)
Cheek swab PCR	20	20	19	95 (75–100)
Throat swab PCR	18	18	18	100 (81–100)
Oral fluid PCR	19	19	19	100 (82–100)
Oral fluid IgA	19	17	0	0 (0–20)
Urine PCR	15	12	7	58 (28–85)
Whole-blood PCR	12	12	5	42 (15–72)
Whole-blood IgM	12	12	3	25 (5–57)
Whole-blood IgA	12	12	2	17 (2–48)
Fingerstick IgM	13	11	2	18 (2–52)
IgG titer (4-fold increase)	9	9	3	33 (7–70)
Visit 2[d]				
Vesicle swab PCR	0	0	0	—
Vesicle slide PCR	0	0	0	—
Scab PCR	11	10	9	90 (56–100)
Vesicle swab DFA	0	0	0	—
Vesicle slide DFA	0	0	0	—
Macular and/or papular swab PCR	1	1	0	0 (0–98)
Macular and/or papular slide PCR	1	1	0	0 (0–98)
Cheek swab PCR	17	16	1	6 (0–30)
Throat swab PCR	17	16	3	19 (4–46)
Oral fluid PCR	17	16	5	31 (11–59)
Oral fluid IgA	17	16	0	0 (0–21)
Urine PCR	12	9	1	11 (0–48)
Whole-blood PCR	9	8	2	25 (3–65)
Whole-blood IgM	9	8	2	25 (3–65)
Whole-blood IgA	9	8	1	13 (0–53)
Fingerstick IgM	11	9	2	22 (3–60)

NOTE. Polymerase chain reaction (PCR) results from a vesicle or scab specimen collected at the first visit were defined as the gold standard and used to classify disease status of case-patients. Dashes (—) indicate value was not calculated because adequate sample was not obtained. CI, confidence interval; DFA, direct fluorescent antibody; Ig, immunoglobulin; NA, not applicable; PCR, polymerase chain reaction; VZV, varicella zoster virus.

[a] No. of VZV-positive specimens/no. of adequate specimens.

[b] For 17 (85%) of 20 enrollees, the first visit took place 0–5 days (range, 0–9) after rash onset.

[c] At the first patient visit, diagnosing study nurse or physician rated the clinical certainty of their diagnosis of varicella disease on a 5-point scale, with 1 being unlikely and 5 being very likely.

[d] For 15 (88%) of 17 enrollees having 2 visits, the second visit took place 13–17 days (range, 13–20 days) after rash onset.

There were limitations to our study. It was conducted using experienced staff, in communities with clinicians that had participated in prior varicella surveillance activities. The quality of case finding, clinical recognition, and sample collection may therefore have differed from other settings, which could have affected assay performance, particularly for PCR testing of macular and/or papular lesions, for which proper specimen collection is important. Persons with mild modified varicella disease may not have sought medical attention or the diagnosis may have otherwise not been considered.

As the epidemiology of varicella disease changes, it has become increasingly important to test suspected cases to obtain a clinical diagnosis for case management and outbreak investigation and to monitor the impact of the varicella vaccine

Table 4. Sensitivity of Clinical Diagnosis and Laboratory Testing among 27 Persons with Vaccine-Modified Varicella Disease

Visit no., diagnostic test	No. of specimens collected	No. of specimens found to be adequate	No. of VZV- positive specimens	Sensitivity, %[a] (95% CI)
Visit 1[b]				
Clinical diagnosis (rated 4–5)[c]	26	NA	22	85 (65–96)
Vesicle swab DFA	6	1	0	0 (0–98)
Vesicle slide DFA	1	0	0	—
Macular and/or papular swab PCR	24	24	24	100 (86–100)
Macular and/or papular slide PCR	20	18	18	100 (81–100)
Cheek swab PCR	27	26	16	62 (41–80)
Throat swab PCR	27	27	19	70 (50–86)
Oral fluid PCR	27	26	22	85 (65–96)
Oral fluid IgA	27	27	0	0 (0–13)
Urine PCR	26	24	8	33 (16–55)
Whole-blood PCR	3	3	0	0 (0–71)
Whole-blood IgM	3	3	1	33 (1–91)
Whole-blood IgA	3	2	0	0 (0–84)
Fingerstick IgM	22	18	3	17 (4–41)
IgG titer (4-fold increase)	1	1	1	100 (3–100)
Visit 2[d]				
Vesicle swab PCR	1	1	0	0 (0–98)
Vesicle slide PCR	0	0	0	—
Scab PCR	14	13	9	69 (39–91)
Vesicle swab DFA	0	0	0	—
Vesicle slide DFA	1	0	0	—
Macular and/or papular swab PCR	5	5	3	60 (15–95)
Macular and/or papular slide PCR	5	4	1	25 (1–81)
Cheek swab PCR	25	24	0	0 (0–14)
Throat swab PCR	24	23	1	4 (0–22)
Oral fluid PCR	25	25	4	16 (5–36)
Oral fluid IgA	25	24	0	0 (0–14)
Urine PCR	22	19	0	0 (0–18)
Whole-blood PCR	1	1	1	100 (3–100)
Whole-blood IgM	1	1	0	0 (0–98)
Whole-blood IgA	1	1	0	0 (0–98)
Fingerstick IgM	19	14	4	29 (8–58)

NOTE. Polymerase chain reaction (PCR) results from a vesicle or scab specimen collected at the first visit were defined as the gold standard and used to classify disease status of case-patients. Dashes (—) indicate value was not calculated because adequate sample was not obtained. CI, confidence interval; DFA, direct fluorescent antibody; Ig, immunoglobulin; NA, not applicable; PCR, polymerase chain reaction; VZV, varicella zoster virus.

[a] No. of VZV-positive specimens/no. of adequate specimens.

[b] For 21 (78%) of 27 enrollees, the first visit took place 0–3 days (range, 0–5 days) after rash onset.

[c] At the first patient visit, diagnosing study nurse or physician rated the clinical certainty of their diagnosis of varicella disease on a 5-point scale, with 1 being unlikely and 5 being very likely.

[d] For 20 (80%) of 25 enrollees having 2 visits, the second visit took place 12–17 days (range, 12–19 days) after rash onset.

program. We have shown that PCR testing of skin lesions is highly sensitive and specific for detecting VZV, and oral specimens can play a supporting diagnostic role in certain settings. However, PCR testing is not universally available, and better tests would be useful for public health workers to diagnose varicella disease after transient lesions have cleared. This is particularly important in outbreak situations, in which accurate case ascertainment is critical for evaluating the impact and effectiveness of varicella vaccine. For now, clinicians and public health workers should be encouraged to request PCR testing of suspected cases of varicella disease, and public and commercial laboratories should be encouraged to conduct such testing. Lastly, the research community should be encouraged to develop new methods for diagnosing varicella and ascer-

Table 5. Specificity of Clinical Diagnosis and Laboratory Testing among 15 Persons with Rash-Illnesses for Whom Varicella Disease Was Excluded

Visit no., diagnostic test	No. of specimens collected	No. of specimens found to be adequate	No. of VZV- negative specimens	Specificity, %[a] (95% CI)
Visit 1				
Clinical diagnosis (not likely varicella or certainty diagnosis rated 1–3)[b]	10	NA	7	70 (30–93)
Vesicle swab DFA	7	0	0	—
Vesicle slide DFA	0	0	0	—
Macular and/or papular swab PCR	13	11	11	100 (72–100)
Macular and/or papular slide PCR	11	9	9	100 (66–100)
Cheek swab PCR	15	15	15	100 (78–100)
Throat swab PCR	15	15	15	100 (78–100)
Oral fluid PCR	15	15	15	100 (78–100)
Oral fluid IgA	15	14	14	100 (77–100)
Urine PCR	11	11	11	100 (72–100)
Whole-blood PCR	3	3	3	100 (29–100)
Whole-blood IgM	4	3	3	100 (29–100)
Whole-blood IgA	4	4	4	100 (40–100)
Fingerstick IgM	13	9	9	100 (66–100)
IgG titer (4-fold increase)	3	3	3	100 (29–100)
Visit 2				
Vesicle swab PCR	7	5	5	100 (48–100)
Vesicle slide PCR	7	7	7	100 (59–100)
Scab PCR	9	9	9	100 (66–100)
Vesicle swab DFA	0	0	0	—
Vesicle slide DFA	0	0	0	—
Macular and/or papular swab PCR	2	1	1	100 (3–100)
Macular and/or papular slide PCR	2	1	1	100 (3–100)
Cheek swab PCR	12	11	10	91 (59–100)
Throat swab PCR	12	11	11	100 (72–100)
Oral fluid PCR	12	11	10	91 (59–100)
Oral fluid IgA	12	12	12	100 (74–100)
Urine PCR	11	11	11	100 (72–100)
Whole-blood PCR	3	3	3	100 (29–100)
Whole-blood IgM	3	2	2	100 (16–100)
Whole-blood IgA	3	3	3	100 (29–100)
Fingerstick IgM	10	9	9	100 (66–100)

NOTE. Polymerase chain reaction (PCR) results from a vesicle or scab specimen collected at the first visit were defined as the gold standard and used to classify disease status of case-patients. These 15 enrollees include all enrollees, regardless of varicella vaccination and disease history, who were laboratory-confirmed as not being a varicella case. Dashes (—) indicate value was not calculated because adequate sample was not obtained. CI, confidence interval; DFA, direct fluorescent antibody; Ig, immunoglobulin; NA, not applicable; PCR, polymerase chain reaction; VZV, varicella zoster virus.

[a] No. of VZV-negative specimens/no. of adequate specimens.

[b] Represents the number of non-cases with available data on physician diagnosis and a rating for certainty of diagnosis if the physician thought that varicella might be a likely diagnosis on a 5-point scale, with 1 being unlikely and 5 being very likely. One case was not diagnosed as likely varicella by the clinician; nine cases were thought to be likely varicella by the clinician and had available data on the rating of certainty of diagnosis; and 5 cases were missing clinician diagnosis

taining recent VZV exposure (eg, the analysis of VZV-specific T and B cells, memory B cells, and activation markers in peripheral blood lymphocytes) in the absence of remaining lesions.

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