Laboratory Diagnosis of Sexually Transmitted Infections in Cases of Suspected Child Sexual Abuse

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ABSTRACT Laboratory diagnosis of microbial agents associated with sexually transmitted infections plays an important role in both the care of victims of child sexual abuse (CSA) and the investigation of suspected CSA incidents, with law enforcement implications. Rapid and sensitive test results prompt immediate actions to treat and protect the victimized children. The development and maturation of automated nucleic acid amplification tests (NAATs) has greatly improved the assay sensitivity and specificity, with only a 1- to 2-h turnaround time. Unfortunately, the performance characteristics of NAATs have been determined largely with a few limited specimen types and evaluated in adults only. This minireview attempts to cover the scope of infectious agents potentially implicated in CSA, specimen collection, laboratory test modalities, and laboratory report constraints, further complicated by infrequently collected specimen types from prepubertal children <13 years of age.

KEYWORDS child sexual abuse, CSA, NAAT, genital, extragenital

The U.S. Centers for Disease Control and Prevention (CDC) defines child maltreatment as “any act or series of acts of commission or omission by a parent or other caregiver (e.g., clergy, coach, teacher) that results in harm, potential for harm, or threat of harm to a child” (1). Under this definition, there are three types of abuse involving acts of commission: physical abuse, sexual abuse, and psychological abuse. Child sexual abuse (CSA) is further defined as “Any completed or attempted (noncompleted) sexual act, sexual contact with, or exploitation (i.e., noncontact sexual interaction) of a child by a caregiver.” Sexual acts include contact involving penetration, however slight, between the mouth, penis, vulva, or anus of the child and another individual (1). Laboratory diagnosis provides critical evidence of sexually transmitted infections (STIs) highly likely to be associated with direct sexual contact.

The prevalence of CSA has been difficult to determine, although the classification for clinical certainty of CSA has been developed and refined over the last 30 years (2). An evidence-based review and guidance for best practice published by the Royal College of Paediatrics and Child Health provided an updated physician handbook “*The Physical Signs of Child Sexual Abuse*,” in 2015 (3). However, the definitions and terminology regarding age range, signs of abuse, and procedures or scope of investigation continue to evolve, adding complexities in interpreting and reporting (4). Moreover, the age range of the victimized children spans multiple cognitive developmental levels; thus, CSA data rely heavily on adult recall (4). CSA frequently starts when the victims are younger than 13 years of age, and there are many factors contributing to data inaccuracy in this age group (5, 6). According to the most recent National Child Abuse and Neglect Data System report, *Child Maltreatment 2017*, the national rate of child maltreatment was estimated at 9.1 per 1,000 children (including ages 0 to 21 years), or 674,000 victims in 2017 (7). Of these victimized children, 8.6% were sexually abused (7).

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TABLE 1 Infectious agents potentially transmitted by CSA and laboratory notification responsibility^a

Infectious agent or condition detected	Evidence for CSA	Timing of notification to:		Comment
		Medical provider	Health jurisdiction of patient	
<i>Neisseria gonorrhoeae</i>	Diagnostic	Immediate	As per local regulations, usually within 2 working days	Exclude perinatal transmission
<i>Chlamydia trachomatis</i>	Diagnostic	Immediate	As per local regulations, usually within 2 working days	Exclude perinatal transmission
Syphilis	Diagnostic	Immediate	As per local regulations, usually within 2 working days	Exclude perinatal transmission
HIV	Diagnostic	Immediate	As per local regulations, usually within 2 working days	Exclude perinatal transmission or blood transfusion
<i>Trichomonas vaginalis</i>	Highly suspicious	Immediate	As per local regulations, usually not required	Exclude perinatal or nonsexual transmission
Genital herpes	Highly suspicious (especially HSV-2)	Immediate	As per local regulations, usually within 2 working days	Exclude perinatal or nonsexual transmission
Others (<i>Mycoplasma genitalium</i> , <i>Campylobacter</i> , <i>Shigella</i> , <i>Neisseria meningitidis</i>)	Suspicious	Immediate	As per local regulations ^b	Need additional evidence to support suspicion of CSA
Bacterial vaginosis	Inclusive	Standard report	Usually not reportable	Need medical follow-up

^aAdapted from references 10 and 33.^b*M. genitalium*, usually not reportable; *Campylobacter*, usually within 2 working days; *Shigella* and *N. meningitidis*, usually within 24 h.

Based on a U.S. Department of Justice report in 2000, a third (34%) of all sexual assault victims were under 12 years of age and 67% were under 18 (8). The actual incidence may be higher, as about 10% of adult respondents in several large surveys in the United States reported experiencing child sexual abuse prior to 18 years of age (9). The gap between the real incidence and the officially recorded incidence is likely considerable due to lack of disclosure by the victimized children (4). Children older than 13 years of age are highly vulnerable to sexual assault but are not the focus of this minireview. Survivors of child sexual abuse are at risk for social, behavioral, and health problems, such as anxiety, depression, substance abuse, post-traumatic stress disorder, and engagement in high-risk sexual behaviors (4, 9). Preventative efforts can provide effective interventions only through early recognition of the signs and symptoms of sexual abuse in children (4, 5).

The demand for laboratory tests to support the evaluation of suspected CSA events has grown as a result of increased awareness, strengthened medical subspecialty, and mandatory reporting laws enacted in the United States and many other countries since the 1970s (5). A list of STI agents in the CDC's 2015 *STD Treatment Guidelines* clearly indicated the role of laboratory diagnostics (Table 1) (10). The development of a standard approach is important to inform test choices that could include all relevant anatomic sites, with age and gender considerations, in children <13 years. Testing for exposure and acquisition of STI agents in children is only part of a CSA investigation that also includes forensic investigations. The hospital CSA team or other qualified clinicians should be notified by the immediate health care providers at the point of suspicion, and the CSA team then oversees the medical and legal activities with strict chain-of-custody documentation. Besides testing for STI agents, laboratory findings of sperm in clinical specimens during microscopic examination or results of pregnancy testing may provide potential forensic evidence for CSA. Clinical laboratories are otherwise not actively involved in forensic specimen collection or in CSA legal reporting functions. Appropriate collection and testing of human articles associated with criminal investigations belong to law enforcement authority functions and are thus not covered by this minireview (11).

Laboratory testing for STIs in the evaluation of CSA is complicated by the low prevalence of sexually transmitted infections in prepubertal children (1 to 5% in most reported surveys) (12–14). Laboratory tests suffer from the poor sensitivity of standard culture methods for STI detection and lack of data on the sensitivity and specificity of

targeted nucleic acid amplification testing (NAAT) platforms in this age group, particularly for samples collected from nongenital sites (2, 15). Therefore, a CSA standard of care involving laboratory tests has to be established at the outset in order to secure the optimal collection of specimens. In the following sections, we focus on challenges faced by laboratories with regard to specimen collection and testing. We also attempt to cautiously propose best practices by developing a CSA test bundle in order to both serve the vulnerable patients and adhere to the latest guidelines.

MICROORGANISMS AND POTENTIALLY AFFECTED BODY SITES

Organisms of interest. The American Academy of Pediatrics (AAP) views nonperinatally transmitted *Neisseria gonorrhoeae*, syphilis, *Chlamydia trachomatis*, and human immunodeficiency virus (HIV) as diagnostic of sexual abuse in prepubertal children (12, 15). The detection of herpes simplex virus (HSV), human papillomavirus (HPV), and *Trichomonas vaginalis* is suspicious or highly suspicious for sexual abuse (6, 10). The diagnostic implications of various STI agents in the context of CSA are summarized in Table 1. Bacterial vaginosis characterized by either traditional microscopy or molecular tests is not considered to be diagnostic for CSA (10).

More recently, *Mycoplasma genitalium*, an emerging STI agent ranking second to *C. trachomatis* in prevalence, has been increasingly recognized (16). However, *M. genitalium* in particular has not been included in the current CDC guidelines, as there are few data on *M. genitalium* in children and its association with CSA is unknown (Table 1). Unusual agents such as *Shigella*, *Campylobacter*, and *Neisseria meningitidis* have been reported to cause sexually transmitted infections in men who have sex with men (17, 18). Although highly unlikely, isolation of such organisms from unconventional body sites during CSA evaluations should not be dismissed without further investigation.

The attribution of STIs in children to CSA is complicated by the fact that gonorrhea, chlamydia, HIV, HPV, syphilis, and HSV can be transmitted from mother to infant during the perinatal period. Thus, the presence of these pathogens may not always be indicative of sexual transmission, depending on the clinical setting. The age of the child, location of infection, and exposure history are helpful in identifying potential perinatal transmission. Outside the neonatal period, *N. gonorrhoeae* is almost always transmitted sexually (19), whereas perinatally acquired *C. trachomatis* has been documented to persist up to age 3 (20). *C. trachomatis* infections identified after age 3 years are more likely to be acquired by sexual contact (19). Children with neonatal HSV infection frequently have recurrent skin lesions even beyond infancy, and thus a history of neonatal infection should be sought for children identified with HSV-2 skin lesions. HSV-2 genital lesions should raise concern for potential sexual abuse (6). Juvenile recurrent respiratory papillomatosis and anogenital warts can result from perinatal HPV transmission, and as the incubation period can be long, children may be older at the time of presentation; however, the likelihood of CSA increases with increasing age of the child (21). HIV and syphilis infection in a child warrant a workup for CSA if perinatal infection can be excluded. Maternal history or testing would identify potential perinatal transmission. There are case reports of perinatal transmission of *T. vaginalis* (22); however, *T. vaginalis* in an older infant or child would be concerning for sexual abuse.

Anatomic sites of interest. The relevant anatomic sites sampled for the diagnosis of STIs in CSA cases are commonly urine and the urogenital tract but can also include the rectum and oropharynx of both male and female children. Genital specimens in girls include those from the vagina and less so the endocervix, depending on age, while specimens in boys included the urethra or, less invasively, the meatus or any penile discharge (23). Ophthalmic infections are not a complete exception for suspected CSA when children are well over the age of perinatal transmission (1 month for *N. gonorrhoeae* and 3 years for *C. trachomatis*) (24). Clinicians should follow the CDC and the American Academy of Pediatrics (AAP) guidelines when deciding what specimens should be included (2, 3, 23).

TABLE 2 Commonly used NAAT platforms for STI agents with 1- to 2-h turnaround times

NAAT	Company	Agent(s) detected						
		<i>N. gonorrhoeae</i> , <i>C. trachomatis</i>	<i>N. gonorrhoeae</i> , <i>C. trachomatis</i> , <i>T. vaginalis</i> , <i>M. genitalium</i>	<i>N. gonorrhoeae</i> , <i>C. trachomatis</i> , <i>T. vaginalis</i>	<i>T. vaginalis</i> , <i>M. genitalium</i>	<i>T. vaginalis</i>	<i>M. genitalium</i>	HSV-1, HSV-2
Gen-Probe Aptima	Hologic	X				X	X	
GeneXpert ^a	Cepheid	X				X		
BD Max	BD Molecular Diagnostics			X				
Cobas 6800/8800 System	Roche Diagnostics	X			X			X
Binx	Binx Health		X					
Simplexa	Focus Diagnostics							X
Solana	Quidel Corporation					X		X
Liaison MDX	DiaSorin Molecular							X

^aRecently cleared for throat and rectal swabs in adults.

TESTING MODALITIES IN A CSA INVESTIGATION

Culture. Due to the need for high specificity in identifying organisms associated with sexually transmitted infections in children, conventional culture has long been chosen as the preferred method of detection for most specimen types. Culture recovery of STI agents provides ultimate concrete evidence for the organism involved and a viable organism(s) for antimicrobial susceptibility and transmission investigations. The CDC states that “culture remains the preferred method for urethral specimens from boys and extragenital specimens (pharynx and rectum) in boys and girls” (23). Culture is also the preferred method of detection for *T. vaginalis*.

While isolation of viable organisms remains the gold standard for establishing laboratory evidence of sexual assault in principle, there are several limitations to the use of culture as the only or preferred diagnostic modality for STIs in prepubertal children. A key limitation is the low sensitivity of culture for any STI agent, particularly from extragenital sites. The sensitivity of culture from extragenital sites ranges from 27 to 44% for *C. trachomatis* and from 41 to 43% for *N. gonorrhoeae* in adults (23). In a study in prepubertal girls, the sensitivity of vaginal culture for *C. trachomatis* was only 20% (13). Although culture for *N. gonorrhoeae* is commonly available at most clinical labs, culture for *C. trachomatis*, HSV, or *T. vaginalis* requires special transport media (universal transport medium [UTM] for *C. trachomatis* and HSV and InPouch [Biomed Diagnostics, Inc., OR, USA] for *T. vaginalis*) and is routinely performed only by reference centers. Cultures for *N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis* also require additional swabs along with NAAT and involve different transport media and temperature requirements (6). In addition, culture is not recommended for *C. trachomatis* from urethral swabs for boys due to the invasiveness of specimen collection or from pharyngeal specimens for boys or girls due to the low yield and potential use of culture systems that do not distinguish between *C. trachomatis* and *Chlamydia pneumoniae* (10, 23). Use of only culture would therefore limit the ability to obtain specimens from all relevant sites, thus decreasing the potential yield (25).

NAAT. There have been a number of rapid NAAT platforms with proven outstanding performance characteristics for STI agents entering and maturing in the setting of the clinical diagnostic theater within the last 10 to 20 years (Table 2), several of which can be run as near-patient/point-of-care assays (frequently available in small community medical services). The sensitivity and specificity for *N. gonorrhoeae* and *C. trachomatis* of currently available NAAT platforms are generally >90% for both urogenital and extragenital specimens in adults (23). CDC recommends NAATs for detection of *C. trachomatis* and *N. gonorrhoeae* in genital tract infections in adults, stating that “NAATs are far superior in overall performance compared with other *C. trachomatis* and *N. gonorrhoeae* culture and nonculture diagnostic methods” (e.g., enzyme immunoassay [EIA] and direct fluorescent-antibody assay [DFA]) (10, 23). With regard to laboratory testing in children, the CDC does acknowledge that “no evidence suggests that

performance of NAAT for detection of *T. vaginalis* in children would differ from that in adults" (10). Therefore, the use of commercially available NAATs for the initial STI rapid testing has been widely adopted in current practice because of their superior sensitivity and results frequently being available within 1 to 2 h (Table 2) (12, 13, 26). For these reasons, some guidelines (e.g., those from the Royal College of Paediatrics and Child Health) and other widely accepted clinical standards recommend NAAT for all specimen types from children being evaluated for STIs due to concern for sexual abuse (2, 3, 23). The CDC guidelines on STI testing allow NAAT for *C. trachomatis* from vaginal swabs and urine in girls (10).

Regulatory and technical constraints. However, there are both regulatory and practicality issues with NAAT in prepubertal children, particularly for extragenital sites. Laboratory validation of these NAATs has been done using limited specimen types sampled from the adult male and female genital and urinary tracts only (13, 23). Until only recently, most NAATs had not been cleared by the FDA for *C. trachomatis* and *N. gonorrhoeae* in rectal and oropharyngeal specimens, and worse, no NAAT assays have been cleared for use in any sample type from prepubertal boys and girls (10, 23). Without other options, most laboratories resort to including disclaimers in NAAT test reports regarding the off-label use of sample types, typically, e.g., "This assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. The performance of this test has not been evaluated in patients less than 14 years of age or in pregnant women" (refer to relevant package insert of commercial platforms).

Even with highly specific NAATs, the positive predictive value of a positive result can be low due to the low prevalence of STIs in children undergoing a sexual abuse evaluation (12, 13). The lack of appropriate collection and testing for confirmatory samples can lead to false allegations of abuse and unnecessary treatment in children (27). For a rapid NAAT approach, there has been a general consensus that confirmatory testing with an alternate NAAT platform employing a different DNA target(s) should be used when the results could be legally significant (6, 28).

Given the legal implications, testing protocols with built-in redundancy, such as employing more than one specimen type and more than one test modality, can only strengthen laboratory test confidence when the off-label use of NAATs is inevitable and culture is not rapid (6). This standard, however, requires that a complex set of samples be collected for CSA evaluations. This can be best accomplished through the development of a CSA test bundle.

Utility of a CSA test bundle. A basic set of specimens for the initial CSA investigation provides the most critical treatment-naïve materials that are optimal for STI testing. As discussed above, due to the poor sensitivity of culture, NAAT assays for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* should be considered high-priority tests when building a CSA microbiology test bundle. For children <13 years of age, who have a wide range of cognitive developmental levels, the inclusion of all possible anatomic sites potentially involved in sexual contact is important, as is having access to the specific collection kits for various modalities of NAATs and cultures at the point of suspicion. The success of obtaining all specimen types with appropriate collection kits begins with an established institutional CSA standard that includes a test bundle for STI testing. By identifying two different NAAT assays and their specified collection kits along with a culture collection kit, laboratories are set to take advantage of the performance strengths of 3 tests addressing both the clinical urgency and CSA reporting requirements.

Practice standards for CSA investigations in pediatric care systems have been developed more recently at our institution and are not yet mature or complete in all involved aspects. We recommend the use of a CSA test bundle including a minimum collection kit organized specifically for a CSA order template (Table 3) in order to best accommodate the immediate and confirmatory tests as well as any further investigative tests. The choice of tests and reference testing should be determined and standardized

TABLE 3 Example of CSA test bundle based on our local availability^a

CSA test bundle
<ul style="list-style-type: none"> ● Prioritize urine collection if no other collection available. ● A set of 3 swab kits per site will be required, except for urine, which can be sent in a urine cup. Please call the microbiology lab for preassembled collection kits with instructions and submit all specimens immediately after completion of specimen collection.
Urine (a single “dirty urine” is the preferred specimen type for both NAATs) <ul style="list-style-type: none"> ● GeneXpert <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> PCR and <i>T. vaginalis</i> PCR (NAAT) ● Aptima <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> TMA^b and Aptima <i>T. vaginalis</i> TMA (NAAT) ● <i>N. gonorrhoeae</i> screen (when no other specimen is obtained, <i>N. gonorrhoeae</i> culture and Gram stain on urine concentrate) ● Wet mount for <i>Trichomonas</i> and sperm
Vaginal (endocervical when applicable) or penile discharge swabs <ul style="list-style-type: none"> ● GeneXpert <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> PCR and <i>T. vaginalis</i> PCR (NAAT) ● Aptima <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> TMA and Aptima <i>T. vaginalis</i> TMA (NAAT) ● <i>N. gonorrhoeae</i> screen (<i>N. gonorrhoeae</i> culture and Gram stain) ● Wet mount for <i>Trichomonas</i> and sperm
Urethra (or meatus) swabs <ul style="list-style-type: none"> ● GeneXpert <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> PCR and <i>T. vaginalis</i> PCR (NAAT) ● Aptima <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> TMA and Aptima <i>T. vaginalis</i> TMA (NAAT) ● <i>N. gonorrhoeae</i> screen (<i>N. gonorrhoeae</i> culture and Gram stain) ● Wet mount for <i>Trichomonas</i> and sperm
Throat swabs <ul style="list-style-type: none"> ● GeneXpert <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> PCR and <i>T. vaginalis</i> PCR (NAAT) ● Aptima <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> TMA and Aptima <i>T. vaginalis</i> TMA (NAAT) ● <i>N. gonorrhoeae</i> screen (<i>N. gonorrhoeae</i> culture and Gram stain) ● Wet mount for <i>Trichomonas</i> and sperm
Rectal swabs <ul style="list-style-type: none"> ● GeneXpert <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> PCR and <i>T. vaginalis</i> PCR (NAAT) ● Aptima <i>C. trachomatis</i>/<i>N. gonorrhoeae</i> TMA and Aptima <i>T. vaginalis</i> TMA (NAAT) ● <i>N. gonorrhoeae</i> screen (<i>N. gonorrhoeae</i> culture and Gram stain) ● Wet mount for <i>Trichomonas</i> and sperm
Copan flocked swab with UTM (the same collection can be used for <i>C. trachomatis</i> and <i>M. genitalium</i> culture) <ul style="list-style-type: none"> ● HSV-1 and -2 PCR (NAAT and culture; specimen details include swabbing the suspected lesions at any potential sexual contact site).
For HPV, hepatitis B virus, HIV, and syphilis testing, please refer to lab test catalog for specimen requirements.

^aWe do not endorse the use of any particular commercial NAAT platform.

^bTMA, transcription-mediated amplification.

at the institutional level when a CSA test bundle is introduced. Test modality and performance characteristics associated with the laboratory diagnosis of these STIs can be found in recent studies and reviews (26). Because of the off-label use of NAATs and their specified collection complexities, nursing and laboratory personnel familiarity and competency training are critically important to ensure test quality and timeliness. The testing protocol and CSA test bundle should be frequently reviewed by a multidisciplinary decision-making team.

CSA TEST BUNDLE DEVELOPMENT AND SPECIMEN COLLECTION

Quality laboratory test performance relies largely on specimens that are collected and maintained for their biological integrity prior to testing. This section discusses a basic specimen set necessary for the initial laboratory evaluation of CSA. With the increasing ability of many current NAAT platforms to be run *stat.*, clear instructions for specimen collection will ensure that rapid results are produced to inform clinical actions for patient treatment and protection, as well as CSA evidence building.

The choice of two NAAT assay modalities can be determined by each institution's

technology preferences and the availability of a second NAAT assay locally accessible at peer institutions or national reference labs, including state public health laboratories. However, as the NAAT platforms continue to be updated over time, the financial burden cannot be underestimated, as most manufacturers are taking the approach of adding more cartridges to the existing assay platforms rather than adding more pathogen-specific targets to retrofit the existing combination cartridge (Table 2). Moreover, each molecular assay is packaged with manufacture/platform-specific collection kits, thus requiring that multiple swabs be collected at the time of assessment for CSA.

Collection of specimens for culture using nonselective transport media (e.g., the most commonly accepted ESwab transport kit) should always be included for any anatomic site, as recollection of samples for other test modalities after the immediate workup is both logistically difficult and biologically compromised for many reasons. Whenever possible, cross validation of the ESwab kit for NAATs would be ideal, given the challenges of collecting 3 test modalities per site. If cross validation is not possible, there remains uncertainty about whether the residual specimens in ESwab transport medium can be repurposed in other dedicated transport media for culture of *C. trachomatis*, HSV, or *T. vaginalis* immediately after a positive NAAT result. Needless to say, the complexity of specimen collection in various transport systems requires a great deal of coordination between clinical providers and the laboratory before, during, and after each CSA event even in the presence of a well-established CSA test bundle.

Table 3 shows an example of a standard CSA test bundle. For CSA investigations, urine, specifically “first-void” or “dirty” urine, is the most easily obtainable specimen type and should always be used for *N. gonorrhoeae* and *C. trachomatis* NAATs. Although female urine and superficial genital swabs are not ideal for STI testing, urine concentrate should be cultured (including Gram stain and wet mount) regardless of gender if no other sample can be accessed. Culture media used for *N. gonorrhoeae* culture in the event of a CSA investigation should include chocolate and Thayer-Martin *N. gonorrhoeae* selective agars. A Gram stain finding of Gram-negative cocci can be used for growth correlation but cannot be used to suggest *N. gonorrhoeae* in the absence of NAAT or culture evidence. Since not all NAAT STI platforms include all agents in the same test cartridge, a wet mount can be used for a rapid *T. vaginalis* and sperm screening, followed by an InPouch for *T. vaginalis* culture if necessary (29). In addition, specimen retention during a CSA workup is extremely important should there be further investigations (10).

Laboratory support to gynecological services has historically included microscopy standards of semiquantitative reporting of polynucleated cells, clue cells, and yeast in addition to *T. vaginalis* and sperm in many physician clinics and pediatric centers caring for teens and adolescents. Institutional CSA procedures should be able to build on the existing standards. While ruling out sperm by wet mount is technically less arduous, microscopy for *T. vaginalis* and Gram stain determination of Gram-negative diplococci or Nugent score may not always be an easy task, and false-positive findings can have adverse consequences (30, 31). By establishing clear criteria for the intended clinical information, microscopy examination is able to provide additional useful information to guide CSA testing and reporting even though bacterial vaginosis remains “inconclusive” for CSA association (Table 1) (10, 32).

While rapid test platforms for *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis* are widely available, laboratory studies of other STI agents, including reference testing, should also be considered. Serological testing for syphilis and viral agents such as HIV and hepatitis B virus should be done when indicated. Skin lesions due to primary or secondary syphilis (painless chancre versus condyloma lata) can be sampled for *Treponema pallidum* direct identification by direct fluorescent-antibody microscopy (DFAM) or NAAT (6). Since there is no FDA-cleared or standard NAAT methods available for *T. pallidum* and since DFAM requires special reagents and expertise, consultation with CDC STI services can be extremely valuable. In addition, if characteristic genital lesions are detected, herpes simplex viruses (HSV-1 and HSV-2) should be tested by a rapid PCR

with culture confirmation; both tests are supported by a single swab in universal transport medium (UTM). The diagnosis of anogenital warts (condyloma acuminatum) is largely clinical. HPV testing should be done on biopsy specimens from suspicious lesions (6, 10). It has been well documented that multiple infections are common in cases of CSA, and thus further testing for other potential agents is warranted upon initial detection of any single potential STI agent (33).

RESULT REPORTING

Microbiological evidence for STI agents provides critical information for clinical assessment of CSA cases which benefits both patient care and the law enforcement investigation to protect the victims. However, the NAAT report should always include a disclaimer for its lack of validation with nonurogenital specimens from patients <13 years of age, even when multiple test modalities were employed. A NAAT with an indeterminate result should be repeated immediately by the same method. Alternatively, repeat testing could be done with the second NAAT, as some PCR modalities are susceptible to inhibitors intrinsic to specimen materials, such as blood in specimens. Most importantly, when one STI agent is identified, an extensive screening for other possible agents should be done. Finding of additional agents or the same agent at additional anatomic sites increases the confidence of CSA as the mode of transmission, especially as pharyngeal and anorectal infections are not uncommon although patients may not be symptomatic (25).

The use of more than one NAAT modality does not mean that every specimen should be tested by both NAATs before reporting. Instead, all specimens should be immediately tested and reported by the primary NAAT at the home institution, as should the microscopy result. Based on the results from the primary NAAT, positive results should prompt the use of a second NAAT for a rapid confirmation. Health care providers should be notified immediately, and the local health jurisdiction should be notified as per local reporting requirements (Table 1). Antimicrobial susceptibility testing should be performed upon *N. gonorrhoeae* isolation and the isolate submitted to the state public health laboratory for epidemiological investigation.

Laboratory records associated with CSA testing in laboratory information systems (LIS) can be used for legal documentation. The ethics and legal implications regarding releasing sensitive test information, such as a laboratory finding of sperm or pregnancy, on pediatric patients (age 0 to 21 years) to a patient/parent portal are usually determined jointly by hospital and laboratory subject matter experts. Laboratorians are generally not involved under most circumstances in determining the visibility of these results in the local electronic health record or chain-of-custody documentation outside LIS.

SUMMARY

Compared to the diagnostic landscape 10 or 20 years ago, NAATs are now the preferred laboratory approach for the detection of STI agents, as they show superior performance characteristics in test sensitivity and specificity, as well as outstanding design features of automation and near-patient testing. The barrier for their use in CSA settings is the lack of test performance validation data when applied to nonurogenital specimen types and specimens collected in children <13 years of age. By obtaining multiple sample types and employing multiple test modalities, laboratories are able to decrease the uncertainty of test results and improve test sensitivity and specificity through test redundancy.

A practical process of specimen collection remains an area of challenge, even with more than one NAAT platform locally accessible. The commercially available NAATs are packaged with platform-specific swabs and transport media that have not been cross validated among NAAT assays and do not take culture for various STI agents (e.g., ESwab for *N. gonorrhoeae*, UTM for *C. trachomatis*, and InPouch for *T. vaginalis*) into consideration. In order to fulfill the requirements of CSA testing by two independent NAATs and culture (Table 3), both medical providers and laboratories face the conundrum of either the use of multiple collection kits per site up front or a second round of

collection pending primary rapid NAAT results prior to patient discharge. Alternatively, laboratory validation of repurposed residual specimen in later preferred transport kits for reference testing would support a more operable CSA workflow.

It is foreseeable that the current diagnostic state of STI pathogen detection in young children with potential CSA may soon become a memorable past. The rise of meta-genomic sequencing technology, once it becomes more automated, could in theory support both the STI diagnostic tests and forensic investigations, obviating the need for *in vitro* cultivation.

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