CLINICAL MICROBIOLOGY REVIEWS, July 2010, p. 493–506 Vol. 23, No. 3
0893-8512/10/$12.00 doi:10.1128/CMR.00024-09

Medical and Legal Implications of Testing for Sexually Transmitted Infections in Children

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INTRODUCTION

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, in addition to medical implications, can have serious legal implications. The presence of an STI is often used to support the presence or allegations of sexual abuse, and conversely, the identification of an STI in a child will prompt an investigation of possible abuse. The significance of the identification of a sexually transmitted agent in such children as evidence of possible child sexual abuse varies by pathogen. Although the identification of sexually transmissible agents in children beyond the neonatal period suggests sexual abuse, exceptions do occur. Perinatally acquired rectal or vaginal *Chlamydia trachomatis* infection may persist for 2 to 3 years after birth (7). Genital warts have been diagnosed for children who have been sexually abused but also for children who have no other evidence of sexual abuse (50, 56, 73). Bacterial vaginosis (BV) has been diagnosed for children who have been abused, but its presence alone does not prove sexual abuse (31). However, postnatally acquired gonorrhea; syphilis; and non-transfusion-acquired and non-perinatally-acquired HIV are usually diagnostic of sexual abuse.

The purpose of this paper is to review the epidemiology of major STIs, including *Neisseria gonorrhoeae*, *C. trachomatis*,

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syphilis, herpes simplex virus (HSV), *Trichomonas vaginalis*, and human papillomavirus (HPV) and the current recommendations for the diagnostic testing of children being evaluated for suspected sexual abuse.

EPIDEMIOLOGY OF CHILD SEXUAL ABUSE

Over the past decades child sexual abuse has gotten the attention of the medical community and child-protective agencies. This is due largely in part to the media, which have focused on nationally highly publicized stories of children sexually molested by their caretakers, such as the Country Walk case, which involved multiple victims in a an illegal day care center (16). Child sexual abuse is a worldwide problem, occurring in developing as well as developed countries (24, 48). Despite the public awareness of child sexual abuse, it still continues to be a topic that is uncomfortable for both physicians and society. Many physicians do not feel comfortable or competent to conduct a proper medical examination of a child who is a victim of sexual abuse (43). In order to conduct an objective and proper child interview as well as a medical examination, professionals need to understand the dynamics of child development, history, and sexual abuse.

Definition of Child Sexual Abuse

The Committee on Child Abuse and Neglect of the American Academy of Pediatrics defined child sexual abuse as "when a child is engaged in sexual activities that he or she cannot comprehend, for which the child is developmentally unprepared and cannot give consent, and/or that violate the law or social taboos of society" (43, 45). These sexual activities can include all forms of oral-genital, genital, or anal contact by or to the child and nontouching abuses, such as exhibitionism, voyeurism, or use of the child for the production of pornography (43, 45). Some of these activities are more likely to result in the transmission of an STI; for example, the acquisition of an STI would be highly unlikely if the abuse is limited to nontouching activities.

Prevalence of Child Sexual Abuse

Over the past 2 decades, there has been an increase in the number of reports of child sexual abuse to child-protective agencies nationwide. Although the actual numbers of children who are sexually abused is unknown, a recent report from the Administration on Children, Youth, and Families found that over 80,000 cases of child sexual abuse were confirmed by child-protective services in the United States in 2005 (80). This figure is probably an underestimate, as it included only those cases that were reported and determined to have sufficient evidence to be substantiated. In contrast, a population survey of North Carolina and South Carolina suggested that the prevalence of abuse throughout childhood was 1.1% (77). This variation may be secondary to differences in criteria and data collection. Most states also included only abuse by caregivers. Due to the fact that children who are sexually abused often delay disclosing or never disclose the abuse, the actual incidence of child sexual abuse may be underestimated and therefore difficult to ascertain. The perpetrator is usually a male known to the victim, and the abuse may have been going on for months to years before disclosure. Fewer than 10% of sexually abused children are victims of rape by a stranger, and they tend to be older (22). Of the abusers, 84% are men, 60% are nonrelatives (mother's boyfriend, friend, babysitter, or neighbor), 30% are male relatives (father or uncle), and 10% are strangers (22). Child victims of sexual abuse range from infants to adolescents; the average age is between 8 and12 years, although younger children are at a high risk due to their vulnerability and the trust that they have in the perpetrator. Studies have suggested that 12% to 25% of girls and 8% to 10% of boys have been sexually abused by the age of 18 years (66).

Risk Factors

Girls are at a greater risk than boys of being victims of sexual abuse (8, 43). However, boys are less likely to report being sexually abused; this is due in part to the social stigma of homosexuality that is often seen when such disclosures are made. Certain family dynamics tend to be risk factors, such as poor child-parent relationships, parental relationship conflict, the absence of the "protective" parent, and the presence of a "nonbiologically" related male in the home (22, 66). A history of sexual abuse in the parents' families can also increase the risk of sexual abuse for the victim. One of the major reasons why children do not disclose the abuse is fear. Children who are victims of sexual abuse fear continued abuse, hurting the nonabusive parent, shame, and the potential loss of home or the love and attention from the nonabusive parent. Shame, guilt, and a sense of responsibility are other common feelings that these children experience (43).

Clinical Presentation of Child Sexual Abuse

Children who are sexually abused can present with a range of complaints such as genital-rectal medical complaints, changes in behavior, or the disclosure of inappropriate sexual contact. The various genital-rectal and medical complaints that can indicate sexual abuse include acute findings such as genital-rectal bleeding, pain or discharge, pregnancy, bruising to the hard palate, or more chronic problems, including recurrent urinary tract infection, abdominal pain, headaches, enuresis, chronic constipation, and encopresis (66). However, most studies have shown that the majority of children with legally confirmed sexual abuse will have normal or nonspecific genital findings; abnormal findings are rarely seen (24, 35).

EPIDEMIOLOGY OF STIs IN CHILDREN BEING EVALUATED FOR SUSPECTED SEXUAL ABUSE

Studies of STIs in children have demonstrated significant variability in the prevalence of infection. In many earlier studies, only symptomatic children were tested, which often gave higher prevalences of gonococcal infection than studies that tested all children being evaluated for suspected sexual abuse. Studies of *C. trachomatis* infection were not done until the 1980s. However, subsequent studies of STIs in sexually abused children have reported low rates of infection. Ingram et al. (38), in a prospective study published in 1992, reported that among 1,538 children aged 1 to 12 years presenting to a tertiary

TABLE 1. Prevalence of STIs in children being evaluated for sexual abuse according to selected studies published since 2005

Study (reference)	Total no. of children tested (% female)	No. of positive samples/total no. of samples tested (%)[c]						
		N. gonorrhoeae	*C. trachomatis*	Syphilis	HSV	*T. vaginalis*	HPV	HIV
Girardet et al. (26)[a]	536 (90.5)	16/483[e] (3.3)	15/482 (3.1)	1/384 (0.3)	5/12 (42)[f]	5/85 (5.9)	NS	0/384
Kelly and Koh (44)[b]	2,162 (85.8)	11/1,690 (0.7)	20/1,668 (1.2)	0/838	8[d]	6/1,288 (0.5)	67/2,162 (3.1)	0/301
Kohlberger and Bancher-Todesca (47)[b]	180 (100)	1/56 (1.8)	1/62 (1.6)	0/5	NS	1/136 (0.7)	NS	0/27
Simmons and Hicks (72)[b]	2,763 (100)	10/2,007 (0.5)	10/2,007 (0.5)	ND	ND	ND	ND	ND

[a] Prospective study.
[b] Retrospective chart review.
[c] ND, not done; NS, not specified.
[d] The number of children tested was not stated.
[e] Denominator females; none of the males were positive for any STI.
[f] Testing done only for children with lesions suggestive of HSV infection.

referral center with a report of fondling or oral-genital and/or genital-genital sexual contact, 2.8% (39 females and 2 males) had *N. gonorrhoeae*, 1.2% had *C. trachomatis* (18 females and 0 males), 0.1% had syphilis, and 0.1% had HSV. Robinson and colleagues (65) cultured *N. gonorrhoeae* from 2/105 (1.9%) and *C. trachomatis* from 1/77 (1.3%) girls aged 0 to 10 years attending a sexual abuse clinic. No boy in the series reported by Robinson et al. tested positive for an STI. Similarly, De Villiers and colleagues detected *N. gonorrhoeae*, *C. trachomatis*, and syphilis in fewer than 2% of girls, no STI infections in boys, and no cases of HIV or HSV among 191 children presenting with sexual abuse; however, specific diagnostic methods were not described (20).

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 one each from Vienna, Austria (47); Auckland, New Zealand (44); and Miami, FL (72). Despite differences in populations and methodologies, the results of the retrospective studies were fairly consistent. Charts were examined for a total of 4,350 children, who were seen over periods ranging from 4 to 7 years. The ages of the children ranged from 0 to 17 years. 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. As these were retrospective chart reviews, they had a number of major limitations. There were different inclusion criteria: Simmons and Hicks (72) included only girls who were seen within 72 h following an assault and who were younger than 11 years of age, whereas the other studies included adolescents. Not every child or adolescent was tested for every STI. Only 5 of the 180 subjects reviewed by Kohlberger and Bancher-Todesca 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 (47). In contrast, testing for STIs was done for 73% of the subjects reviewed by Simmons and Hicks and, depending on the STI, 14.4 to 88.3% of those reviewed by Kelly and Koh (44), with children ≥10 years of age being screened more frequently than those under 10 years of age (97% versus 83.4%). Girardet et al. (26) prospectively examined children 0 to 13 years of age being evaluated for suspected sexual abuse/assault at four tertiary-care centers in the United States (Houston, TX; Atlanta, GA; Harrisburg, PA; and Brooklyn, NY). All children were tested at multiple sites for *N. gonorrhoeae* and *C. trachomatis* by culture, and vaginal and urethral swabs and urine were also tested by using two nucleic acid amplification tests (NAATs). Wet mounts were performed for *T. vaginalis*, and cultures for HSV were done if lesions were present. Sera were also obtained for testing for syphilis, HIV, and type-specific antibody for HSV-1 and -2. A total of 536 children were enrolled; 485 (90.5%) were female. None of the 51 boys enrolled were positive for any STI. Overall, 40 (8.2%) of the girls were found to have one or more STIs. *C. trachomatis* and *N. gonorrhoeae* were detected by culture and/or NAAT in 15 (3.1%) and 16 (3.3%) of the girls enrolled, respectively. *T. vaginalis* was detected by wet mount in 5 of 85 (5.9%) symptomatic girls tested. Serological evidence of syphilis was found for only 1 of 384 (0.3%) children. This child was positive for *N. gonorrhoeae*. None of the children were positive for HIV. Cultures for HSV were obtained from only 12 children who had compatible lesions: 5 (41.7%) were positive, but only 1 of the culture-positive children had type-specific HSV-2 antibody. Although girls who presented with vaginal discharge were more likely to have a positive test result (24.5% versus 6.3%), 67.5% of the children with a confirmed STI had normal or nonspecific anogenital findings. The prevalence of STIs also varied from site to site, ranging from 1.7% in Texas to 7.8% in Atlanta, GA.

N. GONORRHOEAE

The identification of *N. gonorrhoeae* in a child beyond the immediate neonatal period (defined as beginning at birth and including the first month of life) is indicative of some kind of sexual contact (14, 43). Gonococcal infection can occur in the pharynx and rectum in addition to genital sites. Most girls with vaginal infection will have clinical vaginitis, but asymptomatic infections do occur. Ascending infection, including pelvic inflammatory disease, is rare but can also occur. Infections of the pharynx and rectum are usually asymptomatic. Depending on the type of sexual contact, children may be infected only at extragenital sites. The Centers for Disease Control and Prevention (CDC) 2006 sexually transmitted disease (STD) treatment guidelines and the American Academy of Pediatrics Committee on Child Abuse and Neglect recommend that spec-

imens 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 being evaluated for suspected sexual abuse (14).

Direct Examination of Clinical Specimens

The demonstration of intracellular Gram-negative diplococci in a smear prepared from urethral discharge may have a sensitivity of 90 to 95% and a specificity of 95 to 100% for the diagnosis of gonorrhea in adult men with urethritis. However, the sensitivity of this method when used with endocervical secretions from women, obtained with direct visualization of the cervix, is only 50 to 70%. There are no data on performance with vaginal secretions from adults. In adult patients with symptomatic proctitis, the sensitivity of a Gram stain of a rectal smear is only 40 to 60%. The presence of other Gram-negative coccobacilli and bipolar-staining bacilli present in rectal and endocervical specimens also has the potential to lead to false-positive results. There are no published data for anogenital sites in children, but anecdotal experience suggests very poor specificity. The CDC specifically recommends that Gram-stained smears not be used for the diagnosis of gonorrhea in children (14). However, Gram-stained smears are still routinely done for children in many laboratories.

Culture

Culture remains the preferred method for the detection of *N. gonorrhoeae* in children, primarily because it has the highest specificity. Specimens for culture should be collected with Dacron- or rayon-tipped swabs; calcium alginate may be toxic to *N. gonorrhoeae*. If possible, specimens should be plated directly onto selective growth medium after collection; however, this is generally not feasible in most practice situations. There are swab transport systems that can be used, including several commercially available systems that use selective media. Specimens should then be plated onto enriched selective media. These media contain antibiotics (vancomycin, colistin, trimethoprim, nystatin, amphotericin B, or anisomycin) that suppress endogenous bacteria and fungi that are present in the anatomical sites sampled. Presumptive isolates need to be confirmed as *N. gonorrhoeae*; reliance on Gram stain morphology and oxidase are not sufficient, especially when selective media are not used (83). The 2006 CDC STD treatment guidelines recommend that that all presumptive isolates of *N. gonorrhoeae* obtained from children being evaluated for suspected sexual abuse be confirmed by at least two tests that involve different principles: biochemical, enzyme substrate, serological, or nucleic acid hybridization test methods (14). The use of multiple confirmatory tests is necessary, as some of these confirmatory methods, including biochemical and serological methods, can misidentify other *Neisseria* species as *N. gonorrhoeae* (2, 10, 21, 60). This is especially important when specimens from the rectum and pharynx are tested. An example is a case reported by Dossett et al. (21) of an 8-year-old boy with proctitis originally diagnosed as gonorrhea based on use three commercial kits in four different laboratories. All the kits used biochemical methods, and on the basis of Gram staining, colonial morphology, oxidase reaction, and the above-described biochemical tests, the organism was identified as *N. gonorrhoeae*. The organism was sent to the CDC and to the *Neisseria* Reference Laboratory at the University of Washington for further testing and was identified as *Neisseria cinerea* based on coagglutination, monoclonal antibody test, and DNA homology. Subsequently, Whittington et al. reported that 14 of 40 (35%) isolates from children less than 15 years of age that had been identified as *N. gonorrhoeae* and submitted to the CDC for confirmation were not *N. gonorrhoeae* isolates (83). In each instance the diagnosis of gonococcal infection was based primarily on the identification of oxidase-positive Gram-negative diplococci. Confirmatory testing with biochemical or serological tests was performed for only three of these isolates. Most of the specimens were from the throat and eye and were frequently not plated onto selective media. The bacteria misidentified as *N. gonorrhoeae* included *Moraxella catarrhalis*, *Neisseria lactamica*, *N. cinerea*, *Neisseria meningitis*, and *Kingella denitrificans*, all of which are oxidase positive. *K. denitrificans*, which is a Gram-negative coccobacillus, may appear to be a diplococcus on a Gram-stained smear. All these species can be frequently found in the respiratory and genital tracts of children (46).

Nucleic Acid Amplification Tests

The introduction of NAATs for the detection of *N. gonorrhoeae* and *C. trachomatis* has been a major advance in the diagnosis of STIs. The use of NAATs has supplanted standard culture methods for *N. gonorrhoeae* in many laboratories. Currently, there are three commercially available NAATs for *N. gonorrhoeae* and *C. trachomatis*: PCR (Amplicor; Roche Molecular Diagnostics), strand displacement amplification (SDA) (ProbeTec; Becton Dickinson), and transcription-mediated amplification (TMA) (Aptima C2; GenProbe). PCR and SDA are DNA amplification assays, and TMA is an RNA amplification assay. All three assays have FDA approval for use on genital sites (cervix, vagina, and urethra) and urine from adolescents and adults. None are currently approved for extragenital sites (pharynx or rectum) or have approval for any site in children.

Although NAATs offer several advantages over culture-based methods, including higher sensitivity and enabling the use of noninvasive specimens (urine and vaginal swabs), these assays have some limitations, especially for the detection of *N. gonorrhoeae* (76, 81, 82). The gonococcus has the capacity for genetic variation and recombination that can affect the genetic sequences that are targets for amplification, leading to false-negative results (39, 81, 82). In addition, *Neisseria* species also have the relatively rare characteristic of being fully competent for exogenous DNA uptake throughout their life cycle (39, 81, 82). This enables the frequent horizontal interspecies exchange of genetic material between *Neisseria* species, leading to commensal *Neisseria* species acquiring gonococcal sequences and visa versa (76, 81, 82). All NAATs do not have the same performance parameters. 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* (59). This has important implications, especially when testing extragenital sites.

Three recent studies have examined the performance of

NAATs for the detection of *N. gonorrhoeae* from the pharynx and rectum, primarily in men who have sex with men (MSM). McNally et al. (55) reported that SDA had a low positive predictive value for oral (30.4%) and rectal (73.7%) specimens in a population of MSM in Australia. Similar findings were reported by Schachter et al. (69), who compared results of PCR, SDA, and TMA on oral and pharyngeal specimens from MSM in California. Although the sensitivities of the NAATs were better than that of culture for the detection of *N. gonorrhoeae* from both sites, PCR had a specificity of 78.9% with oropharyngeal swabs. Specificities of SDA and TMA were ≥99.4% for both anatomical sites. The overall prevalences of pharyngeal and rectal gonococcal infections in this population were 8.3% and 8.2%, respectively. Bachmann et al. (3) examined the performances of PCR, SDA, and TMA compared to that of culture of pharyngeal specimens from males and females ≥15 years of age who acknowledged performing fellatio or cunnilingus. PCR was found to have a specificity of 73.0%, compared to 96.3% for SDA and 98.6% for TMA. The prevalences of oral infection in this population were 7.0% in men and 9.1% in women. These prevalences are significantly higher than what one would observe for children being evaluated for suspected sexual abuse.

To date, there have been three published studies that compared NAAT results to those of *N. gonorrhoeae* culture for children being evaluated for suspected sexual abuse (9, 35, 42). All these studies included urine specimens as well as vaginal swabs. Although the results of two of these studies suggested that the sensitivity of NAATs for *N. gonorrhoeae* was similar to that of culture, they both had several serious limitations (35, 42). The populations studied included adolescents up to18 and 20 years of age. In a study by Girardet et al. (25), only 48 of 203 (23.7%) of the children enrolled were prepubertal, and only 13 were male. Kellogg et al. (42) evaluated girls only: 58.5% were ≥13 years of age, a large proportion of whom reported consensual sexual activity. Both studies utilized ligase chain reaction (LCR) (LCx; Abbot Diagnostics), which was taken off the market in 2002 because of specificity concerns for the detection of *N. gonorrhoeae* (13). Kellogg et al. also evaluated PCR in addition to LCR (42). Other study limitations included the failure to use an independent reference standard in estimating test performance and the failure to separately analyze test performance by age and gender (when applicable). The prevalences of *N. gonorrhoeae* infection in both studies were 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. Black et al. (9) recently evaluated the use of SDA and TMA on urine and genital swabs versus culture for the diagnosis of *N. gonorrhoeae* and *C. trachomatis* in children 0 to 13 years of age evaluated for sexual abuse in four U.S. cities. All children were tested at multiple sites for *N. gonorrhoeae* and *C. trachomatis* by culture, and vaginal and urethral swabs and urine were also tested with SDA and TMA. Culturing of *N. gonorrhoeae* was performed for all sites by using Thayer-Martin agar medium, and positive results were confirmed at all sites by Gram staining, oxidase test, enzyme detection, and/or biochemical tests according to the site's standard protocol. Positive NAATs for *N. gonorrhoeae* were confirmed by an in-house PCR using an alternate target, the HinfI fragment of the 4.2-kb cryptic plasmid (85). Of the 536 participants with complete data, none of the male children ($n = 51$) were positive for *N. gonorrhoeae* by any test at any site. Of the 485 female participants with complete data, 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 NAAT results. There were discrepant results in two cases (both were SDA positive and TMA negative). One of these girls was positive by urine and negative by vaginal swab, and the other was positive by both urine and swab. All SDA-positive results for *N. gonorrhoeae* were confirmed to be true-positive results by a species-specific *N. gonorrhoeae* PCR. Three girls had discrepant results by site: two were vaginal swab positive and urine negative, and one was vaginal swab negative and urine positive.

These data suggest that SDA and TMA may be alternatives to culture for the detection of *N. gonorrhoeae* in vaginal swabs and urine from prepubertal girls. Although false-positive results with SDA were not observed in that study, the number of cases of gonorrhea was small, and false-positive results could be a possibility if this assay is used more widely. As the prevalence of gonorrhea in children being evaluated for suspected sexual abuse is low, confirmatory testing is essential (41). However, it is important to emphasize that one cannot extrapolate these results to other NAATs, specifically PCR, or use in extragenital specimens or in males. At this point, more data are needed before one can recommend the routine use of NAATs for the detection of *N. gonorrhoeae* in this setting.

Typing of *N. gonorrhoeae* Isolates

Not infrequently in sexual abuse cases, in addition to accurately confirming the presence of *N. gonorrhoeae*, it may also be necessary to prove a linkage between the isolates from the child and those from the alleged perpetrator. Several typing systems are available for gonococcal isolates, including pulsed-field gel electrophoresis (PFGE), sequencing of *N. gonorrhoeae* lipoprotein (Lip), and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), which is a sequence-based method examining the diversity of two outer membrane proteins, Por and TdpB (transferring binding protein B) (19, 51, 52, 78). The latter has been used to investigate transmission clusters among adults (51). There are only two reported cases where typing was used in the setting of a sexual abuse investigation (19, 52). DeMattia et al. (19) used PFGE and Lip sequencing of isolates of *N. gonorrhoeae* obtained from the vaginal culture of a 3-year-old girl and the urethra of her 17-year-old half-brother, who was asymptomatic. The child did not disclose abuse, and her brother denied any sexual contact with his sister. Initial PFGE typing of the isolates demonstrated that they were not identical. The isolates were then sent to the CDC for Lip subtyping, which confirmed that they were not the same, which ultimately resulted in the case against the half-bother being dismissed. NG-MAST was used by Martin et al. (52) for an investigation of a young girl from whom *N. gonorrhoeae* was isolated in a vaginal swab culture. Several months later, the forensic laboratory obtained underpants from the suspected perpetrator, and *N. gonorrhoeae* DNA was identified in several stains. Genotyping of the samples from the underpants and the

child's gonococcal isolate using NG-MAST revealed that they were indistinguishable. The perpetrator in this case subsequently pleaded guilty to sexual assault. Although these cases are instructive, the use of *N. gonorrhoeae* typing as a forensic tool is not standardized and not fully validated to provide evidence in court. If typing is being considered, isolates should be sent to a reference laboratory such as the CDC.

C. TRACHOMATIS

The association of *C. trachomatis* and sexual abuse in children is more complex than for gonococcal infection. A defining characteristic of *C. trachomatis* is the ability to cause prolonged, often subclinical, infection. Genital infections in adult women have been demonstrated to last for months if left untreated (54). Approximately 20% of infants born to women with active *C. trachomatis* infection can acquire the infection in the rectum and vagina in addition to the conjunctivae and respiratory tract (67). These infections are asymptomatic and may persist as long as 3 years (7). This could be a significant confounding variable when evaluating suspected sexual abuse of children. However, the introduction of systematic screening and treatment of *C. trachomatis* in pregnant women has resulted in a dramatic decrease in perinatally acquired infections in infants in the United States (14). However, in countries where prenatal screening and treating of pregnant women are not part of routine obstetrical care, *C. trachomatis* remains the most frequent cause of neonatal conjunctivitis (65a). The prevalence of *C. trachomatis* infection in abused children is low, similar to that of *N. gonorrhoeae* (Table 1). *C. trachomatis* genital infection in children is frequently asymptomatic and may persist for months to years (30). The CDC currently recommends that children being evaluated for suspected sexual abuse be tested for *C. trachomatis* from the anus in both boys and girls and from the vagina in girls (14). The CDC does not recommend obtaining urethral specimens from boys, as available data suggest that the likelihood of recovering *C. trachomatis* is too low to justify the trauma of obtaining an intraurethral specimen, but does recommend obtaining a specimen from the meatus if obvious discharge is present. Testing of pharyngeal specimens from children of either sex is also not recommended, as the prevalence of infection at this site is very low.

Culture

Culture currently remains the method of choice for the detection of *C. trachomatis* in children being evaluated for suspected sexual abuse. However, culturing of *C. trachomatis* is not regulated in any way, and sensitivity may vary from laboratory to laboratory. The CDC specifies that only standard tissue culture systems should be used. Cycloheximide-treated McCoy cells are used by most laboratories (14). The isolation of *C. trachomatis* in tissue culture should be confirmed by the microscopic identification of characteristic intracytoplasmic inclusions, preferably by staining with a species-specific fluorescein-conjugated monoclonal antibody (14). The use of a genus-specific antibody for culture confirmation can lead to a misidentification of *Chlamydia pneumoniae* as *C. trachomatis* in pharyngeal specimens (6). Enzyme immunoassays (EIAs) are not acceptable as confirmatory tests and have been associated with false-positive results, especially when used on vaginal and rectal specimens (12, 32, 33, 61). Currently available EIAs are based on polyclonal antibodies that will cross-react with bacteria present in the anogenital tract and nonspecifically react with fecal material (12, 32, 33, 61). The methods used for culture confirmation became an issue when several large commercial laboratories started using an EIA instead of fluorescent-antibody (FA) staining and visual identification of inclusions for culture confirmation. This has resulted in at least one "outbreak" of *C. trachomatis* infection in an evaluation of suspected sexual abuse among residents and staff of an institution for the mentally retarded in Ohio in 1990 (12). All the "positive" cultures, mostly rectal specimens, were subsequently determined to be false-positive cultures resulting from the 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 the visual identification of inclusions, there is still a subjective component that could lead to a misidentification of artifacts as chlamydial inclusions. This appears to have happened in another pseudo-outbreak in a residential institution in another state (28). A CDC investigation found that *C. trachomatis* culture was performed infrequently at the original laboratory, at a rate of 1 to 2 specimens/month, and there were deviations from "standard" procedures, especially in the preparation of positive controls. Positive controls were prepared by growing an L_2 serovar strain on BGMK cells on coverslips for 4 to 5 days until a cytopathic effect was seen. The cells were then scraped off, diluted, dispensed onto multiple slides, fixed, and stained. Slides contained only chlamydial elementary and reticulate bodies; there were no typical inclusions.

Nonculture Methods

Antigen detection tests. Several nonculture tests are approved for the diagnosis of chlamydial conjunctivitis in infants, specifically EIAs and direct fluorescent-antibody tests (DFAs). The only DFA and EIA still available in the United States are Pathfinder Chlamydia DFA and EIA Microplate (Bio-Rad Laboratories). The use of EIAs and DFAs for vaginal and rectal specimens for the evaluation of children suspected of being sexually abused has been associated with false-positive test results (32, 33, 61). Fecal material can give false-positive reactions with any EIA. Common bowel organisms, including *Escherichia coli*, group B streptococcus, and even some respiratory flora such as group A streptococcus, can also give false-positive reactions in EIAs (61). Another potential problem can occur with the use of an EIA for respiratory specimens. As the only currently available EIA, Pathfinder Chlamydia EIA Microplate (Bio-Rad Laboratories), uses a genus-specific antibody, this test will also detect *C. pneumoniae* if used for respiratory specimens.

There are also several point-of-service tests, which are rapid immunoassays designed to be used at the point of care in the clinic or physician's office. While these assays have good specificity with endocervical specimens (>98%), they are insufficiently sensitive compared to the sensitivities of culture and NAATs (5). There are no data on the use of these assays on

samples from rectogenital sites in children, and these assays should not be used in this population.

NAATs. As described above in the section on gonorrhea, there are currently three FDA-approved NAATs for the simultaneous detection of *N. gonorrhoeae* and *C. trachomatis*: PCR, SDA, and TMA. NAATs are currently approved by the FDA for the detection of *C. trachomatis* from genital sites (cervix, vagina, and urethra) and urine from adolescents and adults. None are approved for extragenital sites (pharynx or rectum) or have approval for any site in children. These methods have been found to have excellent sensitivity for the detection of *C. trachomatis*, usually well above 90%, in genital specimens and urine from adult men and women while maintaining high specificity. However, in the CDC 2006 STD treatment guidelines, the CDC states that NAATs can be used on children being evaluated for suspected sexual abuse if culture is not available *and* if positive results can be confirmed (14). Confirmation was specified as the use of another NAAT that utilized a different genetic target; however, not all NAATs perform equally well (68). Use on extragenital specimens was not discussed. Studies of MSM have found SDA and TMA to be more sensitive than culture for the detection of *C. trachomatis* in the oropharynx and rectum (69). Schachter et al. found the prevalence of chlamydial infection at these sites was lower than that of *N. gonorrhoeae*: 0.8% in the oropharynx and 6.1% in the rectum (69).

A new genetic variant of *C. trachomatis* was discovered in Sweden in 2006, which was found to have a mutation in the sequence of the cryptic plasmid, at the target site for PCR, rendering the organism undetectable by this assay (36). Recent data from Sweden showed that this variant is now responsible for 20 to 65% of all detected chlamydial infections in counties where PCR was used. So far, this variant appears to be limited primarily to Sweden, with a few isolates being identified in Norway and Denmark. The spread of the variant in Sweden was associated with the use of PCR as the NAAT for the diagnosis of *C. trachomatis* infection.

As with *N. gonorrhoeae*, data on the use of available NAATs for the detection of *C. trachomatis* in children are limited. To date, there have been four published studies that compared NAATs to *C. trachomatis* culture for children being evaluated for suspected sexual abuse (9, 25, 42, 53). These studies include two studies that evaluated LCR for the detection of *N. gonorrhoeae* (25, 42), an additional study that evaluated PCR (53), and a recently published study by Black et al. (9), which evaluated SDA and TMA. As described above in the section on the diagnosis of *N. gonorrhoeae*, Girardet et al. (25) and Kellogg et al. (42) evaluated primarily girls, most of whom were postpubertal. Both studies utilized LCR, which is currently not available in the United States. Kellogg et al. also evaluated PCR in addition to LCR (42). The same limitations that applied to *N. gonorrhoeae* apply to the evaluation of these assays for the 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 (when applicable). A third study, conducted by Matthews-Greer et al., evaluated PCR and an EIA compared to culture for the detection of *C. trachomatis* (53). The ages of the children ranged from 1 month to 17 years, with a mean age of 8.6 years. The majority of the children were female (84.2%). 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. The numbers of specimens from nongenital sites were too small to accurately assess the performance of the assays at these sites. Two of three positive rectal specimens were positive by culture and negative by PCR.

A recent multicenter study by Black et al. (9) also evaluated the use of SDA and TMA on urine and genital swabs (vagina and urethra) compared to culture for the diagnosis of *C. trachomatis* in children 0 to 13 years of age. Cultures for *C. trachomatis* were performed at the clinical or hospital laboratories of each center according to their own standard protocols. All sites transported swab specimens at 4°C for *C. trachomatis* culture in either commercial chlamydial or viral transport medium. Culture protocols at all sites included the isolation of *C. trachomatis* in cycloheximide-pretreated McCoy cells, either in shell vials or 24-well or 96-well tissue culture plates. The inoculated cell monolayers were incubated at 35°C to 37°C for 48 to 72 h followed by fixation with ethanol, methanol, or acetone. The fixed monolayers were stained to detect chlamydial inclusions with fluorescein-conjugated *Chlamydia* genus-specific or *C. trachomatis* species-specific monoclonal antibodies. One laboratory also performed a single passage of the inoculated cell monolayers onto a fresh monolayer after 48 h of incubation. The commercial NAATs were performed at the CDC (SDA and TMA). 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 BD ProbeTec sample collection medium. Test results that were positive for *C. trachomatis* by SDA were confirmed by using an in-house PCR targeting the *ompA* gene, performed at the CDC (4). Fifteen (3.1%) of 485 female participants had a positive result for *C. trachomatis* by any test (7 [1.4%] by culture, 11 [2.3%] by vaginal NAAT, and 13 [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 a positive urine NAAT result. Two prepubertal female children had positive *C. trachomatis* cultures from rectal swab specimens but negative vaginal swab specimens by both culture and NAATs and negative urine NAATs. No other participants had positive rectal cultures. There were no discrepant results for any of the participants tested by two commercial NAATs for *C. trachomatis* (ProbeTec and Aptima 2). All *C. trachomatis*-positive results were confirmed to be true-positive results by DNA sequence genotyping. When we compared NAAT results by the type of specimen, only one girl had a discrepant result for *C. trachomatis* (vaginal swab negative and urine positive). The sensitivity of vaginal culture for *C. trachomatis* was 39% for all girls studied ($n = 485$). In contrast, the sensitivities of urine and vaginal swab NAATs were 100% and 85%, respectively, for all female children for the detection of *C. trachomatis*.

The results of Black et al. (9) suggest that NAATs, specifically SDA and TMA, can be used for the detection of *C. trachomatis* in girls being evaluated for suspected sexual abuse. However, the same limitations apply as for the use of these

assays for the detection of *N. gonorrhoeae*. (i) As the prevalence of *C. trachomatis* in this population is low, confirmatory testing is necessary. (ii) One cannot extrapolate these results to other NAATs, specifically PCR and use on specimens other than vagina and urine for girls. (iii) One cannot make any recommendations on the use of these assays for prepubertal boys. The performance of a confirmatory NAAT may be problematic, as most hospital laboratories use only one assay. 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, confirmation of an NAAT-positive result by culture test (requires a separate, invasive specimen), or use of a second, 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 very low, <1% in most studies (20, 26, 38, 44, 47, 65, 84). The majority of these children were asymptomatic. The diagnosis of syphilis was based on serological screening. Cases of symptomatic syphilis appear to be uncommon and are limited mostly to anecdotal reports (17, 84). Clinical findings have included primary chancres, manifestations of secondary syphilis, including rash and condyloma lata, which can be misdiagnosed as genital warts (27). The major confounding variable in the diagnosis of syphilis in children beyond the neonatal period is differentiation 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 clinical manifestations of congenital syphilis may overlap with those of acquired syphilis.

Serology

Serology remains the primary method for diagnosis in children, as in adults. If positive, a nontreponemal test, either the rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL) test, is then confirmed by a *Treponema pallidum*-specific test. Nontreponemal tests detect antibodies to a cardiolipin antigen that is present in tissues. Cardiolipin is the result of spirochetal damage and reliably cross-reacts with *T. pallidum* antigens (14, 84). The RPR is a flocculation assay. The nontreponemal antigens detected by these assays are nonprotein, T-cell-independent antigens that induce little if any immunological memory; thus, the titers will fall with successful treatment (14, 84). Thus, these tests are useful for monitoring the efficacy of treatment. However, a number of other infections and diseases, including malaria, leprosy, and autoimmune conditions (systemic lupus erythematosus), can lead to the induction of anti-cardiolipin antibodies and cause false-positive results with RPR and VDRL (14, 84). The antibodies detected by RPR and VDRL are IgG and are transported across the placenta during pregnancy. The most frequently used *Treponema*-specific test is the fluorescent treponemal antibody absorption (FTA-ABS) test. Other available *Treponema*-specific tests are the *T. pallidum* hemagglutination assay (TPHA) and *T. pallidum* particle agglutination assay (TPPA) (79). Antitreponemal antibodies are usually present for life even after successful treatment; thus, they cannot be used to monitor the efficacy of treatment or to distinguish between active and past infections. These assays detect IgG antibody and, thus, will cross the placenta. Currently, there are no treponeme-specific IgM assays available. An FTA-ABS-IgM test was available in the past but was associated with many false-positive results. Because the performance of the RPR and FTA-ABS is very labor-intensive, many laboratories are now using treponemal EIAs for high-throughput screening and then confirming positive results with a nontreponemal test reversing the traditional syphilis screening sequence (15). Currently, there are more than nine commercially available EIAs for *T. pallidum* antibodies. Some of these assays will also detect IgM antibodies in addition to IgG (79). As with FTA-ABS and other treponeme-specific tests, these antibodies remain throughout life. A study from New York City found that 3% of serum specimens from adults had a reactive EIA but a negative nontreponemal test result that would not have been detected by use of the traditional testing sequence (15). However, the importance of these test results was unclear, as no specific prognostic information exists to guide patient evaluation. Some of these test results might be false-positive results, as treponemal tests will detect antibodies to other treponemal species and may be positive in Lyme disease, autoimmune disorders, and dermatological disorders and even with febrile illness. Several recent studies demonstrated significant variation in the performances of these assays; some may not be appropriate as stand-alone tests for screening or confirmatory syphilis serology (15). None of these assays have been evaluated for the diagnosis of syphilis in children and are not recommended for the diagnosis of congenital syphilis in infants.

Direct Identification of *T. pallidum*

If a child has lesions of primary or secondary syphilis, i.e., chancre or condyloma lata, *T. pallidum* can be visualized in tissue exudates by using dark-field microscopy and a direct fluorescent-antibody-*T. pallidum* (DFA-TP) test or by PCR, if available. Epidermal or mucosal lesions in primary and secondary syphilis tend to contain high numbers of treponemes. Because of the technical challenges of dark-field microscopy and DFA-TPA, they should be performed only in an experienced laboratory. As of this writing, there are no FDA-approved, commercially available NAATs for the detection of *T. pallidum*.

HERPES SIMPLEX VIRUS

The presence of genital herpes in a prepubertal child can also raise the probability of sexual abuse. However, available data are too limited to allow an estimation of the likelihood of sexual transmission after an episode of abuse. The overwhelming majority of published studies of STIs in children being

evaluated for sexual abuse have tested for HSV only in children who presented with suggestive genital lesions. The prevalence of HSV, mostly HSV-2, infection in these studies was low, <5% (20, 26, 38, 44, 47, 63, 65). In a recent multicenter study reported by Girardet et al., only 12 girls were found to have genital ulcers, 5 of whom were culture positive for HSV-2 (26). Only one study from South Africa performed cultures for HSV for all the children being evaluated ($n = 227$); none were positive for HSV (20). As most studies tested only 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.

Culture

If active genital lesions are present, the vesicle should be unroofed for the sampling of vesicular fluid for culture by swabbing the base of the lesion. However, the overall sensitivity of viral culture of genital lesions in adults is only approximately 50% compared to that of PCR (63). The diagnostic yield is highest in the early stages of disease, when lesions are typically vesicular, rather than in the later stages, when lesions have largely crusted. Viral isolation rates are also higher with primary than with recurrent genital herpes, particularly in the setting of asymptomatic recurrences with subclinical shedding.

Specimens for culture should be placed directly into viral culture media and transported rapidly to the laboratory. HSV readily grows on many cell lines, including MRC-5, WI-38, Hep-2, and A549 cells. Viral isolates usually grow in tissue culture by 5 days and are typed as HSV-1 or -2 by antibody staining.

NAATs

While viral culture has remained the standard diagnostic method for the isolation of HSV, real-time HSV PCR assays have emerged as a more sensitive method to confirm HSV infection in clinical specimens obtained from genital ulcers, mucocutaneous sites, and cerebrospinal fluid (74). PCR is particularly useful for the detection of asymptomatic HSV shedding in adults. The main limiting factor in adopting real-time HSV PCR as the primary diagnostic tool in many clinical reference laboratories is the cost of the assay, which substantially exceeds that of viral isolation culture techniques. In addition, the lack of uniform validation of the PCR assay as a diagnostic method for the detection of HSV in clinical specimens other than cerebrospinal fluid has limited its availability in some commercial laboratories. These are all in-house assays; there are no commercially available, FDA-cleared PCR assays, and PCR has not been evaluated for testing of anogenital sites in children (74).

Direct Fluorescent Antibody

Many diagnostic laboratories provide a rapid type-specific DFA test to detect HSV in clinical specimens. This test is specific and reproducible for genital specimens from adults but has not been evaluated or cleared for use on samples from children.

Tzanck Smear

The Tzanck smear may demonstrate the cytopathic effect of the virus (multinucleate giant cells) and can be performed on samples of lesion scrapings from patients with active genital lesions. However, it has limited utility since it has low sensitivity and specificity and should not be used on anogenital specimens from children. Furthermore, only a viral culture can determine whether the infection is due to HSV-1 or HSV-2 or differentiate HSV from varicella-zoster virus.

Serology

Type-specific antibodies to HSV develop during the first several weeks after infection and persist indefinitely (49, 62). The availability of type-specific serology using surface glycoproteins (Gg2 and Gg1 for HSV-2 and HSV-1, respectively) to distinguish HSV-1 and HSV-2 enables the clinician to determine if the patient is at risk of acquisition or has evidence of prior infection with either subtype. The presence of HSV-2 indicates anogenital infection, but the presence of HSV-1 can be consistent with either anogenital or orolabial infection. The sensitivities of HSV IgG type-specific tests vary from 80 to 98% in adults. False-negative results may occur at early stages of infection; the specificity of these assays is greater than 96%. Several FDA-approved type-specific HSV serological tests are commercially available to clinicians evaluating genital ulcer disease in adults. Data on the use of these assays for children are very limited. A preliminary study evaluated two assays (Gull/Meridian enzyme-linked immunosorbent assay [ELISA] and HerpeSelect HSV-1 and HSV-2 ELISAs; Focus Technologies) compared to Western blotting (WB) in a population of healthy children, 1 to 13 years of age, in Texas (49). WB was performed at the University of Washington. The Gull/Meridian ELISA was withdrawn from the market during the study. The seroprevalences of HSV-1 and HSV-2 by WB were 49% and 6%, respectively. The sensitivity of HerpeSelect HSV-1 compared to WB was 80%, with a specificity of 97% and a positive predictive value of 95%. HerpeSelect HSV-2 compared to WB had a sensitivity of 80%, a specificity of 100%, and a positive predictive value of 100%. Subsequently, Ramos et al. compared HerpeSelect HSV-2, Biokit HSV-2 Rapid Test (Biokit), and WB results for sera from 150 children, 1 to 18 yeas of age; 81% were female, seen in a sexual abuse clinic (62). Fifty-one percent of the children were HSV-1 seropositive by WB. The HSV-2 seroprevalence was <1%; eight patients were positive for HSV-2 by at least one test, and only one individual was positive by all three tests, an adolescent who reported consensual sexual activity. Six children were positive by HerpeSelect HSV-2 but negative by Biokit and WB. One patient was positive by WB with atypical bands and also positive by HerpeSelect HSV-2; 137 samples were consistently negative by all tests. Twelve percent of these children had one or more STIs at the time of evaluation, including chlamydia, gonorrhea, and HPV. None had genital herpes. Girardet et al. (26) tested sera from 283 children for HSV-1 and -2 antibodies by using an immunodot enzyme assay with monoclonal antibody inhibition for confirmation performed at the CDC. Antibody to HSV-1 was detected in 45.6% of samples, antibody to HSV-2 was detected in seven (2.5%) children, and three chil-

dren had antibody to both HSV-1 and -2. Cultures for HSV were obtained from only 12 children who had compatible lesions: 5 (41.7%) were positive, but only 1 of the culture-positive children had HSV-2 antibody. These data suggest that type-specific serology for HSV has a poor predictive value for the diagnosis of HSV infection in children being evaluated for suspected child abuse. Furthermore, as demonstrated by Ramos et al. (62), 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. Most published studies of STIs in sexually abused children that tested for *T. vaginalis* were limited to girls presenting with vaginal discharge (20, 26, 38, 44, 47, 63, 65). However, an identification of *T. vaginalis* appears to be strongly associated with sexual activity. In one study where all the participants were evaluated for *T. vaginalis* infection, Hammerschlag et al. (29) obtained vaginal specimens for wet mounts and *T. vaginalis* cultures from 98 healthy girls 2 months to 15 years of age. *T. vaginalis* was identified in wet mounts and by culture for two 13-year-old girls, both of whom had vaginal discharge and reported consensual sexual activity. Rarely, *T. vaginalis* can be transmitted vertically from mother to infant (vaginal and urine) during parturition (70). These infections may persist for up to 9 months after birth.

Microscopy

The presence of motile trichomonads on wet mounts of vaginal secretions is diagnostic of infection, but this occurs for only 50 to 70% of culture-confirmed cases in adult women (23, 58). The organisms remain motile in saline for 10 to 20 min after collection of the sample. Other findings that are almost invariably present with trichomonas infection, but nondiagnostic, include an elevated vaginal pH (>4.5) and an increase in polymorphonuclear leukocytes on direct microscopy. However, there are no standards for vaginal pH in children. Care should be taken in interpretations when trichomonads are reported to be present in urine specimens collected from children for another purpose. As 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 (1). This could present a problem for children that are not toilet trained and are in diapers and with bagged urine specimens.

Culture

Culture on Diamond's medium has a high sensitivity (95%) and a high specificity (>95%). Incubation periods of 2 to 7 days are needed to indentify *T. vaginalis* in culture (23). There are very few reports of the use of culturing of specimens from children (29). A commercial "in-pouch" *T. vaginalis* culture system can be used and is readily available (BioMed Diagnostics, White City, OR).

Rapid Antigen and Nucleic Acid Amplification Tests

Several rapid diagnostic kits using DNA probes and monoclonal antibodies have been developed commercially, with a sensitivity of 90% and a specificity of 99.8%, for vaginal specimens from women with clinical vaginal discharge. The Affirm VP III microbial identification system (Becton Dickinson) test is a direct nucleic acid probe hybridization test for the detection of *T. vaginalis*, *Gardnerella vaginalis*, and *Candida* spp. It was reported to have sensitivities of 80 to 90% for adult women with vaginitis (11). The Affirm VP III test has not been validated or approved for use on genital specimens from prepubertal girls or urethral specimens from men. False-positive results have been reported when the test was used on specimens from children and adult men, with serious legal and social consequences (M. R. Hammerschlag, personal communication). The OSOM TV (Genzyme Diagnostics) test is an objective rapid antigen detection test that has been demonstrated to perform very well in adolescent women, with sensitivities significantly higher than that of wet mount or culture (23). Again, this test has not been validated or approved for use on samples from children.

No FDA-approved NAAT for *T. vaginalis* is currently available in the United States. GenProbe has introduced analytes for a TMA-based NAAT for *T. vaginalis* (APTIMA TV) (37). Preliminary studies have demonstrated that this assay is highly sensitive and specific compared to wet-mount microscopy, culture, and an in-house PCR for the diagnosis of trichomoniasis in women and men (37). However, this is not an FDA-cleared kit, and there are no data on its use on samples from 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 the possibility of nonsexual transmission, either vertically during delivery or horizontally after birth. Criteria for the diagnosis of human papillomavirus (HPV) infection in children, clinical versus detection of HPV DNA, is 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 (40, 50, 71, 72, 73, 75). Given the possible role of perinatal transmission for anogenital and respiratory HPV (laryngeal papillomatosis) infection, an important issue has been 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 (73). Sinclair et al. (73) found that the mean age of children with HPV seen in several clinics, including a child sexual abuse clinic, was 4.5 years, which was similar to the mean ages reported in two studies of children born to mothers with known genital HPV infection. HPV infection was diagnosed by clinical examination and/or biopsy of suspicious lesions. Anogenital HPV lesions in children can also be caused by HPV-2 and HPV-3, which are the serotypes generally associated with cu-

TABLE 2. Recommended tests for STIs in children

Infection	Reportable as sexual abuse	Site(s) and specimen(s)	Recommended test(s)
N. gonorrhoeae	Diagnostic	Vagina, urethra (males), rectum, pharynx	Culture on selective media, isolates confirmed by at least 2 methods that use different principles; NAATs[b] may be used, but some NAATs may cross-react with other *Neisseria* species; specimens should be retained for additional testing; NAATs are not approved for rectal or pharyngeal specimens
C. trachomatis	Diagnostic[a]	Vagina, urethra (males), rectum, urine (if NAAT is used)	Culture (tissue culture, confirmation by staining with FA-conjugated species-specific monoclonal antibody with visualization of characteristic intracytoplasmic inclusions); NAATs[b] may be used if culture is not available; specimens should be retained for further testing; NAATs are not approved for rectal specimens
Syphilis	Diagnostic[a]	Serum, active lesions	Serology (initial screening with nontreponemal test, confirmation with treponemal test); dark-field microscopy to identify treponemes in lesions
HSV	Suspicious	Lesions on vagina, urethra (males), rectum	Culture; screening using serology is not recommended
T. vaginalis	Highly suspicious	Vagina	Examination of vaginal wet mount; culture
HPV	Suspicious	Vagina, urethra (males), rectum	Physical examination; biopsy and HPV typing of lesions
HIV	Diagnostic[a]	Serum	EIA, followed by Western blot, viral load

[a] If perinatal acquisition can be ruled out.
[b] Data on the use of NAATs in children are limited to SDA and TMA with vagina and urine from females.

taneous warts (34). This finding suggests that anogenital HPV in children may be acquired through casual contact and fomites, although abuse through fondling may be an explanation in some cases. Current recommendations for use of the quadrivalent HPV vaccine, which contains HPV-6, -11, -16, and -18, may potentially reduce the risk of HPV infection in children who have been sexually abused. The Advisory Committee on Immunization Practices currently recommends that girls receive the quadrivalent HPV vaccine at 11 to 12 years of age (14a). The vaccine can be administered to girls as young as 9 years of age.

Detection of HPV DNA

As described above, the diagnosis of HPV infection in children has been primarily clinical. Misdiagnosis can happen; there are several reported cases where condyloma lata was mistaken for genital warts (27). Lesions suspicious for HPV should be biopsied and tested for HPV (27, 73). As of this writing, there is only one FDA-approved test that is available for the detection of HPV DNA in cervical specimens from adult women (Hybrid Capture II; Digene Corporation), and several more are currently being evaluated (18). The primary use of these assays is for the detection of high-risk types of HPV in the management and prevention of cervical cancer. HPV DNA testing is not recommended for persons with anogenital warts and has not been evaluated for the detection of HPV in children.

Several studies have evaluated the detection of HPV DNA in children being evaluated for suspected sexual abuse; however, the results have been contradictory. Studies published since 2000 have demonstrated a poor correlation of detection of HPV DNA by PCR and the presence of genital warts (56, 64, 71, 73, 75). 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 the detection of HPV (18). Most assays have used generic primers followed by either probing or sequencing of the products. Nested-PCR assays were used in some studies. The 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 from 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 (56, 64, 71, 73, 75). Siegfried et al. (71) evaluated the presence of subclinical HPV infection in children with suspected sexual abuse by sampling swabs from the throat, vaginal introitus, and/or rectum for HPV DNA by using an in-house PCR. HPV-16 was detected in vaginal swabs from 2 (5%) of 40 children. The girls were 8 and 10 years of age, and the diagnosis of sexual abuse was based on physical examination; however, no details of the physical findings were given. Both girls also had negative cultures for *C. trachomatis* and *N. gonorrhoeae*. These data suggest that routine screening of children suspected of being sexually abused for HPV DNA is not indicated at this time.

CONCLUSION

Current recommendations for testing for STIs in children being evaluated for suspected sexual abuse/assault are summarized in Table 2. Although the introduction of nonculture

methods, especially NAATs, has greatly facilitated the diagnosis of genital infections due to *C. trachomatis* and *N. gonorrhoeae* among adults, data on the use of these tests for children are limited. One cannot extrapolate the performance of NAATs on samples from genital sites from adults to samples from genital and extragenital (rectum and pharynx) sites from children. The prevalence of STIs, including *C. trachomatis* and *N. gonorrhoeae* in children being evaluated for suspected sexual abuse, is low, often below 3%, which influences the positive predictive value of NAATs. A misidentification of an STI in a child can have serious legal ramifications. A dramatic example is the Country Walk case, where a couple running an illegal babysitting service was accused of multiple counts of child sexual abuse (16). One of the defendants remains in prison, serving a 165-year sentence. The putative identification of *N. gonorrhoeae* from a throat culture from the defendant's 6-year-old son was used to support the contention that abuse occurred; however, the method used for culture confirmation, RapID-NH, can misidentify other *Neisseria* spp. as *N. gonorrhoeae* (2, 10, 60).

Because of the legal ramifications, specimens obtained from children should be retained for confirmatory 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. This was evident in a 1990 *C. trachomatis* testing investigation where the commercial laboratory involved used an EIA for culture confirmation because it was semiautomated and required less technician effort (12). Many laboratories have difficulty in appropriately triaging specimens from children. Recently, the National Academy of Sciences released a report describing major problems and deficiencies in the forensic science system in the United States (57). That report stated that the current system is badly fragmented and that rigorous and mandatory certification programs for forensic scientists as well as strong standards and protocols for analyzing and reporting evidence are lacking. The same issues apply to medical microbiology laboratories that perform STI testing for children being evaluated for suspected sexual abuse, which has forensic implications. There is a clear need for more studies of diagnostic testing in this population. In addition, we should consider establishing a network of designated laboratories to do this testing.

REFERENCES

1. **Adu-Sarkodie, Y., B. K. Opuku, T. Crucitti, H. A. Weiss, and D. Mabey.** 2007. Lack of evidence for the involvement of rectal and oral trichomonads in the aetiology of vaginal trichomoniasis in Ghana. Sex. Transm. Dis. **83:**130–132.
2. **Alexander, S., and C. Ison.** 2005. Evaluation of commercial kits for the identification of *Neisseria gonorrhoeae*. J. Med. Microbiol. **54:**827–831.
3. **Bachmann, L. H., R. E. Johnson, H. Cheng, L. E. Markowitz, J. R. Papp, and E. W. Hook III.** 2009. Nucleic amplification tests for diagnosis of *Neisseria gonorrhoeae* oropharyngeal infections. J. Clin. Microbiol. **42:** 902–907.
4. **Bandea, C. I., K. Kubota, T. M. Brown, P. H. Kilmarx, V. Bhullar, S. Yanpaisarn, P. Chaisilwattana, W. Siriwasin, and C. M. Black.** 2001. Typing of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (omp1). Sex. Transm. Infect. **77:**419–422.
5. **Bandea, C. I., E. H. Koumans, M. K. Sawyer, J. Dover, A. O'Connor, J. R. Papp, E. R. Unger, J. Braxton, and C. M. Black.** 2009. Evaluation of the rapid BioStar optical immunoassay for detection of *Chlamydia trachomatis* in adolescent women. J. Clin. Microbiol. **47:**215–216.
6. **Bauwens, J. E., M. S. Gibbons, M. M. Hubbard, and W. E. Stamm.** 1991. *Chlamydia pneumoniae* (strain TWAR) isolated from two symptom-free children during evaluation for possible sexual assault. J. Pediatr. **119:**591–593.
7. **Bell, T. A., W. E. Stamm, S. P. Wang, K. K. Holmes, and J. T. Grayston.** 1992. Chronic *Chlamydia trachomatis* infections in infants. JAMA **267:**400–402.
8. **Berkoff, M. C., A. J. Zolotor, K. L. Makoroff, J. D. Thackeray, R. A. Shapiro, and D. K. Runyan.** 2008. Has this prepubertal girl been sexually abused? JAMA **300:**2779–2792.
9. **Black, C. M., E. M. Driebe, L. A. Howard, N. N. Fajman, M. K. Sawyer, R. G. Girardet, R. L. Sautter, E. Greenwald, C. M. Beck-Sague, E. R. Unger, J. U. Igietseme, and M. R. Hammerschlag.** 2009. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. Pediatr. Infect. Dis. J. **28:**608–613.
10. **Boyce, J. M., and E. B. Mitchell, Jr.** 1985. Difficulties in differentiating *Neisseria cinerea* from *Neisseria gonorrhoeae* in rapid systems used for identifying pathogenic *Neisseria* species. J. Clin. Microbiol. **22:**731–734.
11. **Briselden, A. M., and S. L. Hillier.** 1994. Evaluation of Affirm VP Microbial Identification Test for *Gardnerella vaginalis* and *Trichomonas vaginalis*. J. Clin. Microbiol. **32:**148–152.
12. **Centers for Disease Control.** 1991. False-positive results with the use of chlamydia tests in the evaluation of suspected sexual abuse—Ohio, 1990. MMWR Morb. Mortal. Wkly. Rep. **39:**932–935.
13. **Centers for Disease Control and Prevention.** 2002. Recall of LCx *Neisseria gonorrhoeae* assay and implications for laboratory testing for *N. gonorrhoeae* and *C. trachomatis*. MMWR Morb. Mortal. Wkly. Rep. **51:**709.
14. **Centers for Disease Control and Prevention.** 2006. Sexually transmitted diseases treatment guidelines 2006. MMWR Recommend. Rep. **55**(RR11)**:** 1–100.

14a. **Centers for Disease Control and Prevention.** 2007. Quadrivalent human papillomavirus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recommend. Rep. **56**(RR2)**:** 1–32.

15. **Centers for Disease Control and Prevention.** 2008. Syphilis testing algorithms using treponemal tests for initial screening. Four laboratories, New York City, 2005–2006. MMWR Morb. Mortal. Wkly. Rep. **57:**872–875.
16. **Cheit, R. E., and D. Mervis.** 2007. Myths about the Country Walk case. J. Child Sex. Abuse **16:**95–116.
17. **Christian, C. W., J. Lavelle, and L. M. Bell.** 1999. Preschoolers with syphilis. Pediatrics **103:**e4. http://www.pediatrics.org/cgi/content/full/103/1/e4.
18. **Coutlée, F., D. Rouleau, A. Ferenczy, and E. Franco.** 2005. The laboratory diagnosis of genital human papillomavirus infections. Can. J. Infect. Dis. Med. Microbiol. **16:**83–91.
19. **DeMattia, A., J. S. Kornblum, J. Hoffman-Rosenfield, D. L. Trees, A. J. Tumpey, and D. Laraque.** 2006. The use of combination subtyping in the forensic evaluation of a three-year old girl with gonorrhea. Pediatr. Infect. Dis. J. **25:**461–463.
20. **De Villiers, F. P. R., M. A. Prentice, A. M. Bergh, and S. D. Miller.** 1992. Sexually transmitted disease surveillance in a child abuse clinic. S. Afr. Med. J. **81:**84–86.
21. **Dossett, J. H., P. C. Appelbaum, J. S. Knapp, and P. A. Totten.** 1985. Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. J. Clin. Microbiol. **21:**575–577.
22. **Finkelhor, D., G. Hotaling, I. A. Lewis, and C. Smith.** 1990. Sexual abuse in a national survey of adult men and women: prevalence characteristics and risk factors. Child Abuse Negl. **14:**19–28.
23. **Garber, G. F.** 2005. The laboratory diagnosis of *Trichomonas vaginalis*. Can. J. Infect. Dis. Med. Microbiol. **16:**35–38.
24. **Gilbert, R., C. S. Widom, K. Brown, D. Fergusson, E. Webb, and S. Janson.** 2009. Burden and consequences of child maltreatment in high-income countries. Lancet **373:**68–81.
25. **Girardet, R. G., N. McClain, S. Lahoti, K. Cheung, B. Hartwell, and M. McNeese.** 2000. Comparison of the urine-based ligase chain reaction test to culture for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pediatric sexual abuse victims. Pediatr. Infect. Dis. J. **20:**144–147.
26. **Girardet, R. G., S. Lahoti, L. A. Howard, N. N. Fajman, M. K. Sawyer, E. M. Driebe, F. Lee, R. L. Sautter, E. Greenwald, C. Beck-Sagué, M. R. Hammerschlag, and C. M. Black.** 2009. The epidemiology of sexually transmitted infections in suspected child victims of sexual assault. Pediatrics **124:**79–86.
27. **Goldenring, J. M.** 1989. Secondary syphilis in a prepubertal child. Differentiating condyloma lata from condyloma acuminata. N. Y. State J. Med. **89:**180–181.
28. **Gust, D. A., S. A. Wang, C. M. Black, T. M. Brown, M. E. St. Louis, K. A. King, M. P. Quinlisk, and W. C. Levine.** 2002. A pseudo-outbreak of *Chlamydia trachomatis* in a state residential facility: implications for diagnostic testing. J. Infect. Dis. **185:**841–844.
29. **Hammerschlag, M. R., S. Alpert, I. Rosner, P. Thurston, D. Semine, D.**

McComb, and W. M. McCormack. 1978. Microbiology of the vagina in children: normal and potentially pathogenic organisms. Pediatrics **62:**57–62.
30. **Hammerschlag, M. R., B. Doraiswamy, E. R. Alexander, P. Cox, W. Price, and A. Gleyzer.** 1984. Are rectogenital chlamydial infections a marker of sexual abuse in children? Pediatr. Infect. Dis. **3:**100–104.
31. **Hammerschlag, M. R., M. Cummings, B. Doraiswamy, P. Cox, and W. M. McCormack.** 1985. Nonspecific vaginitis following sexual abuse in children. Pediatrics **75:**1028–1031.
32. **Hammerschlag, M. R., P. J. Rettig, and M. E. Shields.** 1988. False positive results with the use of chlamydial antigen detection tests in the evaluation of suspected sexual abuse in children. Pediatr. Infect. Dis. J. **7:**11–14.
33. **Hammerschlag, M. R., S. Ajl, and D. Laraque.** 1999. Inappropriate use of nonculture tests for the detection of *Chlamydia trachomatis* in suspected victims of child sexual abuse: a continuing problem. Pediatrics **104:**1137–1139.
34. **Handley, J., E. Hanks, K. Armstrong, A. Bingham, W. Dinsmore, A. Swann, M. F. Evans, J. O. McGee, and J. O'Leary.** 1997. Common association of HPV 2 with anogenital warts in prepubertal children. Pediatr. Dermatol. **14:**339–343.
35. **Heger, A., L. Ticson, O. Velasquez, and R. Bernier.** 2002. Children referred for possible sexual abuse: medical findings in 2384 children. Child Abuse Negl. **26:**645–659.
36. **Herrmann, B.** 2007. A new genetic variant of *Chlamydia trachomatis*. Sex. Transm. Infect. **83:**253–254.
37. **Huppert, J. S., J. E. Mortensen, J. L. Reed, J. A. Kahn, K. D. Rich, W. C. Miller, and M. M. Hobbs.** 2007. Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of *Trichomonas vaginalis* in young women. Clin. Infect. Dis. **45:**194–198.
38. **Ingram, D. L., V. D. Everett, P. R. Lyna, S. T. White, and L. A. Rockwell.** 1992. Epidemiology of adult sexually transmitted disease agents in children being evaluated for sexual abuse. Pediatr. Infect. Dis. J. **11:**945–950.
39. **Ison, C., C. M. Bellinger, and J. Walker.** 1986. Homology of cryptic plasmid of *Neisseria gonorrhoeae* with plasmids from *Neisseria meningitidis* and *Neisseria lactamica*. J. Clin. Pathol. **39:**1119–1123.
40. **Jayasinghe, Y., and S. M. Garland.** 2006. Genital warts in children: what do they mean? Arch. Dis. Child. **91:**696–700.
41. **Katz, A. R., P. V. Effler, R. G. Ohye, B. Brouillet, M. V. C. Lee, and P. M. Whiticar.** 2004. False-positive gonorrhea test results with a nucleic acid amplification test: the impact of low prevalence on positive predictive value. Clin. Infect. Dis. **38:**814–819.
42. **Kellogg, N. D., J. Baillargeon, J. L. Lukefahr, K. Lawless, and S. W. Menard.** 2004. Comparison of nucleic acid amplification tests and culture techniques in the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in victims of suspected child sexual abuse. J. Pediatr. Adolesc. Gynecol. **17:**331–339.
43. **Kellogg, N.** 2005. The evaluation of sexual abuse in children. Pediatrics **116:**506–512.
44. **Kelly, P., and J. Koh.** 2006. Sexually transmitted infections in alleged sexual abuse of children and adolescents. J. Paediatr. Child Health **42:**434–440.
45. **Kempe, C. H.** 1978. Sexual abuse, another hidden pediatric problem: the 1977 C. Anderson Aldrich Lecture. Pediatrics **62:**382–389.
46. **Knapp, J. S.** 1988. Historical perspectives and identification of *Neisseria* and related species. Clin. Microbiol. Rev. **1:**415–431.
47. **Kohlberger, P., and D. Bancher-Todesca.** 2007. Bacterial colonization in suspected sexually abused children. J. Pediatr. Adolesc. Gynecol. **20:**289–292.
48. **Lalor, K.** 2004. Child sexual abuse in Tanzania and Kenya. Child Abuse Negl. **28:**833–844.
49. **Leach, C. T., R. L. Ashley, J. Baillargeon, and H. L. Jenson.** 2002. Performance of two commercial glycoprotein-G-based enzyme immunoassays for detecting antibodies to herpes simplex viruses 1 and 2 in children and young adolescents. Clin. Diagn. Lab. Immunol. **9:**1124–1125.
50. **Marcoux, D., K. Nadeau, C. McCuaig, J. Powell, and L. L. Oligny.** 2006. Pediatric anogenital warts: a 7-year review of children admitted to a tertiary-care hospital in Montreal, Canada. Pediatr. Dermatol. **23:**199–207.
51. **Martin, I. M. C., C. A. Ison, D. M. Aanensen, K. A. Fenton, and B. G. Spratt.** 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J. Infect. Dis. **189:**1497–1505.
52. **Martin, I. M. C., E. Foreman, V. Hall, A. Nesbitt, G. Forster, and C. A. Ison.** 2007. Non-cultural detection and molecular genotyping of *Neisseria gonorrhoeae* from a piece of clothing. J. Med. Microbiol. **56:**487–490.
53. **Matthews-Greer, J., G. Sloop, A. Springer, K. McRae, E. LaHaye, and R. Jamison.** 1999. Comparison of detection methods for *Chlamydia trachomatis* in specimens obtained from pediatric victims of suspected sexual abuse. Pediatr. Infect. Dis. J. **18:**165–167.
54. **McCormack, W. M., S. Alpert, D. E. McComb, R. L. Nichols, D. Z. Semine, and S. H. Zimmer.** 1979. Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*. N. Engl. J. Med. **300:**123–125.
55. **McNally, L. P., D. J. Templeton, F. Jin, A. E. Grulich, B. Donovan, D. M. Whiley, and P. H. Cunningham.** 2008. Low positive predictive value of a nucleic acid amplification test for nongenital *Neisseria gonorrhoeae* infection in homosexual men. Clin. Infect. Dis. **47:**e25–27.
56. **Myhre, A. K., A. Dalen, K. Berntzen, and D. Bratlid.** 2003. Anogenital papillomavirus in non-abused children. Acta Pediatr. **92:**1445–1452.
57. **National Academy of Sciences.** 2009. Strengthening forensic science in the United States: a path forward. National Academies Press, Washington, DC.
58. **Nye, M. B., J. R. Schwebke, and B. A. Body.** 2009. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am. J. Obstet. Gynecol. **200:**188.e1–188.e7.
59. **Palmer, H. M., H. Mallinson, R. L. Wood, and A. J. Herring.** 2003. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **41:**835–837.
60. **Philip, A., and G. C. Garton.** 1985. Comparative evaluation of five commercial systems for the rapid identification of pathogenic *Neisseria* species. J. Clin. Microbiol. **22:**101–104.
61. **Porder, K., N. Sanchez, P. M. Roblin, M. McHugh, and M. R. Hammerschlag.** 1989. Lack of specificity of Chlamydiazyme for detection of vaginal chlamydial infection in prepubertal girls. Pediatr. Infect. Dis. J. **8:**358–360.
62. **Ramos, S., J. L. Lukefahr, R. A. Morrow, L. R. Stanberry, and S. L. Rosenthal.** 2006. Prevalence of herpes simplex virus types 1 and 2 among children and adolescents attending a sexual abuse clinic. Pediatr. Infect. Dis. J. **25:**902–905.
63. **Reading, R., and Y. Rannan-Eliya.** 2007. Evidence for sexual transmission of genital herpes in children. Arch. Dis. Child. **92:**608–613.
64. **Rice, P. S., C. Mant, J. Cason, J. M. Bible, P. Muir, B. Kell, and J. M. Best.** 2000. High prevalence of human papillomavirus type 16 infection among children. J. Med. Virol. **61:**70–75.
65. **Robinson, A. J., J. E. M. Watkeys, and G. L. Ridgway.** 1998. Sexually transmitted organisms in sexually abused children. Arch. Dis. Child. **79:**356–358.
65a. **Rours, G. I. J. G., M. R. Hammerschlag, J. T. H. N. De Faber, R. de Groot, and R. P. Verkooyen.** 2008. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics **121:**e321–326.
66. **Sapp, M. V., and A. M. Vandeven.** 2005. Update on childhood sexual abuse. Curr. Opin. Pediatr. **17:**258–264.
67. **Schachter, J., M. Grossman, R. L. Sweet, J. Holt, C. Jordan, and E. Bishop.** 1986. Prospective study of perinatal transmission of *Chlamydia trachomatis*. JAMA **255:**3374–3377.
68. **Schachter, J., E. W. Hook, D. H. Martin, D. Willis, P. Fine, D. Fuller, J. Jordan, W. M. Janda, and M. Chernesky.** 2005. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: all NAATs are not created equal. J. Clin. Microbiol. **43:**1372–1373.
69. **Schachter, J., J. Moncada, S. Liska, C. Shayevich, and J. D. Klausner.** 2008. Nucleic acid amplification tests in the diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men who have sex with men. Sex. Transm. Dis. **35:**637–642.
70. **Schwandt, A., C. Williamns, and R. H. Beigi.** 2008. Perinatal transmission of *Trichomonas vaginalis*. J. Reprod. Med. **53:**59–61.
71. **Siegfried, E., J. Rasnick-Conley, S. Cook, C. Leonardi, and J. Monteleone.** 1998. Human papillomavirus screening in pediatric victims of sexual abuse. Pediatrics **101:**43–47.
72. **Simmons, K. J., and D. J. Hicks.** 2005. Child sexual abuse examination: is there a need for routine screening for *N. gonorrhoeae* and *C. trachomatis*. J. Pediatr. Adolesc. Gynecol. **18:**343–345.
73. **Sinclair, K. A., C. R. Woods, D. J. Kirse, and S. H. Sinal.** 2005. Anogenital and respiratory tract human papillomavirus infections among children: age, gender and potential transmission through sexual abuse. Pediatrics **116:**815–825.
74. **Singh, A., J. Preiksaitis, A. Ferenczy, and B. Romanowski.** 2005. The laboratory diagnosis of herpes simplex virus infections. Can. J. Infect. Dis. Med. Microbiol. **16:**92–98.
75. **Stevens-Simon, C., D. Nelligan, P. Breese, C. Jenny, and J. M. Douglas.** 2000. The prevalence of genital human papillomavirus infections in abused and nonabused preadolescent girls. Pediatrics **106:**645–649.
76. **Tabrizi, S. N., S. Chen, M. A. Cohenford, B. B. Lentrichia, E. Coffman, T. Schultz, J. W. Tapsall, and S. M. Garland.** 2004. Evaluation of real time polymerase chain reaction assays for confirmation of *Neisseria gonorrhoeae* in clinical samples tested positive in the Roche Cobas Amplicor assay. Sex. Transm. Infect. **80:**68–71.
77. **Theodore, A. D., J. J. Chang, D. K. Runyon, W. M. Hunter, S. I. Bangdiwala, and R. Algans.** 2005. Epidemiologic features of the physical and sexual maltreatment of children in the Carolinas. Pediatrics. **115:**e331–e337.
78. **Trees, D. L., A. J. Schultz, and J. S. Knapp.** 2000. Use of the neisserial lipoprotein (Lip) for subtyping of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **38:**2914–2916.
79. **Tsang, R. S., I. E. Martin, A. Lau, and P. Sawatsky.** 2007. Serological diagnosis of syphilis: comparison of the Trep-Check IgG enzyme immuno-

assay with other screening and confirmatory tests. FEMS Immunol. Med. Microbiol. **51:**118–124.
80. **U.S. Department of Health and Human Services Administration on Children, Youth, and Families.** 2007. Child maltreatment 2005. Department of Health and Human Services, Washington, DC.
81. **Whiley, D. M., J. W. Tapsall, and T. P. Sloots.** 2006. Nucleic acid amplification testing for *Neisseria gonorrhoeae*. An ongoing challenge. J. Mol. Diagn. **8:**3–14.
82. **Whiley, D. M., S. M. Garland, G. Harnett, G. Lum, D. W. Smith, S. N. Tabrizi, T. P. Sloots, and J. W. Tapsall.** 2008. Exploring "best practice" for nucleic acid detection of *Neisseria gonorrhoeae*. Sex. Health **5:**12–23.
83. **Whittington, W. L., R. J. Rice, J. W. Biddle, and J. S. Knapp.** 1988. Incorrect identification of *Neisseria gonorrhoeae* from infants and children. Pediatr. Infect. Dis. J. **7:**3–10.
84. **Woods, C. R.** 2005. Syphilis in children: congenital and acquired. Semin. Pediatr. Infect. Dis. **16:**245–257.
85. **Xu, K., V. Glanton, S. R. Johnson, C. Beck-Sagué, V. Bhullar, D. H. Candal, K. S. Pettus, C. E. Farshy, and C. M. Black.** 1998. Detection of *Neisseria gonorrhoeae* infection by ligase chain reaction testing of urine among adolescent women with and without *Chlamydia trachomatis* infection. Sex. Transm. Dis. **25:**533–538.

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