Testing for sexually transmitted infections (STIs) in children presents a number of problems for the practitioner that are not usually faced when testing adults for the same infections. The identification of an STI in a child can have, in addition to medical implications, serious legal implications. The presence of an STI is often used to support the presence or allegations of sexual abuse and, in some cases, may prompt an investigation of possible abuse. The purpose of this paper is to review the recent data on the epidemiology of child sexual abuse including the epidemiology of major STIs (Neisseria gonorrhoeae, Chlamydia trachomatis, syphilis, herpes simplex virus, Trichomonas vaginalis, and human papillomavirus) and summarize the current recommendations for diagnostic testing in this population.

METHODS

A literature review was performed using PubMed: papers published since 2005 were searched, using the following terms: child sexual abuse, assault, sexually transmitted infections, gonorrhea, Neisseria gonorrhoeae, C. trachomatis, Trichomonas vaginalis, syphilis, herpes simplex virus (HSV) type 2, human papillomavirus (HPV), and HIV. The review also included abstracts of Interscience Conference on Antimicrobial Agents and Chemotherapy, Infectious Diseases Society of America, Pediatric Academic Societies, 11th International Chlamydia Symposium, International Society for Sexually Transmitted Disease Research, and the Canadian STI Treatment Guidelines.

Epidemiology of Sexually Transmitted Infections in Children Being Evaluated for Suspected Sexual Abuse

Four studies examining the epidemiology of STIs in children and adolescents being evaluated for suspected sexual abuse have been published since 2005 (Table 1). Three were retrospective chart reviews, including 1 each from Vienna, Austria [8]; Auckland, New Zealand [9]; and Miami, Florida [10]. Despite differences in population and methodologies, the results of the retrospective studies were fairly consistent. Charts were examined from a total of 4350 children, who were seen...
were found to have enrolled were positive for any STI. Overall, 40 (8.2%) of the girls were enrolled; 485 (90.5%) were female. None of the 51 boys type-specific antibody for HSV-1 and -2. A total of 536 children presented. Sera were also obtained for testing for syphilis, HIV, and T. vaginalis amplification tests (NAATs). Wet mounts were performed for C. trachomatis, Neisseria gonorrhoeae, Chlamydia trachomatis and cultures for HSV were done if lesions were consistent with earlier published studies [11]. As these were retrospective chart reviews, they had a number of major limitations. There were different inclusion criteria and not every child or adolescent was tested for every STI. Only 5 of the 180 subjects reviewed by Kohlberger and Bancher-Todesca [8] were tested for syphilis; 31% were tested for N. gonorrhoeae and 34.4% were tested for C. trachomatis, but 75.5% were tested for trichomonas. In contrast, testing for STIs was done in 73% of the subjects reviewed by Simmons and Hicks [10] and, depending on the STI, prevalence of STIs varied from site to site, ranging from 1.7% in Texas to 7.8% in Atlanta.

### Table 1. Prevalence of Sexually Transmitted Infections in Children Being Evaluated for Sexual Abuse: Selected Studies Published Since 2005

<table>
<thead>
<tr>
<th>Study</th>
<th>No. positive/tested (%)</th>
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<tbody>
<tr>
<td>Simmons &amp; Hicks [10]</td>
<td>2763 (100)</td>
</tr>
<tr>
<td>Girardet et al [12]</td>
<td>536 (90.5)</td>
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<td>Kelly &amp; Koh [9]</td>
<td>2162 (85.8)</td>
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<tr>
<td>Kohlberger &amp; Bancher-Todesca [8]</td>
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</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV, herpes simplex virus; ND, not done; NS, not specified.

a Propective study.

b Denominator represents females; none of the males were positive for any STI.

c Testing only done in children with lesions suggestive of HSV.

d Retrospective chart review.

e Number of children tested not stated.

Diagnosis of Sexually Transmitted Infections in Children N. gonorrhoeae

The identification of N. gonorrhoeae in a child beyond the neonatal period is indicative of some kind of sexual contact. The Centers for Disease Control and Prevention (CDC) 2006 STD Treatment Guidelines recommends that specimens for culture of N. gonorrhoeae be collected from the pharynx and anus of girls and boys, the vagina in girls, and the urethra in boys for evaluation of suspected sexual abuse [7]. The 2006 CDC guidelines also specifically recommend that Gram-stained smears not be used for the diagnosis of gonorrhea in children. A major change in the 2011 guidelines is the inclusion of recommendations for the use of NAATs for detection of N. gonorrhoeae and C. trachomatis in children. Use of NAATs has supplanted standard culture methods for N. gonorrhoeae in many laboratories. All of the currently available assays have US Food and Drug Administration (FDA) approval for use in genital sites (cervix, vagina, urethra) and urine from adolescents and adults; however, none are approved for extragenital sites in adults (pharynx or rectum) or for any site in children.

during periods ranging from 4 to 7 years. Children’s ages ranged from 0 to 17 years and the overwhelming majority was female. The prevalence of STIs, specifically gonorrhea and C. trachomatis, was low, ranging from 0.4 to 1.8%. No child was found to have syphilis or HIV by serology. These findings are consistent with earlier published studies [11]. As these were retrospective chart reviews, they had a number of major limitations. There were different inclusion criteria and not every child or adolescent was tested for every STI. Only 5 of the 180 subjects reviewed by Kohlberger and Bancher-Todesca [8] were tested for syphilis; 31% were tested for N. gonorrhoeae and 34.4% were tested for C. trachomatis, but 75.5% were tested for trichomonas. In contrast, testing for STIs was done in 73% of the subjects reviewed by Simmons and Hicks [10] and, depending on the STI, prevalence of STIs varied from site to site, ranging from 1.7% in Texas to 7.8% in Atlanta.
To date, 3 studies have been published that compared NAATs to *N. gonorrhoeae* culture in children being evaluated for suspected sexual abuse [13–15]. All included urine specimens as well as vaginal swabs. Although the results of the 2 earlier studies suggested that the sensitivity of NAATs for *N. gonorrhoeae* was similar to culture, both studies had several serious limitations [13, 14]. The populations studied were predominantly female and included adolescents up to 18 and 20 years of age, many of whom reported consensual sexual activity; only 23.7% and 41.5% of the children enrolled were prepubertal [13, 14]. Both studies utilized ligase chain reaction (LCR, LCx, Abbott Diagnostics), which was taken off the market in 2002 because of specificity concerns for detection of *N. gonorrhoeae* [16]. One study evaluated polymerase chain reaction (PCR) in addition to LCR [14]. Other study limitations included the failure to use an independent reference standard in estimating test performance and failure to separately analyze test performance by age and gender (when applicable). The prevalence of *N. gonorrhoeae* infection in both studies was low (1.9% and 3.2%), reducing the precision of sensitivity estimates. The number of extragenital specimens was also too low to assess test performance at those sites. A recently published multicenter study evaluated the use of strand displacement amplification (SDA) and transcription-mediated amplification (TMA) versus culture for diagnosis of *N. gonorrhoeae* and *C. trachomatis* in children, 0–13 years of age, evaluated for sexual abuse in 4 US cities [15]. All children were tested at multiple sites for *N. gonorrhoeae* and *C. trachomatis* by culture, and vaginal and urethral swabs were tested with SDA and urine with SDA and TMA. Cultures of *N. gonorrhoeae* were performed at all sites according to standard protocols. Positive NAATs for *N. gonorrhoeae* were confirmed by an in-house PCR using an alternate target, the Hinfl fragment of the 4.2-kb cryptic plasmid [17]. As described earlier, a total of 536 children were enrolled; none of the 51 boys were positive for *N. gonorrhoeae* by any test at any site. Of the 485 female participants, 16 (3.3%) had a positive result for *N. gonorrhoeae* by any test: 12 (2.5%) by culture, 14 (2.9%) by vaginal NAAT, and 14 (2.9%) by urine NAAT. All participants who had a positive vaginal culture for *N. gonorrhoeae* had positive urine NAATs. There were discrepant results in 2 cases (both SDA-positive and TMA-negative). One of these girls was positive by urine testing and negative by vaginal swab, the other was positive both by urine and swab analysis. All SDA-positive results for *N. gonorrhoeae* were confirmed to be true positives by a species-specific *N. gonorrhoeae* PCR. Three girls had discrepant results by site: 2 were vaginal-swab positive and urine negative, and 1 was vaginal-swab negative and urine positive. The results of this study suggest that SDA and TMA may be alternatives to culture for the detection of *N. gonorrhoeae* in vaginal swabs and urine in prepubertal girls.

Not all NAATs perform equivalently. PCR and SDA have both been demonstrated to have cross-reactivity with other *Neisseria* species including *N. cinerea*, *N. flavescens*, *N. lactamica*, *N. sicca*, and *N. subflava* [18–20]. This can have important implications especially when testing extragenital sites, especially the pharynx. Although false-positive results were not observed with SDA in the multicenter study [15], it still remains a possibility, especially if the assay is used more widely. Additional testing is still essential as the prevalence of gonorrhea in children being evaluated for suspected sexual abuse is low; low positive predictive values have been reported in low-prevalence adult populations [21]. It is important to emphasize that one cannot extrapolate from these results to other NAATs, specifically PCR, or use in extragential specimens (pharynx, rectum) or in any site in boys.

**C. trachomatis**

The prevalence of *C. trachomatis* infection in abused children is also low (Table 1). *C. trachomatis* genital infection in children is frequently asymptomatic and may persist for months to years [22]. The 2006 CDC guidelines recommended that children being evaluated for suspected sexual abuse be tested for *C. trachomatis* at the anus in both boys and girls and from the vagina in girls [7]. Pharyngeal specimens from children of either sex are not recommended, as the prevalence of *C. trachomatis* infection at this site is very low in children being evaluated for suspected sexual abuse.

Even though culture is considered the gold standard for detection of *C. trachomatis* in children being evaluated for suspected sexual abuse, *C. trachomatis* culture is not standardized or regulated in any way. Sensitivity may vary significantly from laboratory to laboratory. The isolation of *C. trachomatis* in tissue culture should be confirmed by the microscopic identification of the characteristic intracytoplasmic inclusions, preferably with a species-specific fluorescein-conjugated monoclonal antibody [7]. Use of genus-specific antibody for culture confirmation can lead to misidentification of *Chlamydia pneumoniae* as *C. trachomatis* in pharyngeal specimens [23]. Enzyme immunoassays (EIAs) are not acceptable as confirmatory tests and have been associated with false-positive results, especially when used with vaginal and rectal specimens [24, 25]. The methods used for culture confirmation became an issue when several large commercial laboratories started using EIA instead of fluorescent antibody staining and visual identification of inclusions for culture confirmation. This resulted in at least 1 outbreak of *C. trachomatis* infection in the evaluation of suspected sexual abuse among residents and staff of an institution for the mentally retarded in Ohio in 1990 [26]. All the cultures found to be positive, mostly rectal specimens, were subsequently determined to be false-positives resulting from carryover of fecal material and bacteria in the culture specimens. The major advantage of culture is that it is 100%
specific; however, because confirmation is dependent on visual identification of inclusions, there is still a subjective component that could lead to misidentification of artifacts as chlamydial inclusions. This appears to have happened in another pseudo-outbreak in a residential institution in another state [27].

NAATs are currently approved by the FDA for detection of C. trachomatis from genital sites (cervix, vagina, urethra) and urine from adolescents and adults. None are approved for extragenital sites (pharynx or rectum) in adults or have approval for any site in children. These methods have been found to have excellent sensitivity for detection of C. trachomatis, usually well above 90%, in genital specimens and urine from adult men and women, while maintaining high specificity [28]. The 2006 CDC STD treatment guidelines recommended that NAATs can be used in children being evaluated for suspected sexual abuse if culture is not available and if positive results can be confirmed [7]. Confirmation was specified as use of another NAAT that utilized a different genetic target; however, not all NAATs perform equally [29]. Use in extragenital specimens was not discussed. As with N. gonorrhoeae, data on the use of available NAATs for detection of C. trachomatis in children are limited. To date, there are 4 published studies that compared NAATs to C. trachomatis culture in children being evaluated for suspected sexual abuse [13, 14, 28]. They include the 2 previously described studies that evaluated LCR for detection of N. gonorrhoeae [13, 14], an additional study that evaluated PCR [30], and the recent multicenter study by Black et al [15], which evaluated SDA and TMA. Girardet et al [13] and Kellogg et al [14] primarily evaluated girls, most of who were postpubertal. Both studies utilized LCR, which is currently not available in the United States [16]. Kellogg et al also evaluated PCR in addition to LCR [14]. The same limitations that applied to N. gonorrhoeae apply to the evaluation of these assays for detection of C. trachomatis: failure to use an independent reference standard in estimating test performance and failure to separately analyze test performance by age and gender. A third study compared PCR and EIA to culture [28]. The ages of the children ranged from 1 month to 17 years, with a mean age of 8.6 years; 82.4% were female. Anatomic sites tested included vagina, cervix, rectum, pharynx, male urethra, and urine. The overall prevalence of C. trachomatis infection in these studies ranged from 6.6% to 8.3% by LCR and/or PCR and 0.8 to 5.3% by culture. There were many discrepant results, especially between culture and NAATs, which were more frequent with rectal specimens, but the numbers of specimens from nongenital sites was too small to accurately assess the performance of the assays at these sites.

The recent multicenter study by Black et al [15] evaluated the use of SDA and TMA using urine and genital swabs (vagina and urethra) compared with culture for diagnosis of C. trachomatis in children 0–13 years of age. Cultures for C. trachomatis were performed at the clinical or hospital laboratories of each center, according to their own protocols. Culture protocols at all sites included the isolation of C. trachomatis in cycloheximide-pretreated McCoy cells, either in shell vials or 24- or 96-well tissue culture plates. The fixed monolayers were stained to detect chlamydial inclusions with fluorescein-conjugated Chlamydia genus–specific or C. trachomatis species–specific monoclonal antibodies. All samples were processed and tested according to manufacturers’ protocols except for the TMA tests, which were performed on previously frozen urine or swabs collected in the BD ProbeTec sample collection medium. Test results that were positive by SDA for C. trachomatis were confirmed using an in-house PCR targeting the ompA gene, performed at the CDC [30]. Fifteen of 485 (3.1%) female participants had a positive result for C. trachomatis by any test (1.4% by culture; 2.3% by vaginal NAAT; 2.7% by urine NAAT). None of the male participants had any positive cultures or NAATs for C. trachomatis. All participants who had a positive vaginal culture for C. trachomatis also had positive urine NAATs. Two girls had positive C. trachomatis cultures from rectal swab specimens but negative vaginal swab specimens by both culture and NAATs, and negative urine NAATs. There were no discrepant results in any of the participants tested by SDA and TMA for C. trachomatis. All C. trachomatis-positive results were confirmed by DNA sequence genotyping. The sensitivity of vaginal culture for C. trachomatis was 39% in all girls studied. In contrast, the sensitivities of urine and vaginal swab NAATs were 100% and 85%, respectively, in all female children for detection of C. trachomatis. The results of Black et al [15] suggest that NAATs can be used for detection of C. trachomatis in girls being evaluated for suspected sexual abuse. However, the same limitations apply as those for use of these assays for detection of N. gonorrhoeae: (1) As the prevalence of C. trachomatis in this population is low, additional testing is probably necessary; (2) one cannot extrapolate from these results to other NAATs, specifically PCR, and use in specimens other than vagina and urine in girls; and (3) one cannot make any recommendations on the use of these assays in prepubertal boys. Some of the more recently available commercial NAATs, such as TMA (Aptima 2), offer an alternate target confirmation method that can be used on the same testing platform; however there are no data on the use of this confirmatory test in this setting. Additional options include sending blinded specimens to an independent or reference laboratory for confirmation testing, confirming a NAAT-positive result by culture test (requires a separate, invasive specimen), or use of an additional alternate technology (commercial NAAT). Specimens collected from children for forensic applications should be retained in the laboratory for purposes of additional testing, in accordance with local policies and procedures.
Syphilis
Although children can acquire syphilis through sexual abuse, the prevalence of syphilis in these children has been <1% in most studies [8–12]. Diagnosis of syphilis was based on serologic screening as most of these children were asymptomatic. Cases of symptomatic syphilis appear to be uncommon and are mostly limited to anecdotal reports [16]. Clinical findings have included primary chancres, and manifestations of secondary syphilis including rash and condyloma lata, which can be misdiagnosed as genital warts [31]. The major confounding variable in the diagnosis of syphilis in children beyond the neonatal period is differentiating between acquired and congenital infection. As most pregnant women in the United States are screened for syphilis during pregnancy, congenital infection could be ruled out if maternal records can be accessed. However, this may not always be possible and some the clinical manifestations of congenital syphilis may overlap with those of acquired syphilis.

Herpes simplex virus
The presence of genital herpes in a prepubertal child also raises the probability of sexual abuse. However, available data are too limited to allow estimation of the likelihood of sexual transmission. The overwhelming majority of published studies of STIs in children being evaluated for sexual abuse have limited data on HSV in children who presented with suggestive genital lesions. The prevalence of HSV infection in these studies was low, <5% [8–12]. In the multicenter study reported by Girardet et al [12], only 12 girls were found to have genital ulcers, and only 5 were culture positive for HSV. As most studies have only tested symptomatic children, we have no way of knowing how common asymptomatic infection may be in these children, even as more evidence emerges about the frequency of asymptomatic and subclinical infections in adults.

Data on use of type-specific serology for HSV-1 and HSV-2 in children are very limited. Ramos et al [32] compared HerpeSelect HSV-2, Biokit HSV-2 Rapid Test (Biokit), and Western blot in sera from 150 children being seen in a sexual abuse clinic. The ages ranged from 1 to 18 years, and 81% were female. Fifty-one percent of the children were HSV-1 seropositive by Western blot. In contrast, <1% were seropositive for HSV-2. Although 8 patients were positive for HSV-2 by at least 1 test, only 1 individual was positive by all 3 tests, an adolescent girl who reported consensual sexual activity. Six children were positive by HerpeSelect HSV-2, but negative by Biokit and Western blot, and 137 samples were consistently negative. None of the children had clinical genital herpes. Sera from 283 children enrolled by Girardet et al [11] were tested for HSV-1 and HSV-2 antibodies using an immunodot enzyme assay with a monoclonal antibody inhibition for confirmation performed at the CDC. Antibody to HSV-1 and HSV-2 was detected in 45.6% and 2.5% of the children, respectively. Three children had antibody to both HSV-1 and HSV-2. As stated previously, cultures for HSV were obtained from only 12 children who had lesions suggestive of herpes, but only 1 of 5 culture-positive children had HSV-2 antibody. These data suggest that type-specific serology for HSV has a poor predictive value for diagnosis of HSV infection in children being evaluated for suspected child abuse, on the basis of a single serum sample. Furthermore, as demonstrated by Ramos et al [32], the performance of even FDA-approved tests can be inconsistent in a low-prevalence population.

Trichomonas vaginalis
Although T. vaginalis is probably the most prevalent nonviral STI among adults in the United States, data on this infection in the setting of child sexual abuse are limited. In most published studies of STIs in sexually abused children, testing for T. vaginalis was limited to girls presenting with vaginal discharge [8–12]. Rarely, T. vaginalis can be transmitted vertically from mother to infant (vaginal, urine) during parturition [33]. These infections may persist for several months after birth. Care should be taken in interpretation when trichomonads are found in urine specimens from children collected for another purpose. Because the morphology of Pentatrichomonas (Trichomonas) hominis, a nonpathogenic intestinal flagellate, is very similar to that of T. vaginalis, care must be taken to make sure that specimens are not contaminated with fecal material; this could present a problem with bagged urine specimens.

Culture for T. vaginalis on Diamond medium has a high sensitivity (95%) and specificity (>95%) [34]. Incubation periods of 2–7 days are needed to indentify T. vaginalis in culture [34]. There are several point-of-care tests available including the Affirm VP III Microbial Identification System, which is a direct nucleic acid probe hybridization test for detection of T. vaginalis, Gardnerella vaginalis, and Candida species [35]. It has been reported to have sensitivities of 80%–90% in adult women with vaginitis [35]. The Affirm VP III has not been validated or approved for used in genital specimens from prepubertal girls or urethral specimens from men. False-positive results have been reported when used in children and adult men, with serious legal and social consequences (M. R. Hammerschlag, personal communication). GenProbe has introduced analytes for a TMA-based NAAT for T. vaginalis (APTIMA TV) [36]; preliminary studies have demonstrated that this assay is highly sensitive and specific compared with wet mount microscopy, culture, and in-house PCR for the diagnosis of trichomoniasis in women and men [36]. However, there are no data on use in children of either sex.

Human papillomavirus
The association of genital warts and sexual abuse in children is complicated by the long period of latency before lesions become clinically apparent and possibility of nonsexual transmission, either vertically during delivery or horizontally after birth. The criteria for diagnosis of HPV infection in children, clinical versus
detection of HPV DNA, are also not standardized. Most published studies of HPV infection in children being evaluated for sexual abuse have relied on the presence of clinical lesions consistent with genital warts for the diagnosis of HPV infection [2, 8–12]. Given the possible role of perinatal transmission for anogenital and respiratory HPV (laryngeal papillomatosis) infection, an important issue has been determining at what age is the presence of HPV infection due to sexual abuse. Although studies and reviews of the subject have suggested that 24 months of age was the upper limit for anogenital warts in children to be secondary to perinatal transmission, a significantly longer incubation period, ≥5 years, has been suggested for laryngeal papillomatosis [4]. Current recommendations for use of the quadrivalent HPV vaccine, which contains HPV 6, 11, 16, and 18, may potentially affect the risk of HPV infection in children who have been sexually abused.

The diagnosis of HPV infection in children has been primarily clinical. Lesions suspicious for HPV should be biopsied and tested for HPV. Several studies have evaluated detection of HPV DNA in children being evaluated for suspected sexual abuse; however, the results have been contradictory [2–5, 37]. Studies published since 2000 have demonstrated poor correlation of detection of HPV DNA by PCR and presence of genital warts [3–5]. There is also a great deal of heterogeneity of the PCR methods used. Due to the large number of HPV types involved in genital disease, type-specific PCR assays are not practical for detection of HPV. Most have used generic primers followed by either probing or sequencing of the products. Nested PCR assays were used in some studies. Use of generic HPV PCRs may be less sensitive than specific HPV 16 PCR. Even so, the association of the presence of HPV DNA with abuse is not very strong: although HPV DNA has been detected in genital and rectal swabs in 15% of girls thought to be abused, it has also been detected in vaginal and/or anal specimens from 2.1% of healthy children with no history of abuse [3–5, 37, 38].

**SUMMARY**

Although the introduction of nonculture methods, especially NAATs, has greatly facilitated the diagnosis of genital infections due to *C. trachomatis* and *N. gonorrhoeae* in adults, data on use of these tests in children are limited. One cannot extrapolate from the performance of NAATs in genital sites in adults to genital and extragenital (rectum, pharynx) sites in children. The prevalence of STIs, including *C. trachomatis* and *N. gonorrhoeae*, in children being evaluated for suspected sexual abuse are low, often <3%, which influences their positive predictive value. Misidentification of an STI in a child can have serious legal ramifications. Because of the legal implications, specimens obtained from children should be retained for additional testing, a practice that is not universally followed. Microbiology is also increasingly being outsourced to commercial laboratories, which can complicate testing for STIs in children, especially outside academic medical centers. Unfortunately, accuracy may not be the top concern when these laboratories select tests. Many laboratories have difficulty in appropriately triaging specimens from children. There is a clear need for additional and continuing studies of diagnostic testing in this population, especially as new tests are introduced. We should also consider establishing a national network of designated laboratories to do this testing.

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**References**


