Public Health Laboratory Testing Practices for

Sexually Transmitted Infections

2021 APHL Survey Report

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

July 2024

Executive Summary

This report provides a summary of the 2021 Sexually Transmitted Infection (STI) Testing Practices Survey that was fielded to 110 APHL member public health laboratories. The survey collected data on laboratories' capabilities and capacities related to testing for STIs. The survey was fielded between June and August 2022, and had a response rate of 76.4% (84 out of 110).

Overall, 76 out of 84 (90.5%) public health laboratories provided some kind of STI testing in-house (**Table 1**). In 2021, responding laboratories performed 1,601,031 gonorrhea tests, 1,540,893 chlamydia tests, 793,564 syphilis tests, 182,573 trichomonas tests, 21,323 herpes simplex virus (HSV) tests, 4,865 *Mycoplasma genitalium* tests and 4,176 human papilloma virus (HPV) tests.

Table 1. Public health laboratories that provided pathogen testing by type, 2021 (n=76)

Type of Pathogen	State (n=45)		Local (n=31)		Total (n=76)	
	#	%	#	%	#	%
Syphilis	44	98%	28	90%	72	95%
Gonorrhea	42	93%	31	100%	73	96%
Chlamydia	40	89%	30	97%	70	92%
Herpes Simplex Virus 1 / 2	26	58%	17	55%	43	57%
Trichomonas	13	29%	21	68%	34	45%
Mycoplasma genitalium	3	7%	8	26%	11	15%
Human Papilloma Virus	3	7%	0	0%	3	4%

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Introduction

Background

Sexually transmitted infections (STIs) are rising in the United States. 2021 data from the US Centers for Disease Control and Prevention (CDC) shows large increases in gonorrhea, syphilis and congenital syphilis, following the interruptions to STI testing and surveillance caused by the COVID-19 pandemic in 2020 and likely 2021.[1] Between 2017 and 2021, CDC estimates gonorrhea cases increased by 27%, syphilis cases increased by 74% and congenital syphilis cases increased by 303%. No significant change was noted in chlamydia cases, which decreased 3.75%. CDC also reported that preliminary data reflected 2.5 million cases of chlamydia, gonorrhea and syphilis combined across the US in 2021.[1]

Public health laboratories play an important role in providing high quality testing for STIs and associated antimicrobial resistance. To assess the capabilities and capacities of US public health laboratories to perform STI testing, APHL fielded the 2021 STI Testing Practices Survey. Similar surveys were conducted in 2017, 2016, 2015, 2013, 2011, 2007, 2005 and 2001. The results of this 2021 survey report provide situational awareness and will be used to inform the focus of APHL resources and tools for public health laboratories.

About the 2021 STI Testing Practices Survey

Methods

APHL fielded a 47-question survey to laboratory directors at 110 APHL member public health laboratories, including 56 state and territorial laboratories and 54 local laboratories. APHL's STI Subcommittee developed the survey with input from the Infectious Diseases Committee. The survey was distributed via email to laboratory directors on June 13, 2022 and administered via Qualtrics. The survey remained open through August 16, 2022. The deadline for survey submission was extended several times to provide additional time for completion due to public health laboratories simultaneously responding to the COVID-19 and mpox public health emergencies. Respondents were asked to provide information regarding laboratory testing practices from January 1, 2021 through December 31, 2021.

Responses

Out of 110 laboratories that received the survey, only 84 laboratories (76.4%) responded. State and territorial laboratories had a higher response rate than local laboratories, with response rates of 87.5% (n=49/56) and 64.8% (n=35/54) respectively.

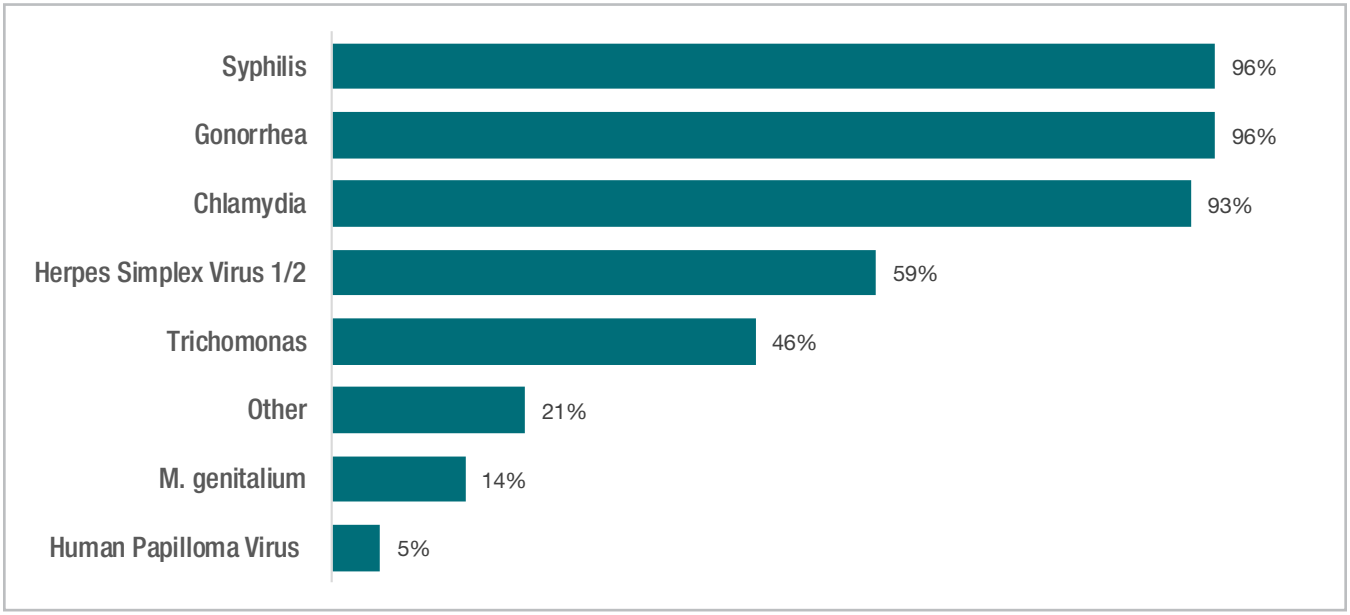
Limitations

The questions for this survey were modeled after questionnaires from previous surveys in an effort to increase the utility of the data and add considerably to the general body of knowledge about STD testing. This survey was also updated to reflect current market availability of STD test technology.

Overview of STI Testing

A majority (90.5%, n=76) of responding public health laboratories offered STI testing (**Figure 1**). Laboratories performing STI testing commonly offered in-house testing for syphilis (n=72/76, 94.7%), gonorrhea (n=73/76, 96.1%) and chlamydia (n=70/76, 92.1%). This is unsurprising, as these three STIs are nationally notifiable. About half of responding laboratories offered in-house testing for herpes simplex virus (HSV) 1 and/or 2 (n=43/76, 56.6%) and trichomonas (n=34/76, 44.7%). One local laboratory noted that referral testing for chlamydia, syphilis, human papilloma virus (HPV) and trichomonas was available, while two additional local laboratories offered referral testing for HSV (**Figure 1**).

Figure 1. Public health laboratories that provide or refer testing for STIs (n=76)



Public health laboratories infrequently offered in-house testing for *M. genitalium* (n=11/76, 14.5%) and HPV (n=3/76, 3.9%). However, based on results from a 2020 *Mycoplasma genitalium* Laboratory Testing Survey, there was an increase among public health laboratories offering *M. genitalium* testing. As of 2020, 6.2% (n=4/65) of responding laboratories offered *M. genitalium* testing, whereas 14.5% (n=11/76) offered this testing in 2021. Of note, however, the 2020 survey had a low (60.2%) response rate, which should be taken into consideration when evaluating this change. "Other" STIs for which laboratories reported testing or referring testing included *Haemophilus ducreyi*, which causes the STI chancroid, HIV, hepatitis C virus (HCV) and *Entamoeba histolytica*, however not every laboratory that performs HIV or HCV testing reported that here.

Collectively, 511 public health laboratory staff were trained to perform STI testing, with an average of 6.7 full time equivalents (FTEs) per laboratory. Three hundred and forty-five staff were routinely assigned to perform STI testing, with an average of 4.5 FTEs per laboratory. Local laboratories had slightly fewer staff trained and assigned to perform STI testing on average compared to state laboratories, however, the difference was less than one FTE.

Self-Collected Specimens & Point-of-Care Tests

Non-traditional avenues for STI testing provide the opportunity to expand testing, reach under-served populations and reduce burdens on clinics and providers. As such, this survey asked several questions related to self-collection of specimens as well as opinions on Clinical Laboratory Improvement Amendments (CLIA)-waived point-of-care tests (POCTs), which are tests that can be performed by non-laboratorians in a non-laboratory setting. One caveat with the data is that no definition of "clinical setting" was provided in the survey and laboratories were not asked to expand upon their answers.

Approximately half of responding laboratories (52.6%, n=40/76) accepted specimens that were self-collected in a clinical setting; 23.7% (n=18/76) did not accept this type of self-collected specimen and another 23.7% (n=18/76) indicated that specimens were not specified as self-collected or clinician-collected (**Figure 2**).

When specimens were self-collected in a clinical setting, laboratories most frequently accepted vaginal and rectal specimens for chlamydia (n=38/40) and gonorrhea testing (n=37/40); however, some laboratories also accepted pharyngeal specimens as well as specimens for trichomoniasis and *M. genitalium* testing (**Figure 3**). Laboratories did not accept specimens self-collected in a clinical setting for HSV or HPV testing. At the time of this survey, several commercially available assays were US Food and Drug Administration (FDA)-cleared for use with vaginal swabs self-collected in a clinical setting, but there were no FDA-cleared options for self-collected extragenital specimens.

Figure 2. Laboratories that accepted specimens self-collected in a clinical setting (n=76)

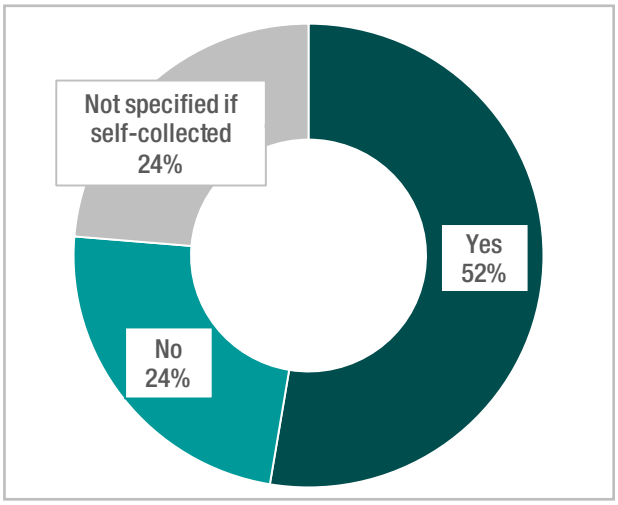
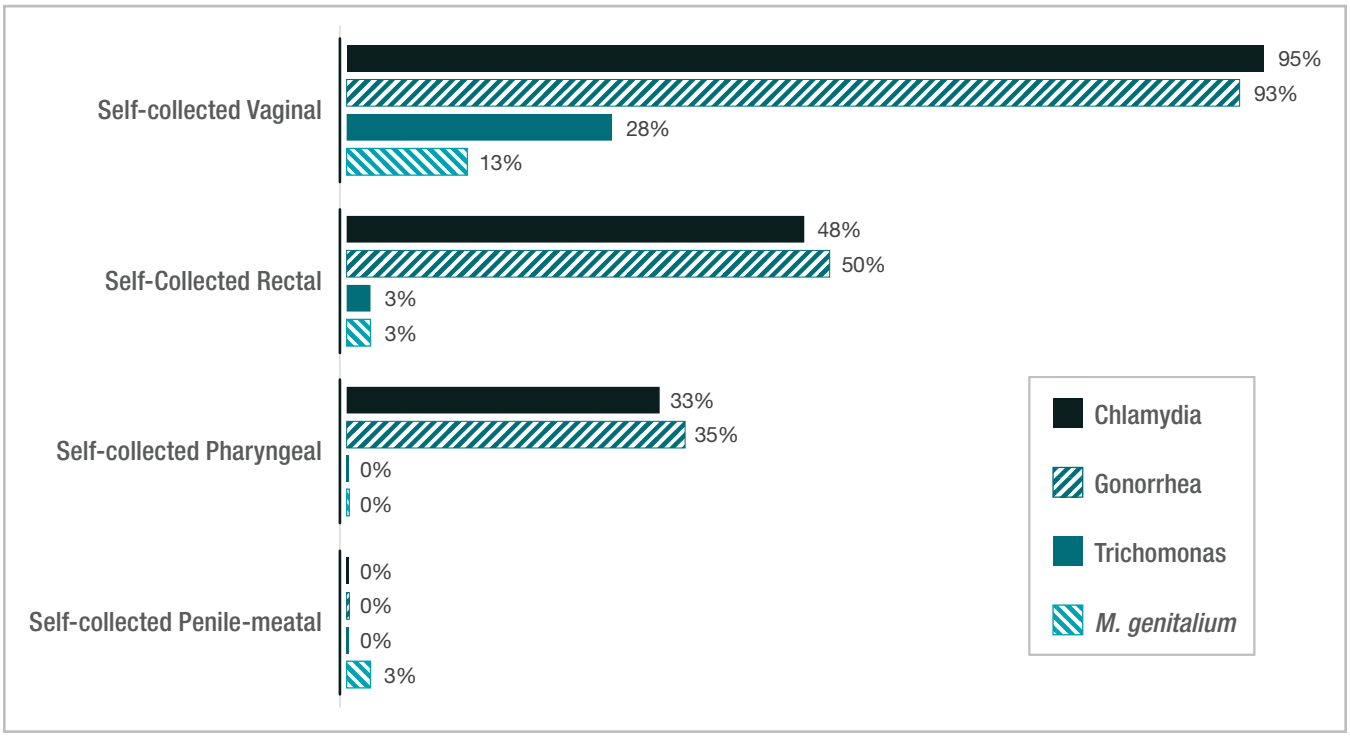


Figure 3. Specimens self-collected in a clinical setting: acceptable specimen types and tests (n=40)



Only 6.6% of laboratories (n= 5/76) accepted specimens self-collected in a non-clinical setting. Three laboratories that do not currently accept specimens self-collected in a non-clinical setting had plans to implement or support such testing in the future. All five laboratories that accepted self-collected specimens in a non-clinical setting accepted self-collected vaginal specimens for chlamydia and gonorrhea testing; two laboratories also accepted self-collected rectal specimens. One laboratory accepted self-collected vaginal and rectal specimens for trichomonas testing. At the time of this survey there were no FDA cleared STI assays available for use in a public health laboratory with any type of specimen self-collected in a non-clinical setting.

Respondents were also asked to provide their opinions on CLIA-waived POCTs. CLIA-waived POCTs are rapid tests that have received FDA approval or clearance to be performed by non-laboratorians in a non-laboratory setting, such as a clinic. These opinion questions were optional for respondents whose laboratories offered STI testing in 2021.

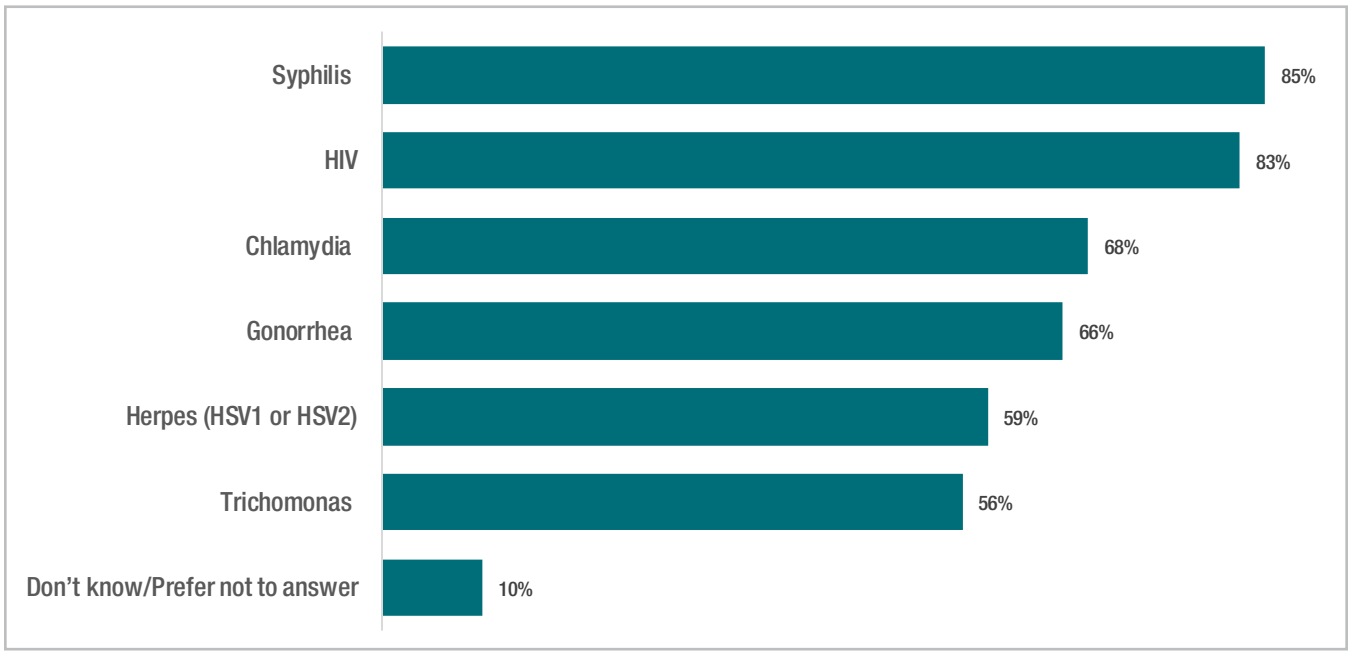
Out of 53 respondents that elected to answer, 45 (84.9%) indicated that, in general, they believed there would be a benefit to patients or public health if clinics or providers were to perform on-site STI POCTs.

Laboratory staff identified quick turnaround time for results and treatment as one of the primary benefits of implementing POCTs. Laboratorians also felt that POCTs could reduce time to link patients and their partners to care, reduce spread of disease, allow for immediate communication and education of patients, and offer a less costly option as compared to laboratory testing. Laboratorians also mentioned that POCTs may be especially useful for patients with whom follow up or scheduling additional appointments are challenging.

When asked which POCTs should be performed respondents cited syphilis (85.4%, n=35/41) and HIV (82.9%, n=34/41) most often (**Figure 4**).

Respondents—both those who supported and opposed POCT for STIs—were asked to identify major challenges to POCT utilization in clinical settings. Laboratorians identified the lack of quality assurance and quality control programs/measures as one of the most challenging factors. Over half of respondents identified the lack of mechanisms to report results to public health authorities/surveillance, mistrust of test results and low test accuracy as major challenges. In addition to highlighting the lack of QA/QC in clinical settings for POCT, laboratory staff opposed to POCT emphasized that laboratory testing is more accurate and reliable and, clinical personnel have high turnover rates, which would require frequent training to uphold quality practices.

Figure 4. Which STI POCTs should be performed (n=41)



Chlamydia trachomatis Testing

Out of the 84 responding public health laboratories, 70 (83.3%) performed in-house *Chlamydia trachomatis* (CT) testing and one laboratory offered referral testing. These laboratories tested 1,540,893 specimens for CT, of which 129,914 (8.4%) were positive. State laboratories tested a majority of the specimens and averaged 28,980 specimens per laboratory (range: 179-134,489) while local laboratories tested an average of 12,723 specimens (range: 0-89,166).

Similar to 2017, nucleic acid amplification testing (NAAT) was by far the most commonly employed method for detecting CT, utilized by all laboratories that offered in-house testing (**Figure 5**). Additionally, 8.5% of laboratories also offered in-house fluorescent antibody (DFA) and /or culture. No laboratories reported offering CT drug susceptibility testing (DST) in-house, but seven laboratories referred specimens for DST to other public health laboratories including CDC.

Laboratories frequently identified the Aptima Combo 2® for CT/GC (Hologic) as their primary method utilized to detect CT (**Figure 6**). This was also the most common test used in 2017. However, a dramatic increase in laboratories reporting this test as their primary testing method was observed, increasing from 66.2% (n=45/68) in 2017 to 90.0% (n=63/70) in 2021.

Figure 5. CT Test Services Offered by Public Health Laboratories (n=71)

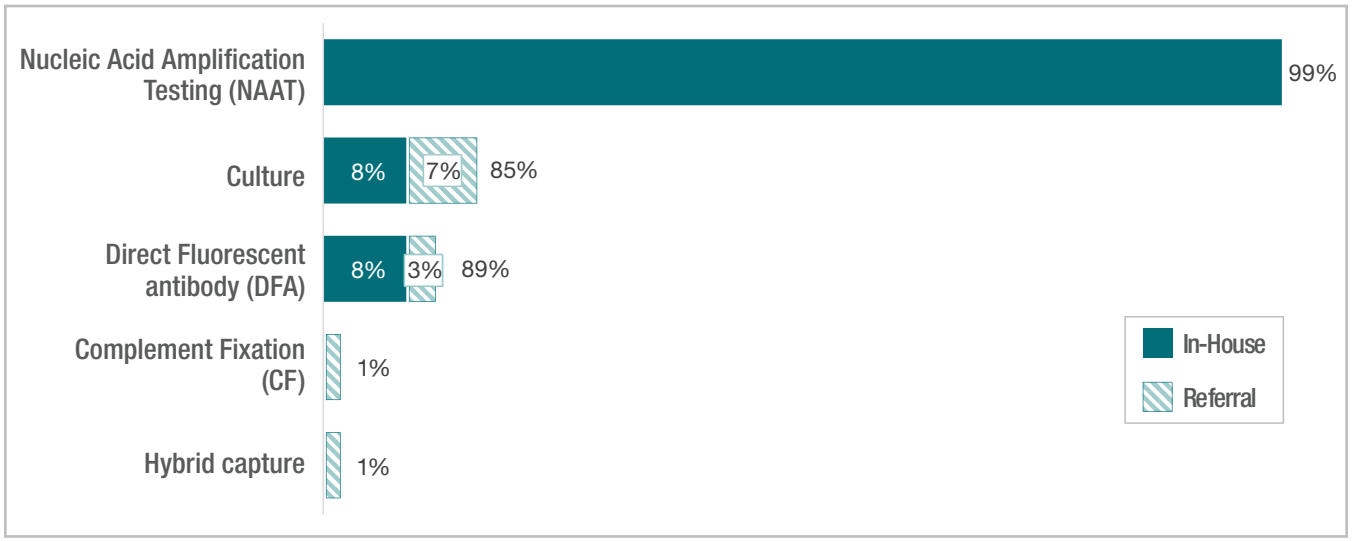
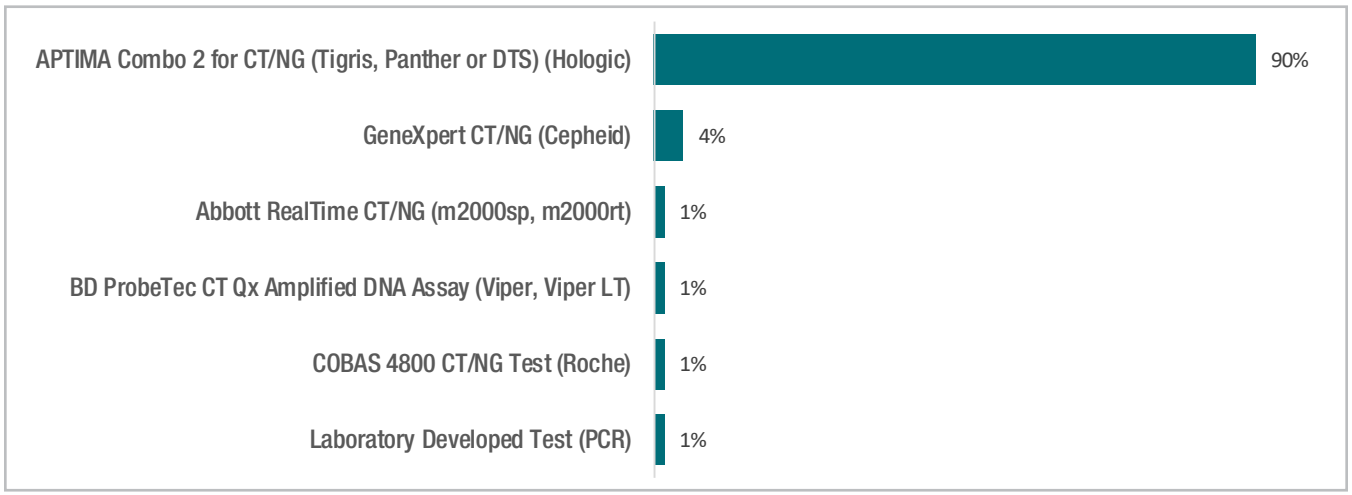


Figure 6. Primary Test Methods Used to Identify CT (n=70)



CT NAAT Testing

As described above, NAAT is the most common method used by public health laboratories to identify CT. Of the 70 public health laboratories that reported conducting CT NAAT testing, 90.0% (n=63) reported that they did not conduct repeat testing on specimens that tested positive for CT by NAAT, 7.1% (n=5) reported that they occasionally conduct repeat testing on positive specimens (e.g., when contamination is suspected), and 2.9% (n=2) reported they routinely conduct repeat testing.

Laboratories accepted a wide variety of specimens for CT NAAT, with urine, ano/rectal swabs, and endocervical swabs being the most widely accepted (**Figure 7**). Urine was the most common specimen type received for both men and women (**Figure 8**). Although first catch urine is the preferable specimen type in males, in females self or clinical collected vaginal swabs are recommended due to the reduced sensitivity of urine.[2]

Figure 7. Accepted specimen types for CT NAAT (n=70)

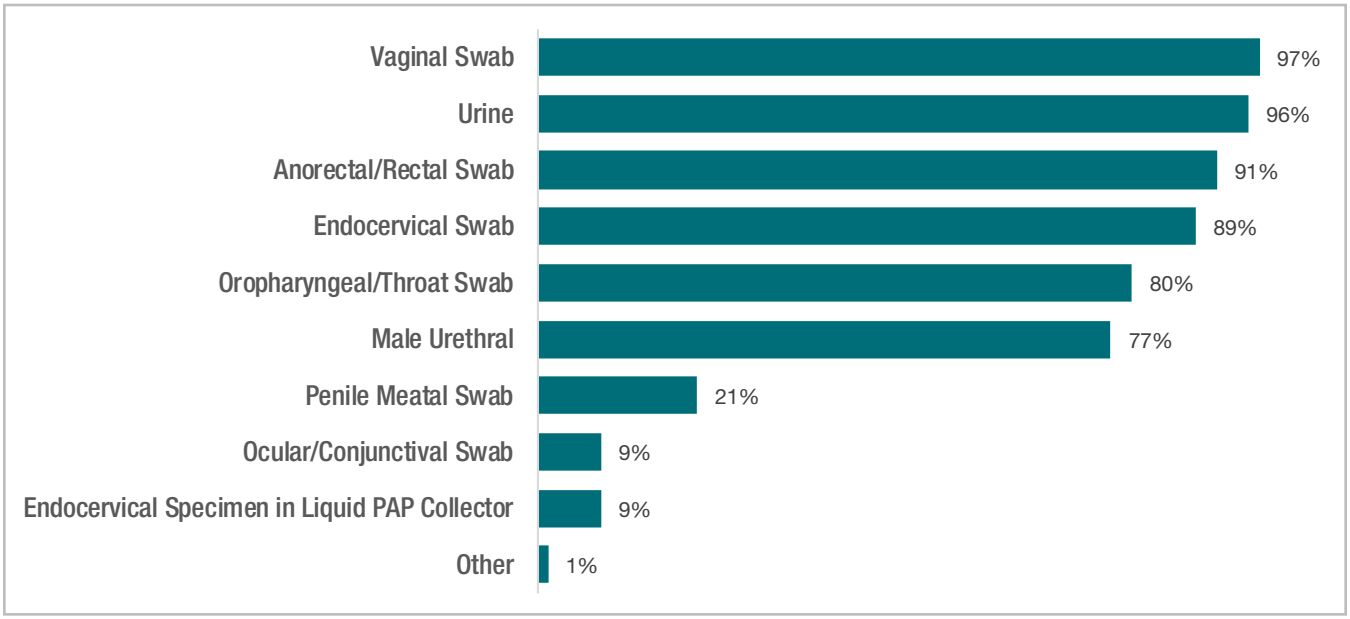
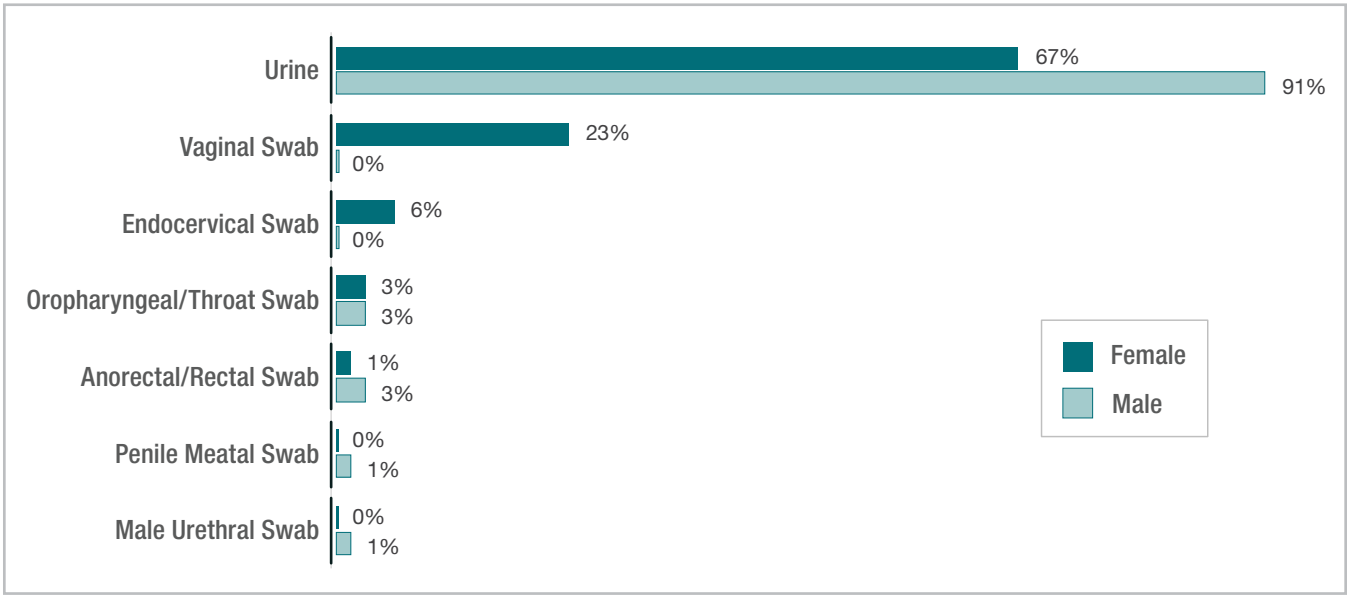


Figure 8. Most Frequently Received Specimen Types for CT Testing (n=70)



Lymphogranuloma Venereum Testing

Lymphogranuloma venereum (LGV) is an infection caused by select strains of CT. LGV can result in severe inflammation and infection, causing lymphadenopathy and proctocolitis.[3] While commonly used CT NAATs detect the strains that cause LGV, they do not differentiate them from other CT strains. Testing for LGV strains specifically is not commonly requested nor performed. Only four of 84 responding laboratories (4.8%) reported receiving specimens specifically for LGV testing in 2021. One of these laboratories performed an in-house laboratory developed LGV test, one laboratory used a NAAT to detect CT and then referred the specimen to a different laboratory for differentiation and two laboratories referred specimens for LGV testing elsewhere.

Neisseria gonorrhoeae Testing

Out of the 84 responding public health laboratories, 73 laboratories (86.9%) provided in-house testing for *Neisseria gonorrhoeae* (GC) in 2021. Nearly all (95.9%, n=70/73) of these laboratories also provided CT testing in-house. These laboratories tested 1,601,031 specimens for GC, of which 85,157 specimens (5.3%) were positive. Over 70% (n=1,158,887) of GC tests were performed by state laboratories, which averaged 27,593 specimens per laboratory (range: 10-134,583). Local laboratories performed 14,263 GC tests on average (range: 0-135,301).

Of the 73 laboratories performing GC testing 94.5% used a NAAT based method (n=69); roughly half offered GC culture (n=38, 52.1%) and gram stain smear (n=33, 45.2%) (**Figure 9**). Gram stain was more likely to be offered by local (67.7%, n=21/31) rather than state public health laboratories (28.6%, n=12/42). Approximately 81% of laboratories performing GC testing utilized the Aptima Combo 2® for CT/NG as their primary method for GC identification (**Figure 10**). Similar to what was observed with CT testing, between 2017 and 2021, there was an increase in laboratories identifying this test as their primary method of identification. In 2017, 65.6% (n=44/67) reported the Aptima Combo 2® as their primary test method and in 2021, this rose to 80.8%.

Figure 9. GC test services offered by public health laboratories (n=73)

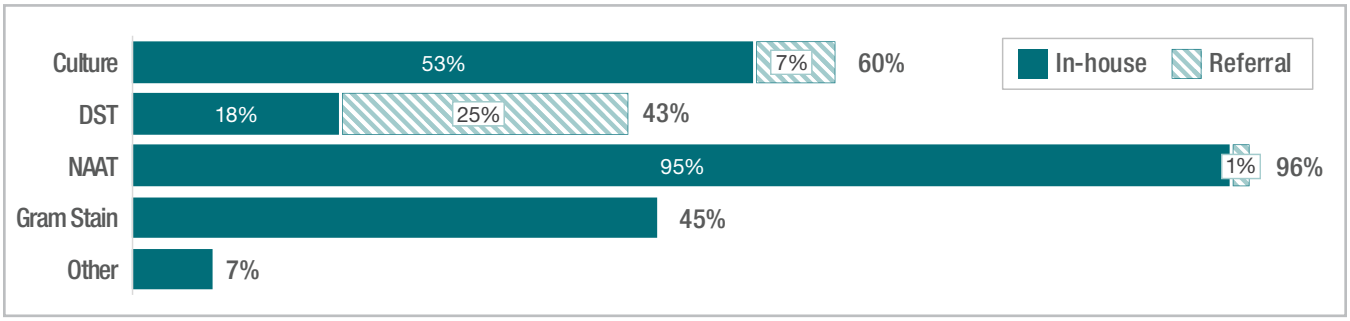
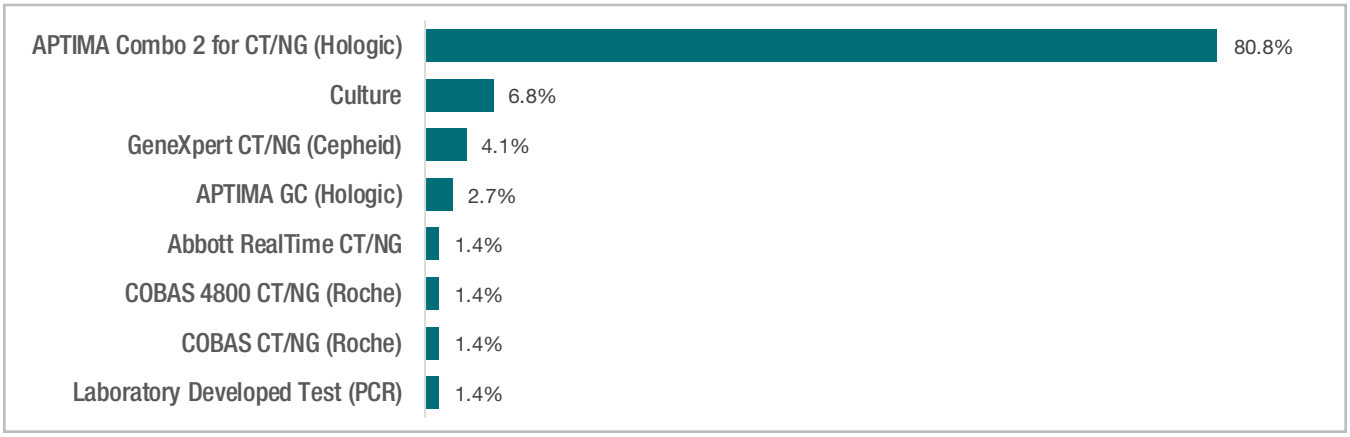


Figure 10. Primary test methods used to identify GC (n=73)



GC NAAT

NAAT was the most common GC diagnostic testing service offered by responding laboratories that performed GC testing (n=69/73, 94.5%). Of these laboratories, 50 (72.5%) reported that CT and GC NAATs can only be ordered in combination. This integrated testing is beneficial as CT and GC often co-occur and this reduces the potential need for patients to return for additional testing. At 23.1% of the public health laboratories, CT and GC NAAT could be ordered as individual tests or in combination (n=16), in 2.9% CT and GC NAAT could only be ordered individually (n=2), and one laboratory (1.5%) had order rules that varied by specimen type.

Of the 69 laboratories performing GC NAAT, most accepted urine (n=65, 94.2%), vaginal and anorectal/rectal swabs (n=64, 92.3%), endocervical swabs (n=61, 88.4%), oropharyngeal/throat swabs (n=59, 85.5%) and male urethral swabs (n=55, 79.7%) (**Figure 11**). Fewer laboratories accepted penile meatal swabs (n=15, 21.7%), endocervical specimens in liquid PAP collector (n=6, 8.7%), ocular/conjunctival swabs (n=5, 7.2%) or "Other" genital swabs (n=2, 2.9%).

Urine was the most commonly received specimen type for both male and female specimens (**Figure 12**). However, as with chlamydia testing, CDC recommends first-catch urine as the optimal specimen for males whereas vaginal swabs (self-collected or clinician collected) are the optimal specimens for females.[4] Only 23.2% of laboratories that performed GC NAT (n=16/69) reported vaginal swabs as the most common specimen types received for females.

Figure 11. Accepted Specimens for GC NAAT (n=73)

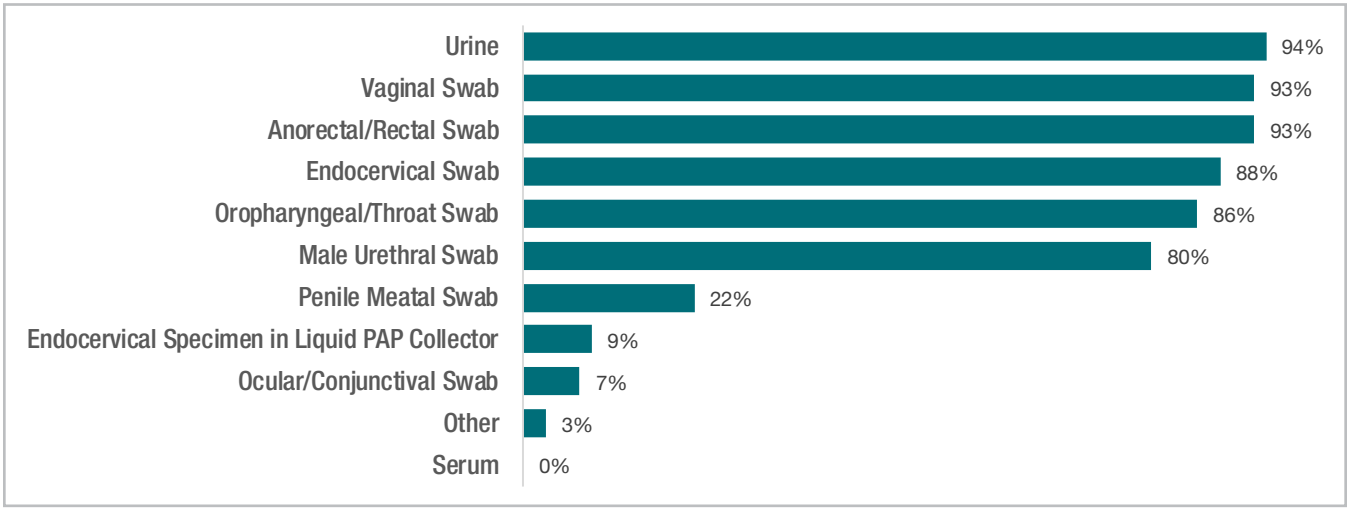
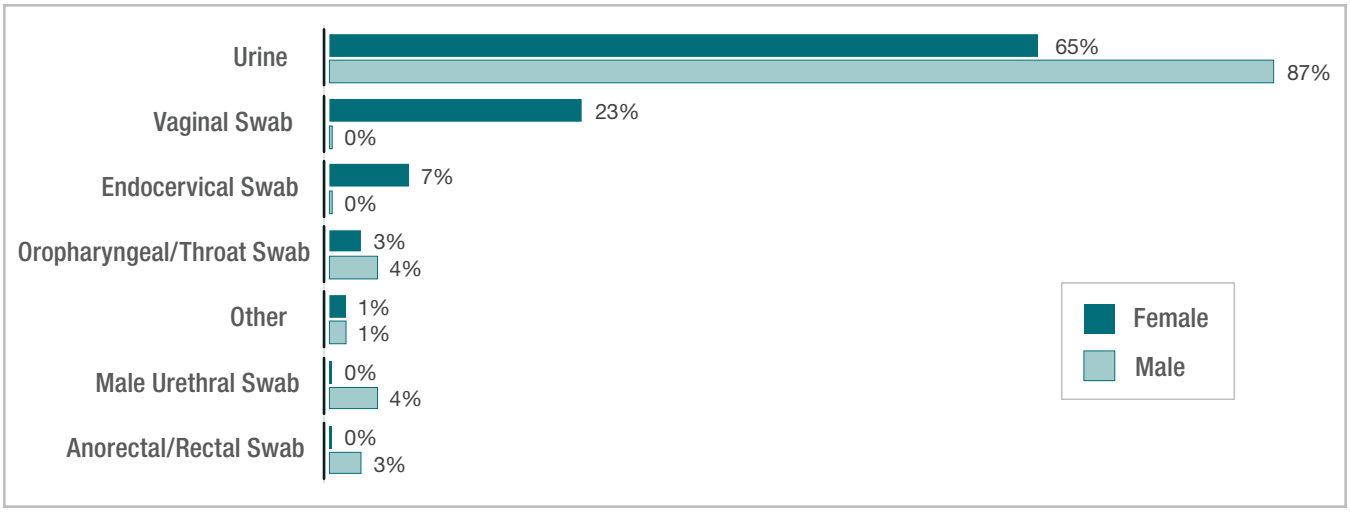


Figure 12. Most Received Specimen Types for GC Testing (n=70)

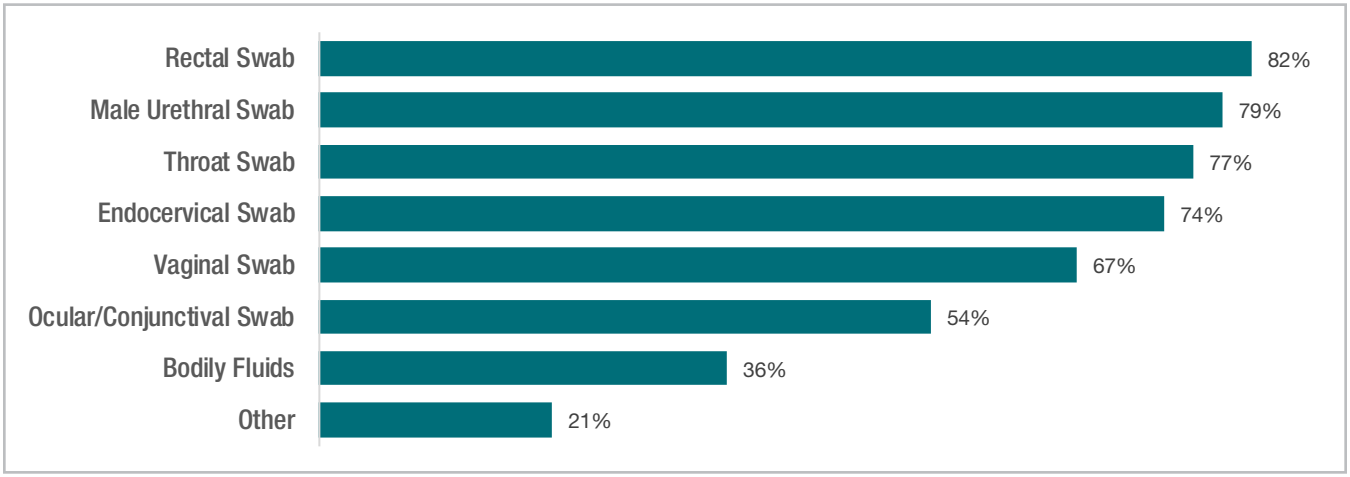


GC Culture

GC culture and identification was the second most offered GC testing service, with 38 laboratories performing in-house culture. Laboratories (n=32, not all provided specimen counts) cultured 20,800 specimens (range: 1-10,000) and averaged 650 specimens per laboratory in 2021.

Laboratories accepted numerous specimen types for diagnostic testing. More than 75% of these laboratories validated rectal swabs (n=32/39, 82.0%), male urethral swabs (n=31/39, 79.5%), throat swabs (n=30/39,76.9%) and endocervical swabs (n=29/39, 74.4%). "Other" specimen types included isolates, urine, blood, pharyngeal swabs and genital swabs streaked onto plates prior to shipping (**Figure 13**). Methods including Gram stain + oxidase (n=31/39, 79.5%), MALDI-TOF (n=21/39, 53.8%) and biochemical/sugars (n=13/39, 33.3%) were commonly paired with culture for GC identification.

Figure 13. Specimen types validated for GC culture (n=39)



As documented in *Antibiotic Resistance Threats in the United States, 2019*, CDC classifies drug resistant gonorrhea as an urgent threat.[5] Gonorrhea has developed resistance to all but one class of antibiotics, making drug susceptibility testing crucial. Overall, 13 laboratories provided in-house DST and 19 laboratories referred specimens for DST; one of these laboratories both offered DST and referred specimens. Unsurprisingly, only laboratories that offered GC culture provided in-house DST. Twelve laboratories reported methods utilized for DST (**Figure 14**). Etest was most utilized (91.7%) followed by disc diffusion (33.3%). DST for a wide array of antimicrobials were offered, but most laboratories provided resistance testing for ceftriaxone (n=12/12, 100%), cefixime (91.7%) and azithromycin (91.7%) (**Figure 15**). Two-thirds of these laboratories' assays included ciprofloxacin. No laboratories performed in-house testing for cefoxitin or spectinomycin. Laboratories referred specimens for testing to another public health laboratory (n=16), CDC (n=2) or a commercial laboratory (n=1).

Figure 14. Methods used for GC drug susceptibility testing (n=12)

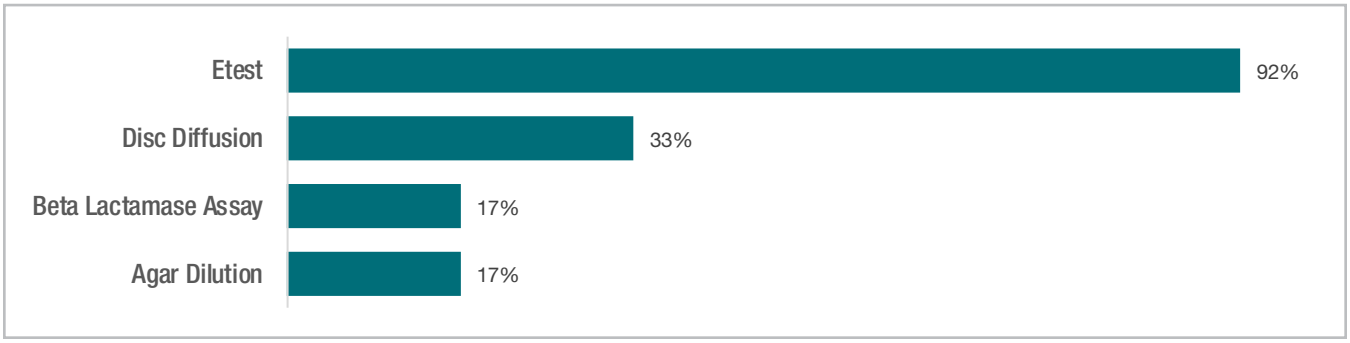
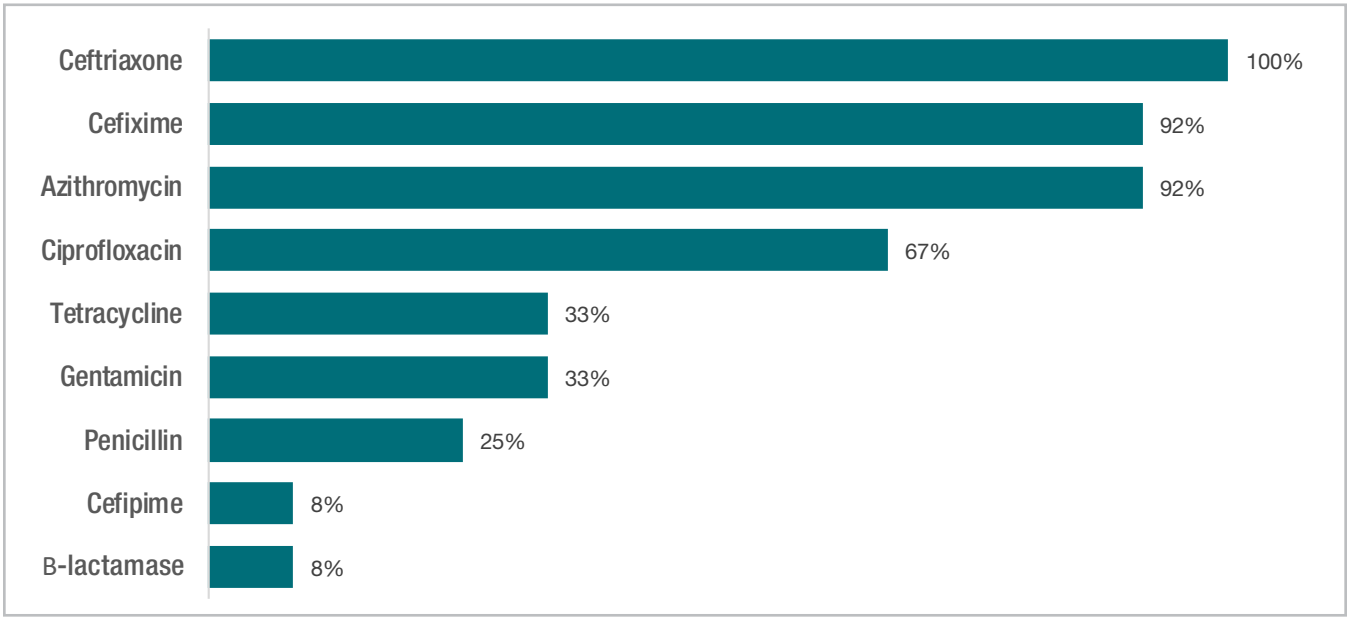


Figure 15. **Antimicrobials included in GC DST at public health laboratories (n=12)**



CT and GC Medical-legal Testing

Laboratories that provided CT and/or GC NAAT (n=70) were asked to describe medical-legal testing practices. Overall, 32 laboratories provided in-house medical-legal testing while 38 did not. One laboratory provided only CT testing and two laboratories provided only GC testing; the remaining 29 laboratories provided medical legal testing for both STIs. NAAT was commonly provided for both CT (n=25/30, 83.3%) and GC (n=25/31, 80.6%) medical legal testing (**Figure 16**). Culture (n=13/31, 41.9%) was more commonly utilized for GC than CT (n=2/30, 6.7%). Among laboratories offering NAAT (either alone or in combination testing), the most utilized assays were the APTIMA Combo and Combo 2 (n=20/27, 74.1%) (**Figure 17**). Laboratories that defined their combination testing methods (n=4) mentioned both culture in combination with NAAT (n=2) and NAAT in combination with another NAAT (n=2).

Figure 16. **Type of testing provided for CT and GC medical-legal testing (n=30 CT, 31 GC)**

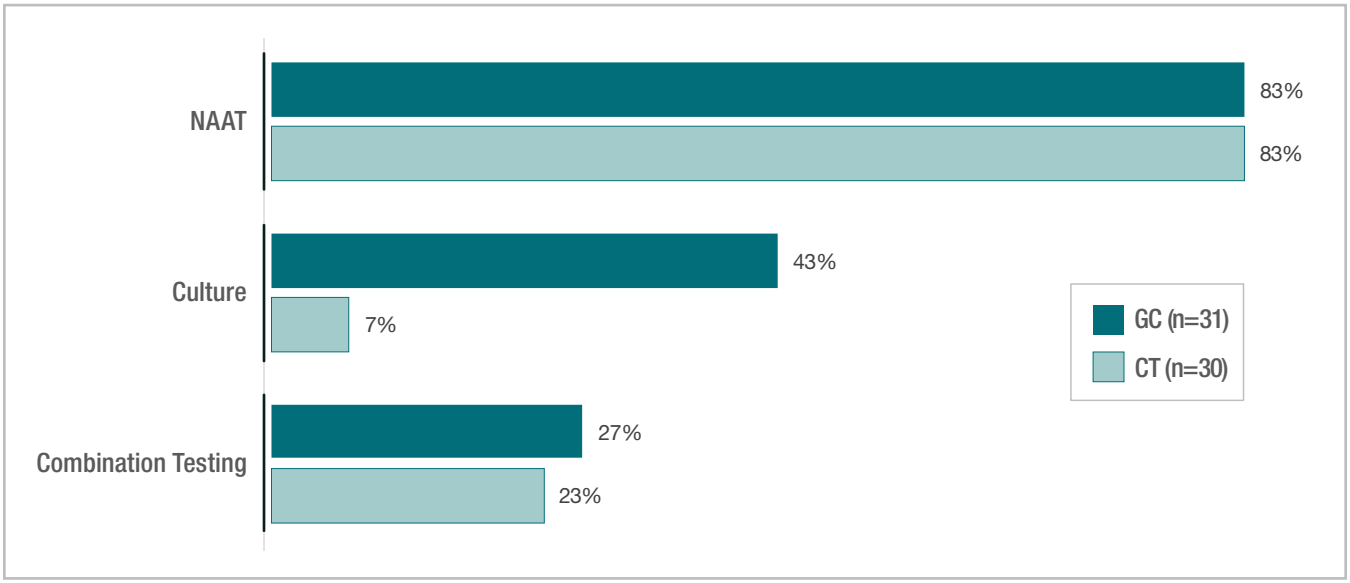
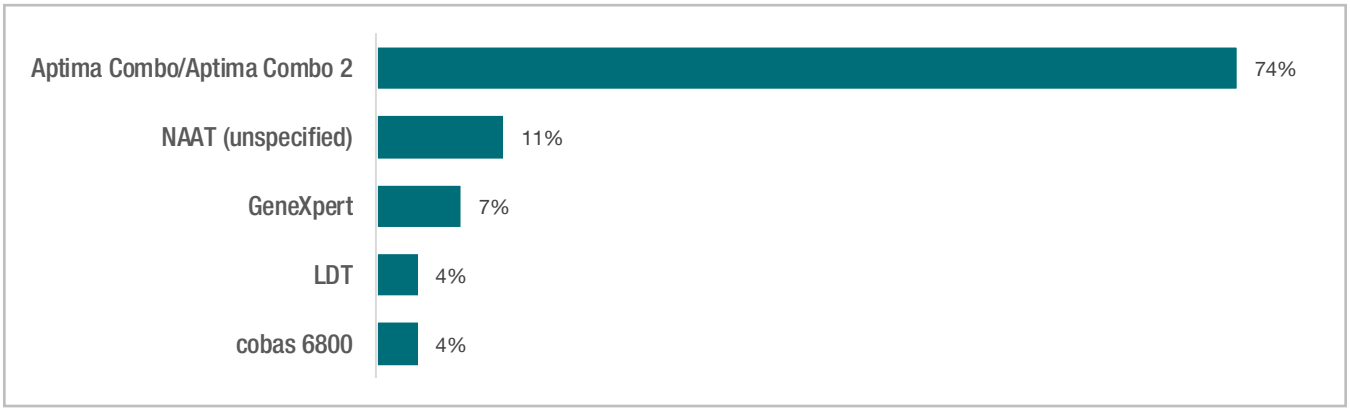


Figure 17. NATs used for CT and/or GC medical-legal testing.



CT and GC Testing in Special Populations

In 2021, 18 laboratories received 1,579 requests to perform non-medical-legal testing on specimens from patients under 14 years old. The majority of requests (n=1,520, 96.3%) were received by state laboratories (n=11) as compared to local laboratories (n=7). Many of the commercially available and FDA cleared NAATs for CT and GC have not been evaluated with specimens from children or adolescents. Therefore, laboratories must validate these specimen types prior to acceptance. About half (n=34/70, 48.6%) of laboratories that perform CT and GC testing reported accepting specimens from adolescents under 14 years of age. Ten of these 34 laboratories reported the ability to accept specimens from patients under 14 years old from other public health laboratories for referral testing.

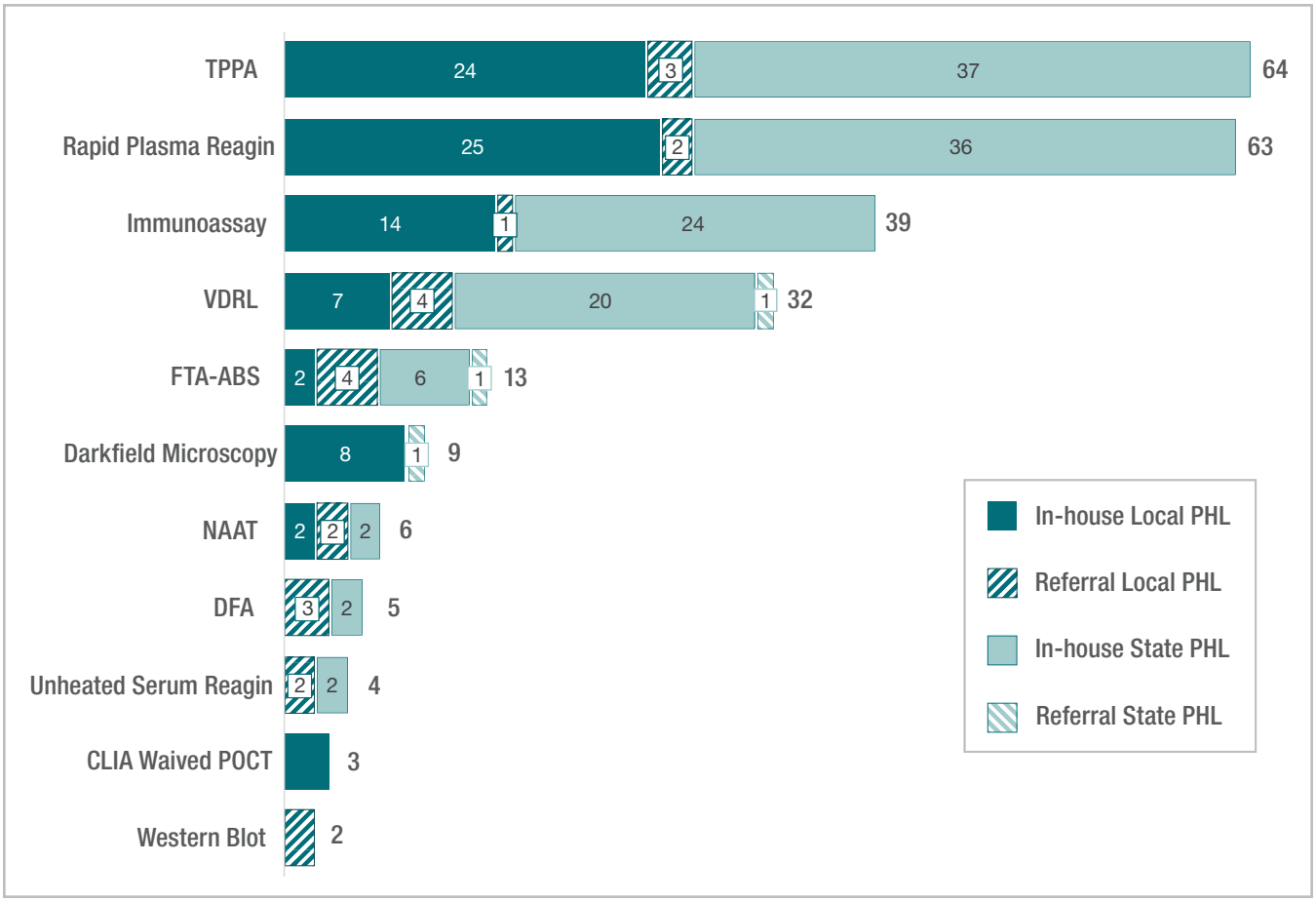
Treponema pallidum (Syphilis) Testing

Out of the 84 responding public health laboratories, 72 laboratories (85.7%) provided some kind of in-house testing for syphilis in 2021. These laboratories tested 793,564 specimens for syphilis with an average positivity rate of 14.3% (n=113,852). An average of 11,022 syphilis specimens (range of 0 to 86,426) were received per laboratory. State laboratories performed over 70% of syphilis tests and averaged 12,913 specimens per laboratory (range: 67-86,426), whereas local laboratories averaged 8049 syphilis tests per laboratory (range: 0-46,768).

The majority of laboratories that performed in-house syphilis testing reported conducting rapid plasma regain (RPR) testing (n=61/72, 84.7%) and an equal number of laboratories offered *Treponema pallidum* particle agglutination (TPPA). 52 of these laboratories performed both RPR and TPPA. Immunoassays (n=38/72, 52.8%) and venereal disease research laboratory (VDRL) tests (n=27/72, 37.5%) were also commonly performed in-house. Few laboratories performed in-house fluorescent treponemal antibody absorption (FTA-ABS), darkfield microscopy, NAAT, direct fluorescent antibody (DFA), unheated serum regain or CLIA-waived POCT and no respondents reported using an in-house Western blot (**Figure 18**).

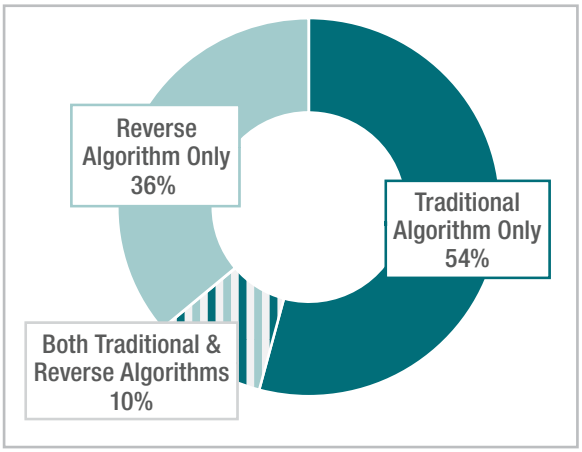
Twenty-seven laboratories reported performing VDRL testing. The majority (n=25, 92.6%) indicated that they used VDRL as it was the only test still recommended for cerebral spinal fluid testing. We did not confirm which specimen types were tested with VDRL in this survey. Other cited reasons for performing VDRL included costs associated with having more than one syphilis method available (n=3), costs associated with verification of new methods (n=1), low test volume (n=1), low cost of the VDRL test (n=1), reliability of VDRL quantitative values for serum (n=1), and the ability to offer a gold standard test that is not found in most clinical laboratories (n=1).

Figure 18. Syphilis Testing Methods Used by Public Health Laboratories (n=72)



Laboratories reported using a variety of different algorithms (**Figure 19**) with 54.2% (n=39/72) using only the traditional algorithm (i.e. starting with a lipoidal (nontreponemal) assay as the first test in the testing algorithm, **Figure 20A**), 36.1% (n=26/72) using only the reverse algorithm (i.e. starting with a treponemal assay as the first test in the testing algorithm, **Figure 21A**) and 9.7% (n=7/72) using both the traditional and reverse algorithms. A higher percentage of local laboratories (n=12/28, 42.9%) used only the reverse algorithm compared to state laboratories (14/44, 31.8%). However, both local (13/28, 46.4%) and state public health laboratories (n=26/44, 59.1%) were more likely to rely on only the traditional algorithm.

Figure 19. Syphilis Testing Algorithms Used by Public Health Laboratories (n=72)



Traditional Algorithm

The lipoidal (nontreponemal) assays used by the 46 public health laboratories that follow the traditional algorithm are displayed in Figure 20B. Eight laboratories reported using more than one lipoidal (nontreponemal) test method and cited reasons including: use of VDRL for CSF only, different methods used for screening and quantitation, customer choice, and maintenance of backup methods in case of instrument failure or receipt of low-volume specimen. About 37.0% (n=17/46) of laboratories used the Syphilis RPR Card Test (Arlington Scientific), 28.3% (n=13/46) used the Macro-Vue™ RPR Card Test (BD) and 21.7% (n=10/46) used the BD BBL™ VDRL Antigen test (Fisher).

For treponemal testing, about 69.6% (n=32/46) of laboratories following the traditional algorithm reported using the SeroDia® TP-PA (Fujirebio) assay and 15.2% (n=7/46) used the ARCHITECT Syphilis TP (Abbott) assay (Figure 20C). Nine laboratories reported using more than one non-treponemal test method and cited reasons including: changed testing platform in 2021, customer preference, use of rapid tests in STAT lab settings, use of additional test to resolve discrepancies, maintenance of backup methods in case of instrument failure or staffing challenges and differential use by community clinics due to test build.

Figure 20A. The traditional algorithm

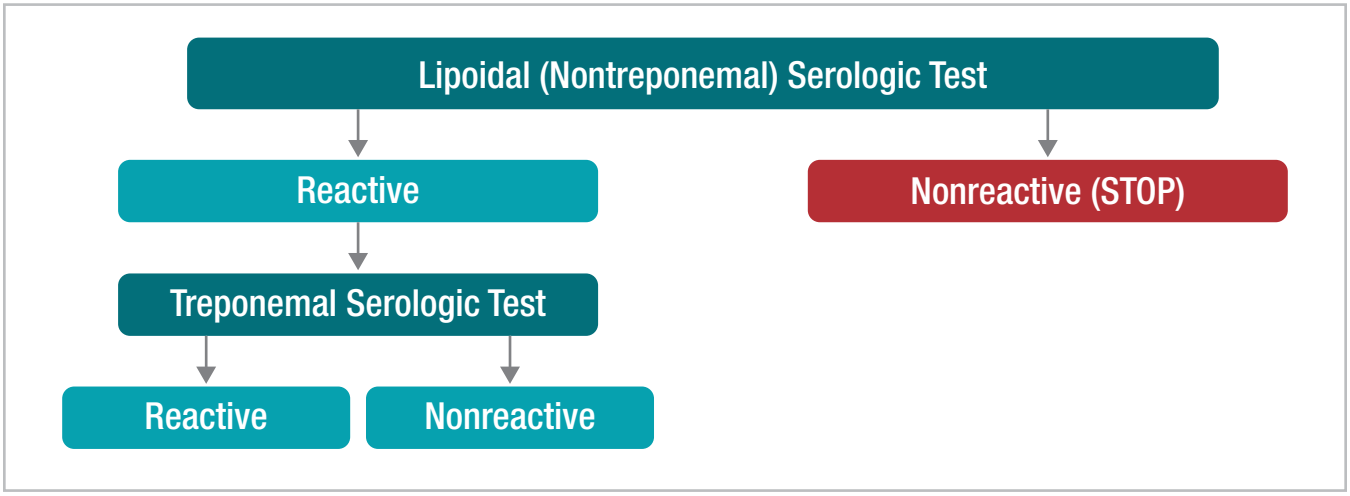


Figure 20B. Lipoidal (nontreponemal) assays used by public health laboratories in the traditional algorithm (n=46)

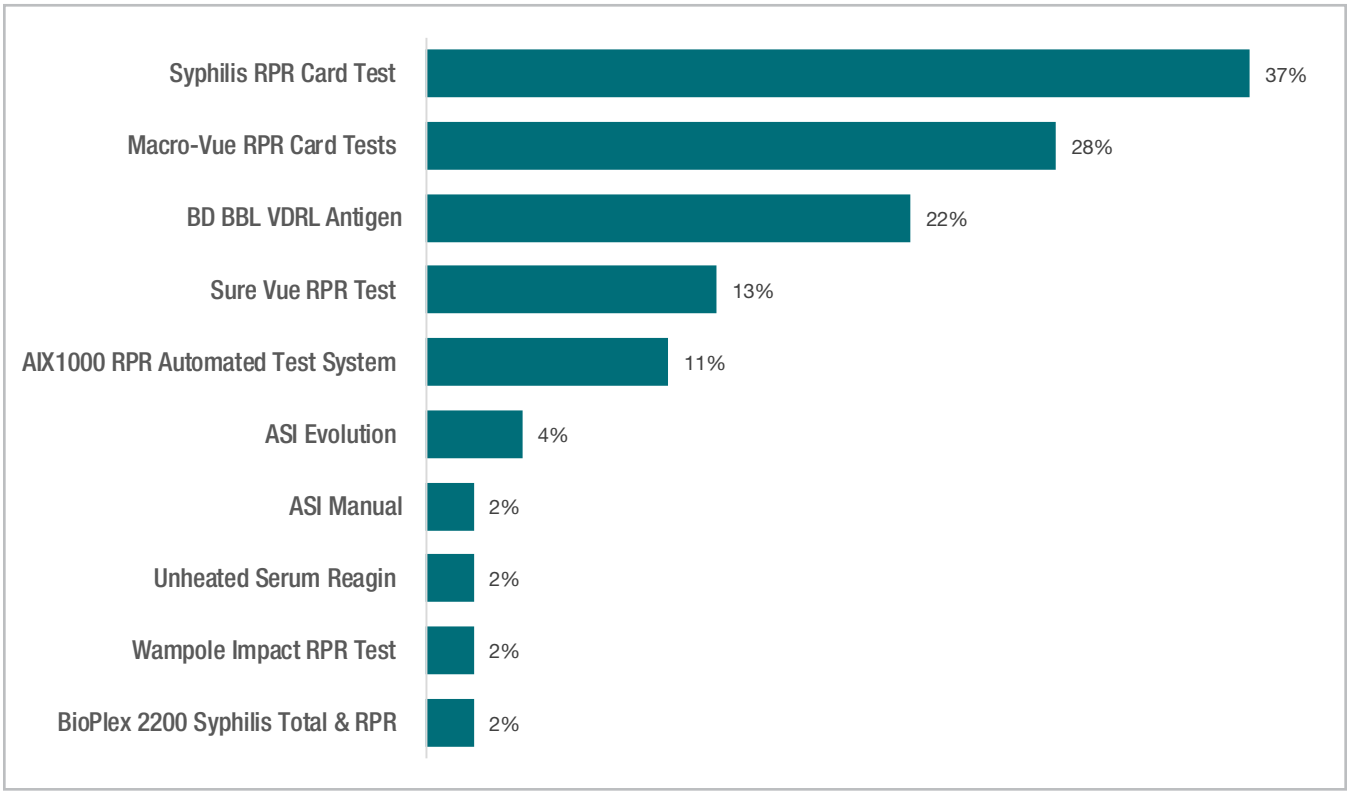
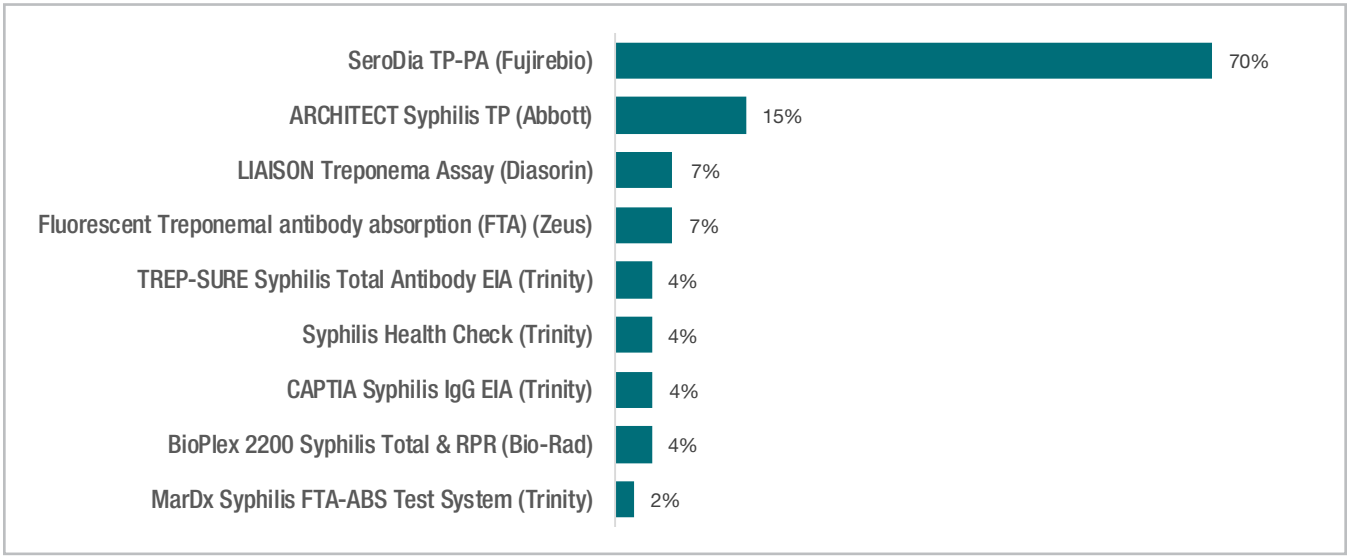


Figure 20C. Treponemal assays used by public health laboratories in the traditional algorithm



Reverse Algorithm

Of the 33 respondents that reported following the reverse algorithm (i.e., starting with a treponemal assay as the first test in the testing algorithm), 27.3% (n=9/33) started with the ARCHITECT Syphilis TP (Abbott), 24.2% (n=8/33) used the BioPlex 2200 Syphilis Total & RPR (Bio-Rad) first and 15.2% (n=5/33) first used the LIAISON® Treponema assay (DiaSorin). Other assays used as the first step in the reverse algorithm are shown in **Figure 21B**.

The lipoidal (nontreponemal) assays used by respondents for the reverse algorithm are displayed in Figure 21C. Three laboratories reported using more than one non-treponemal test method and cited reasons including: use of VDRL for CSF only, different methods used for screening and quantitation and verification of a new method in 2021. About 39.4% (n=13/33) of laboratories used the Macro-Vue™ RPR Card Test and 30.3% (n=10/33) used the Syphilis RPR Card Test (Arlington Scientific). Other lipoidal (nontreponemal) assays used in the reverse algorithm are shown in **Figure 21C**.

The majority (81.8%, n=27/33) of reverse algorithm users rely upon the SeroDia® TP-PA assay as the third step in the algorithm. Additional tests used in the third step of a reverse algorithm are shown in **Figure 21D**.

Figure 21A. The reverse algorithm

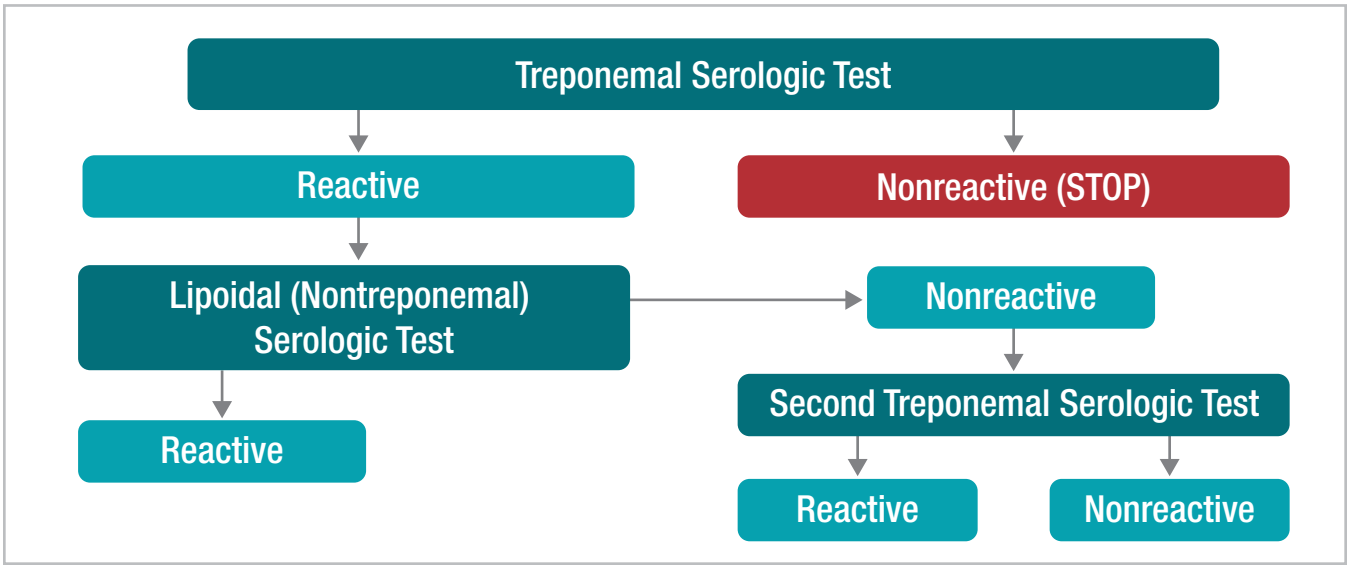


Figure 21B. Treponemal assays used by public health laboratories as the first step in the reverse algorithm (n=33)

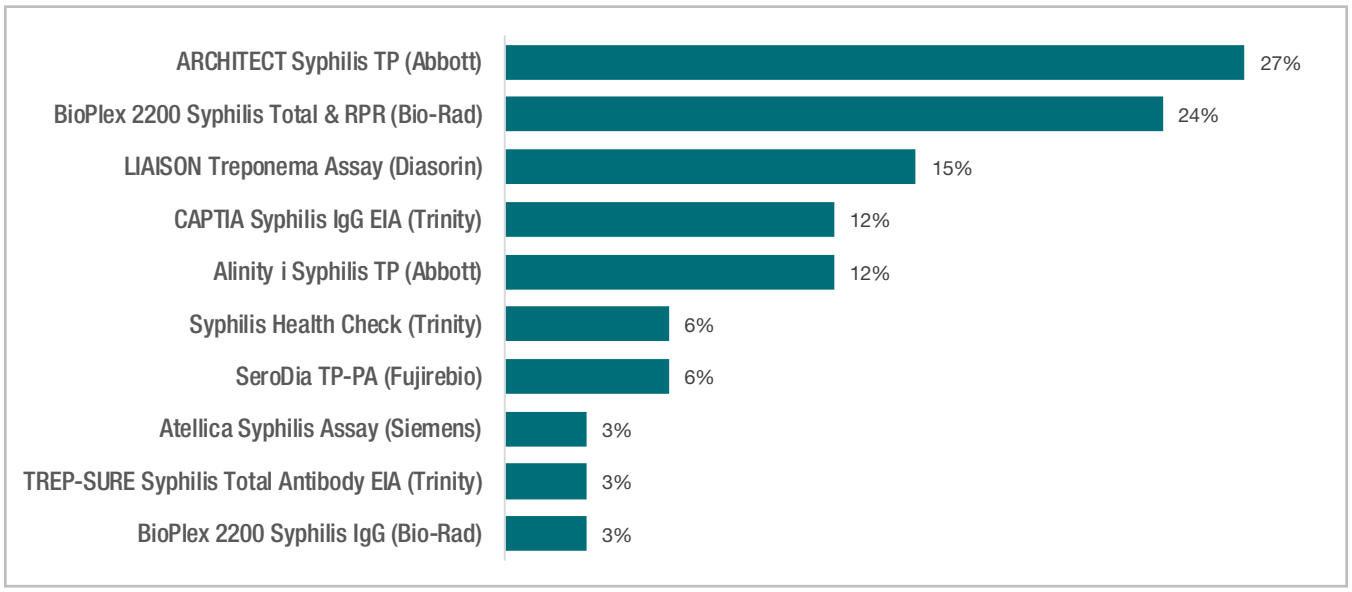


Figure 21C. Lipoidal (nontreponemal) assays used by public health laboratories in the reverse algorithm (n=33)

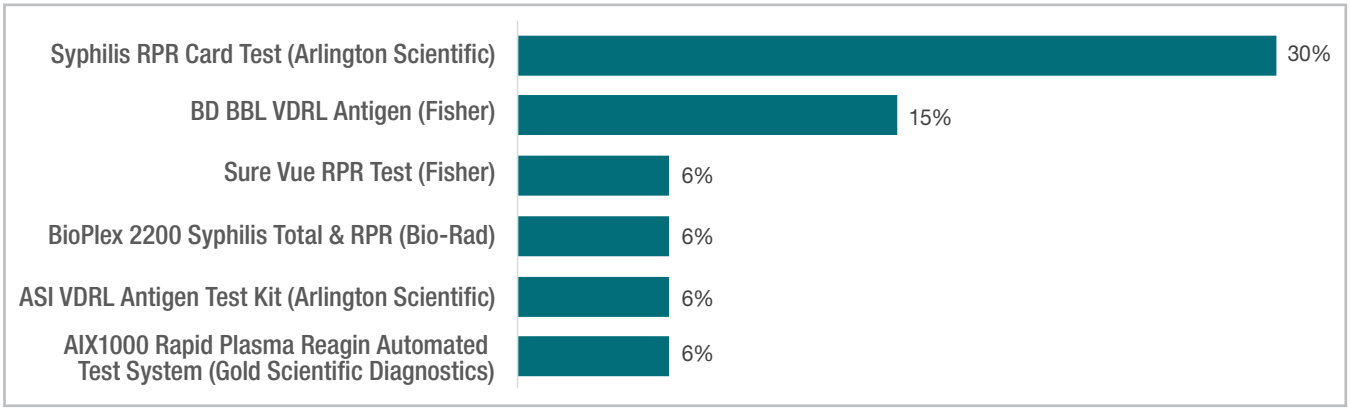
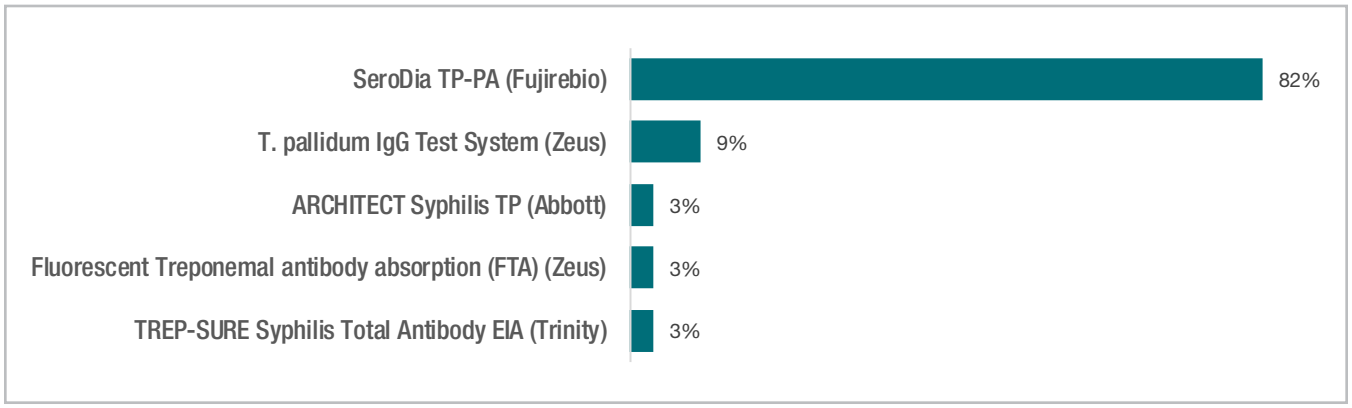


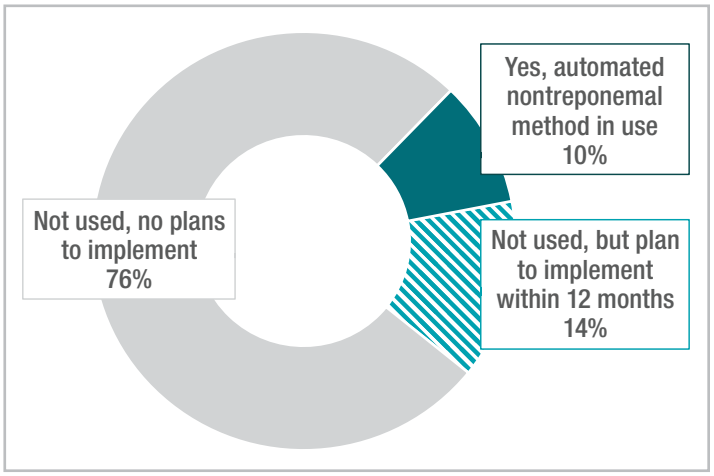
Figure 21D. Treponemal assays used by public health laboratories in the third step of the reverse algorithm



Most (86.1%, n=62/72) laboratories reported use of manual lipoidal (nontreponemal) testing. Automated nontreponemal testing was used by 13.9% (n=10/72) of laboratories and another 9.7% (n=7/72) had plans to implement an automated method in the next 12 months (**Figure 22**). When laboratories used automated methods for lipoidal (nontreponemal) testing, 57.1% (n=4/7) relied upon a manual RPR to obtain an endpoint dilution when the titer was initially outside the range of the automated instrument, 28.6% (n=2/7) performed no further testing but reported results using a greater than or equal to notation, and 14.3% (n=1/7) performed dilutions off the instrument and then obtained an endpoint titer using the automated instrument.

Of the laboratories that test for syphilis, 44.4% (n=32/72) reported having an alternative protocol for testing samples from patients with a past history of syphilis and most commonly cited performance of nontreponemal testing only as the alternative approach (75.0%, n=24/32). Such an alternative protocol was used by the same percentage of laboratories that employed only the traditional (46.2%, n=18/39) and only the reverse (46.2%, n=12/26) algorithms, but a lower percentage of laboratories that used both algorithms (28.6%, n=2/7) reported use of alternative methods. Eleven laboratories (15.3%) reported having been asked to test a sample to determine if the cause of a chancre was syphilis or herpes.

Figure 22. Use of automated methods for lipoidal (nontreponemal) testing by public health laboratories



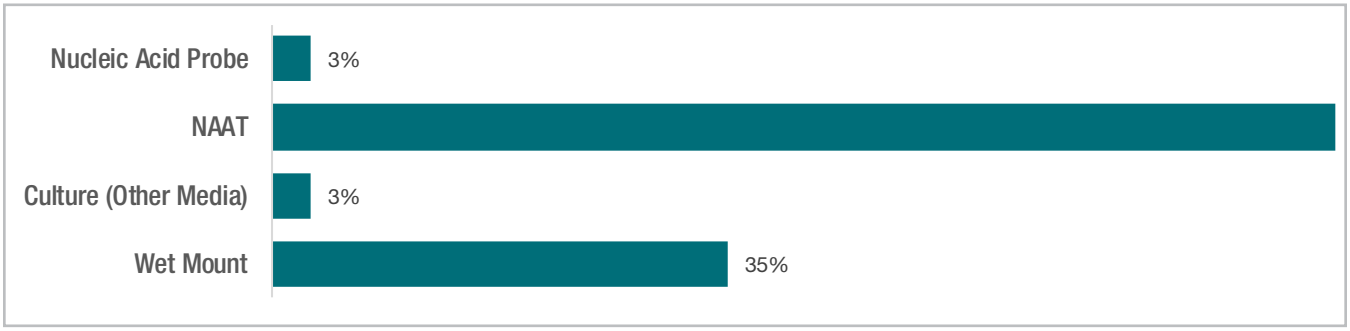
Trichomonas vaginalis Testing

Trichomonas vaginalis (TV) testing was performed in house by 34 out of the 84 responding laboratories (40.5%) and one laboratory that didn't test in-house referred samples for TV testing to a different laboratory. TV testing was more likely to be offered by local (62.9%, n=22/35) than by state public health laboratories (26.5%, n=13/49).

A total of 182,573 specimens were tested for TV and 8,958, or about 5%, were positive. The average number of samples tested per laboratory was 5,370 with a range of 0 to 62,441.

The most common types of TV tests used for in-house testing were NAAT (n=28, 82.4%) and wet mount (n=12, 35.3%) (**Figure 23**). Few laboratories performed any type of culture for TV testing. Of the 28 laboratories that offered TV NAAT testing, 22 (78.6%) said that TV NAAT can be ordered as an individual test, 20 (71.4%) said that TV NAAT can be ordered in combination with CT/GC NAAT and one laboratory (3.6%) said TV NAAT can be ordered in combination with testing for *M. genatalium*.

Figure 23. TV services provided by public health laboratories (n=34)



Herpes Simplex Virus Testing

Of the 84 responding public health laboratories, 43 (51.2%) reported offering any type of herpes simplex virus (HSV) testing in-house, although one of these laboratories discontinued testing in June of 2021 and did not provide further information. About 31.5% (n=17/54) of local and 46.4% (n=26/56) of state public health laboratories offered HSV testing. A total of 21,477 samples were tested for HSV, with a range of 0–3,330 per laboratory. About 33.9% of specimens (n=7,285) tested positive for HSV.

Types of HSV testing offered by laboratories are displayed in **Figure 24**. The majority of laboratories that performed HSV testing used some type of NAAT (83.7%, 36/43). Most frequently, a type specific NAAT was used (97.2%, n=35/36); in fact, three of the four laboratories that utilized a non-type specific NAAT also utilized a type specific NAAT. About a quarter of laboratories that perform HSV testing reported offering some type of HSV culture (25.6%, n=11/43). The majority of these (81.8%, n=9/11) offered culture with typing; one state and one local laboratory only offered culture without typing.

Twelve laboratories (27.9%, n=12/43) offered some type of serological test for HSV. Ten of these laboratories (83.3%, n=10/12) utilized an HSV-2 type specific immunoassay and eight laboratories (66.7%, n=8/12) also utilized and HSV-1 type specific immunoassay. Two laboratories (16.7%, n=2/12) used a non-type specific immunoassay only. Only a few laboratories (7.0%, n=3/43) utilized DFA.

The NAATs used by public health laboratories are shown in **Figure 25**. Of the 36 laboratories that used HSV NAAT, 27.8% (n=10/36) used a laboratory developed NAAT, 25.0% used the Aptima® Herpes Simplex Viruses 1&2 Assay (Hologic) and 19.4% used the Solana® HSV 1+2/VZV Assay (Quidel). The remaining 27.8% used another method (**Figure 25**).

Figure 24. HSV testing offered by location (n=43)

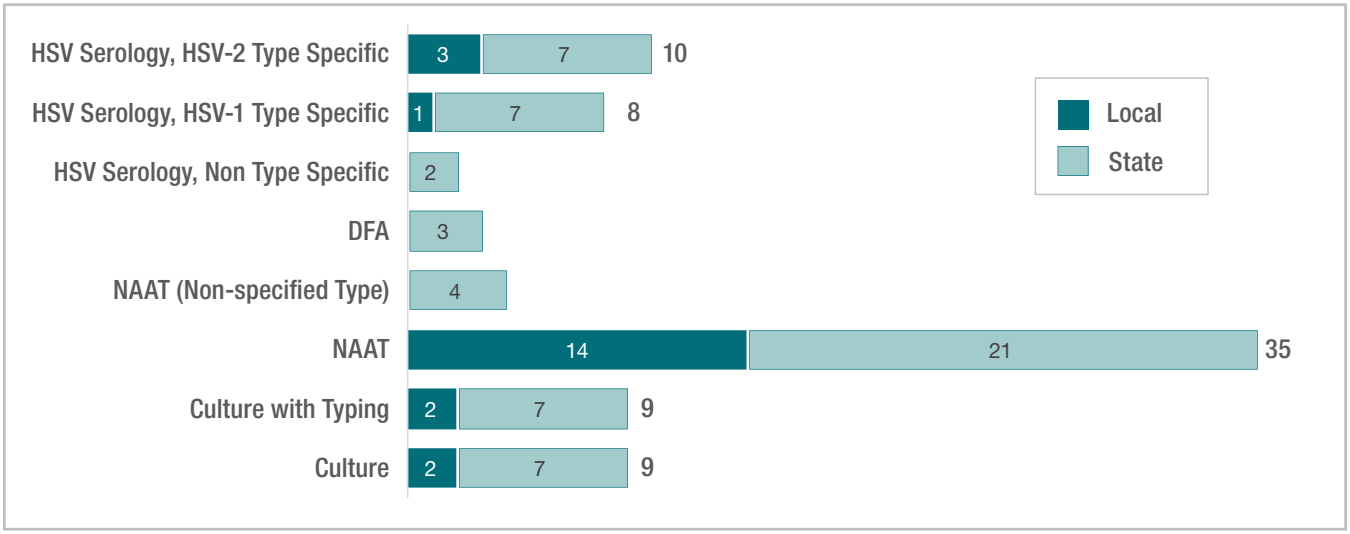
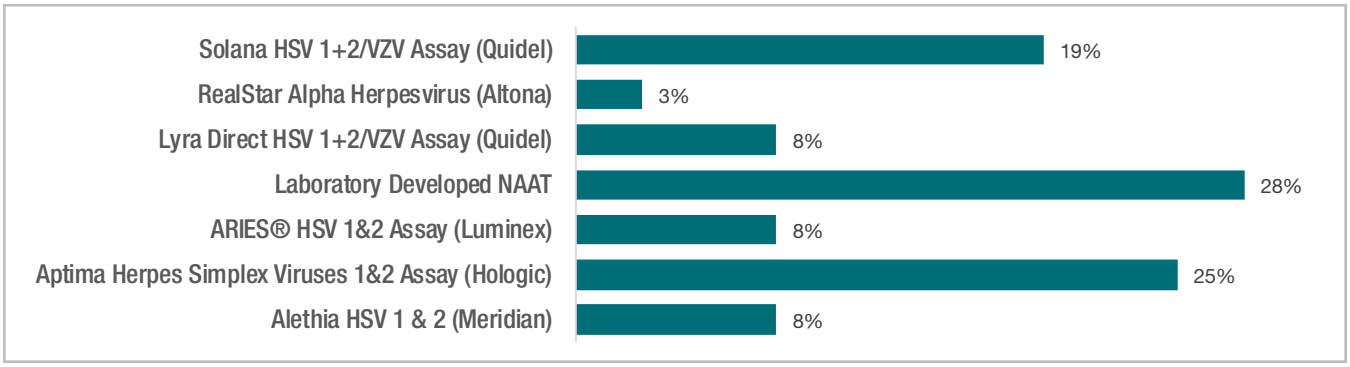


Figure 25. HSV NAATs used by public health laboratories (n=36)



Of the 11 laboratories that reported using culture for HSV, the majority (72.7%, n=8/11) reported using standard cell culture (**Figure 26**).

There was little consistency in the methods used for HSV serology testing. **Table 2** displays the variety of HSV serology methods performed, with some laboratories using more than one test. No laboratories performed HSV-2 confirmatory testing (Biokit or Western blot) following an initial positive in a type-specific test, however one laboratory had plans to implement confirmatory testing as recommended in *STI Treatment Guidelines, 2021*.[6]

Figure 26. HSV Culture methods used by public health laboratories (n=11)

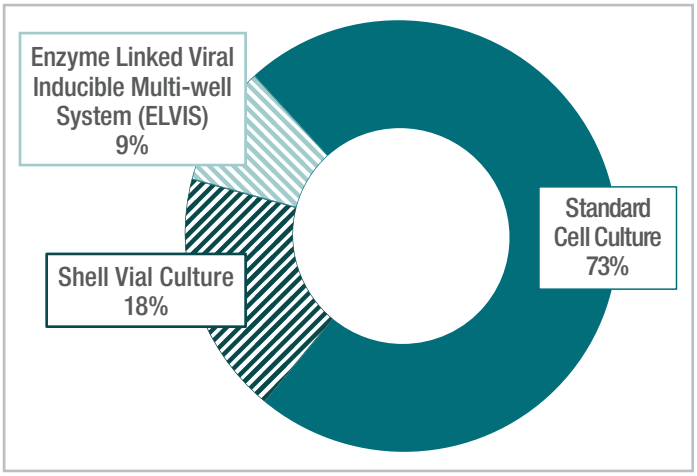


Table 2. HSV serology methods used by public health laboratories

HSV Serology Tests	Laboratory Type	
	Local	State
BioPlex 2200 HSV-1 and HSV-2 IgG (Bio-Rad)	0	1
Captia Herpes Simplex Virus 1 Type Specific IgG EIA (Trinity)	0	1
Captia Herpes Simplex Virus 2 Type Specific IgG EIA (Trinity)	0	1
HerpeSelect 1 ELISA IgG Herpes Simplex Virus-1 (Gold Standard Diagnostics)	1	0
HerpeSelect 2 ELISA IgG Herpes Simplex Virus-2 (Gold Standard Diagnostics)	2	0
LIAISON HSV-1 Type Specific IgG (Diasorin)	0	2
HSV 1 & 2 IgG (Gold Standard Diagnostics)	0	1
ZEUS ELISA HSV gG-1 IgG Test System (Zeus Scientific)	0	1
ZEUS ELISA HSV gG-2 IgG Test System (Zeus Scientific)	0	1
ZEUS ELISA HSV-1 &2 IgM Test System (Zeus Scientific)	0	1
Focus Diagnostics HerpesSelect 1 and 2 Immunoblot IgG	0	1
Liaison HSV-2 Type specific IgG	1	1
HSV1 & HSV 2 – Diasorin	0	1
LDT HSV EIA	0	1

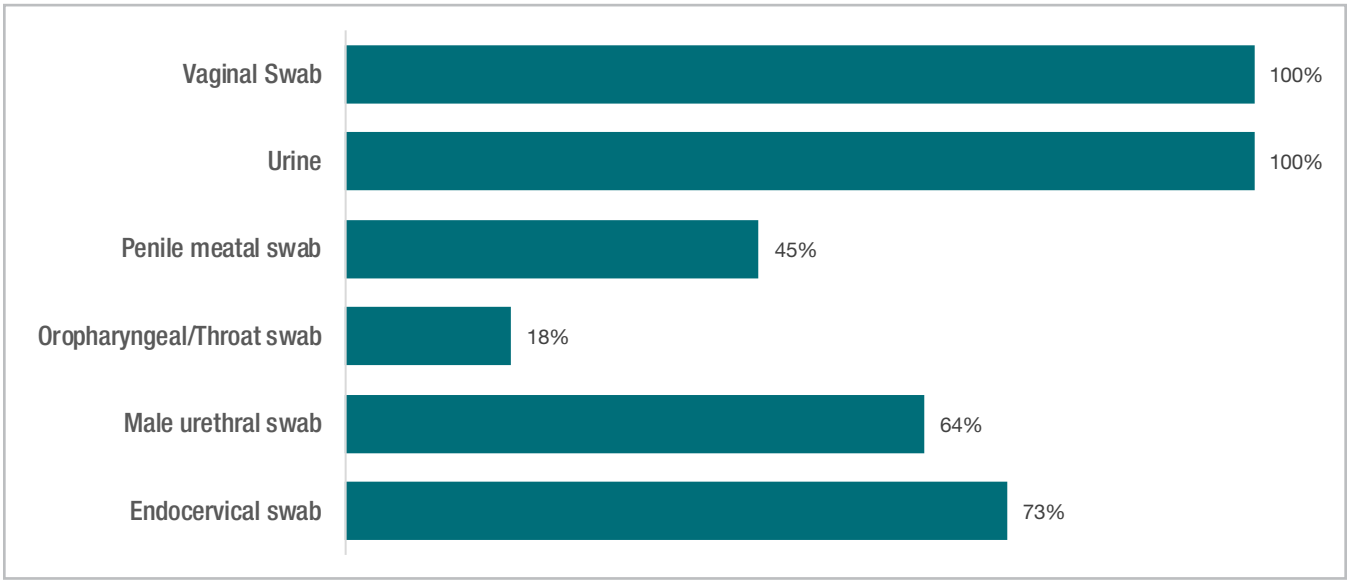
Mycoplasma genitalium

Mycoplasma genitalium (MG) testing was performed in house by 11 out of the 84 responding laboratories (13.1%). Like TV, MG testing was more likely to be offered by local (22.9%, n=8/35) than by state public health laboratories (6.1%, n=3/49). In total, 4,865 specimens were tested for MG by the 11 respondents, with an average of 811 samples per laboratory (range 0–1,699). The positivity rate was about 13.9% with 674 samples testing positive.

All 11 laboratories that offered MG testing services reported use of NAATs. Most commonly (90.9%, n=10/11) the Aptima® *Mycoplasma genitalium* assay (Hologic) was used and one laboratory used the cobas® TV/MG assay (Roche). None performed drug susceptibility testing for MG.

In five (45.5%, n=5/11) of the laboratories, MG testing could be ordered as either a standalone test or in combination with other STI tests; in four (36.4%, n=4/11) MG testing was only offered in combination with other STI tests; in two (18.2%, n=2/11) MG testing could only be ordered as a standalone test. The specimen types accepted by the laboratories that perform MG testing are shown in **Figure 27**. All 11 laboratories accepted urine and vaginal swabs, while other specimen types were accepted less frequently.

Figure 27. Specimen types accepted at public health laboratories for MG testing (n=11)



Human Papilloma Virus Testing

Testing for human papilloma virus (HPV) in public health laboratories is not very common, with only three state public health laboratories offering any HPV testing method in-house (3.6%, n=3/84) and one additional public health laboratory offering referral services. It may be that few public health laboratories offer HPV testing because HPV is not a notifiable STI, and HPV testing is normally associated with clinical care rather than public health. Moreover, HPV testing is frequently bundled with cervical cytology testing services, which is also uncommon in public health laboratories. Interestingly, two responding public health laboratories offered liquid-based cervical cytology services and both also offered HPV testing.

The three responding public health laboratories that offered HPV testing in-house tested a total of 4,176 samples, with 843 (20.2%) testing positive. An average of 1,392 HPV specimens were collected per laboratory, ranging from 253 to 2,823 samples each. All three laboratories offered NAAT testing and one of them additionally offered genotyping.

Plans to Add/Drop STI Testing Services

Over a quarter (27.6%, n=21/76) of the responding public health laboratories that currently perform STI testing said they planned to add additional testing services within the next 12 months (from summer 2022 when the survey was conducted), 42.1% (n=32/76) said they had no plans to add services, and 30.3% (n=23/76) said they were unsure. Services that laboratories planned to add are detailed in **Table 3**. Most commonly, respondents planned to add MG (n=10) or TV (n=8) testing services.

No laboratories planned to eliminate STI testing services, although one local laboratory had eliminated HSV testing in 2021. One laboratory planned to reduce the use of the traditional syphilis testing algorithm, with the goal of fully transitioning to the reverse algorithm for screening and use of a quantitative RPR for patients with a past history of syphilis.

Table 3. Testing public health laboratories planned to add in the next 12 months

Tests	Laboratory Type	
	Local	State
Extragenital NAA Testing for CT/GC	1	0
Gonorrhea antimicrobial susceptibility testing	2	1
Treponemal Syphilis Assays (e.g., EIA, Rapid, Darkfield)	1	2
Other Syphilis testing (unspecified)	1	0
Any Trichomonas testing	7	1
Any Mycoplasma genitalium testing	7	3
HSV Serology	1	0
Any HPV testing	0	1
Any Next Generation Sequencing Methods for STIs	0	2
Any point-of-care tests	0	2

Testing Facilities, Funding and Billing

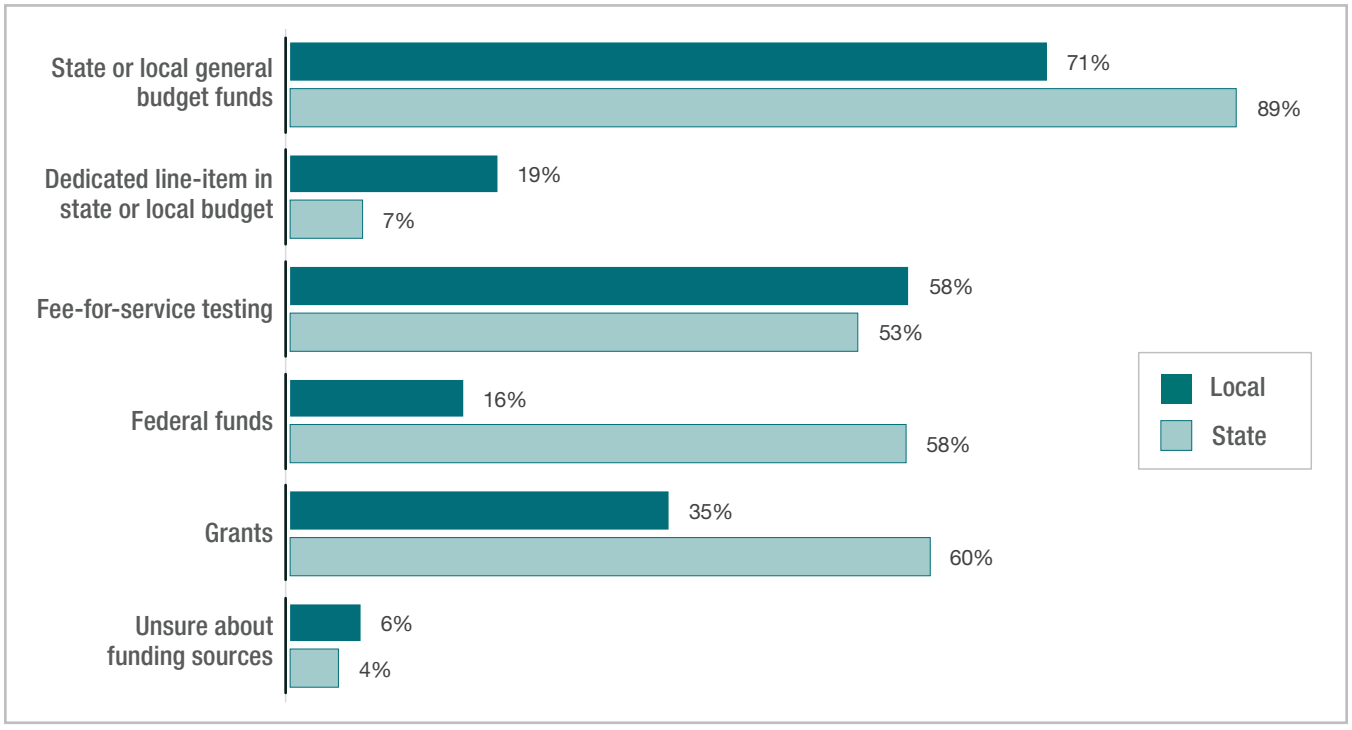
Public health laboratories reported receiving specimens from a variety of facilities for STI testing. The majority reported receiving specimens from public clinics (81.6%) and corrections facilities (71.1%). Types of facilities that submit patient specimens to laboratories for STI testing are listed in **Table 4** and stratified by laboratory type. Submission from "other" facilities most frequently included college student health centers (n=5), but also involved academic research institutions (n=1), brothels (n=1), children's assessment facilities (n=1), commercial laboratories (n=1) and coroners (n=1).

Nearly three-quarters (81.6%) of laboratories received funding from state or local general budget funds for STI testing and roughly half received funding from fee-for-service testing (55.3%) or grants (50.0%). **Figure 28** shows the breakdown of funding sources for state and local laboratories.

Table 4. Facilities that submit specimens to public health laboratories

Facilities	Laboratory Type	
	Local	State
Public clinic/ Federally Qualified Health Center (FQHC)	40 (88.9%)	22 (71.0%)
Correctional facilities	38 (84.4%)	16 (51.6%)
Private physician's offices or clinics	21 (46.7%)	2 (6.5%)
Clinical laboratories in your jurisdiction	25 (55.6%)	7 (22.6%)
Hospitals	34 (75.6)	10 (32.3%)
Non-profit agencies (e.g., Planned Parenthood)	34 (75.6%)	10 (32.3%)
Other federal, state or local department or agency	35 (77.8%)	13 (41.9%)
Other	4 (8.9%)	6 (19.4%)

Figure 28. Funding sources for STI testing at public health laboratories (n=76)



Public health laboratories charged fees to a wide range of submitters for STI testing. About 18.4% charged fees to all submitters, 13.2% charged other state or local departments or agencies, 10.5% each charged private physicians' offices and public clinics and 6.6% charged corrections facilities (**Figure 29**). About 9.2% of laboratories directly charged individual clients based on a sliding scale.

Almost half (48.7%, n=37/76) of respondents reported that they currently billed third party payers for STI testing when this survey was fielded in the summer of 2022. Another 5.3% (n=4) had plans in place to implement third party billing by the end of the 2022 calendar year (**Figure 30**).

Figure 29. Types of Submitters Charged Fees for STI Testing (n=76)

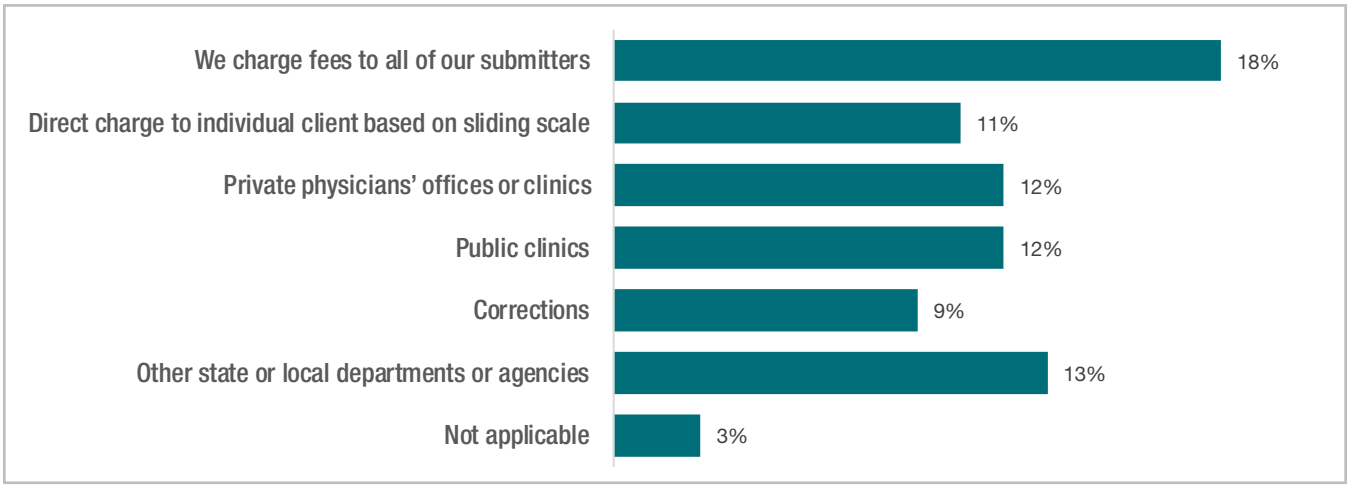
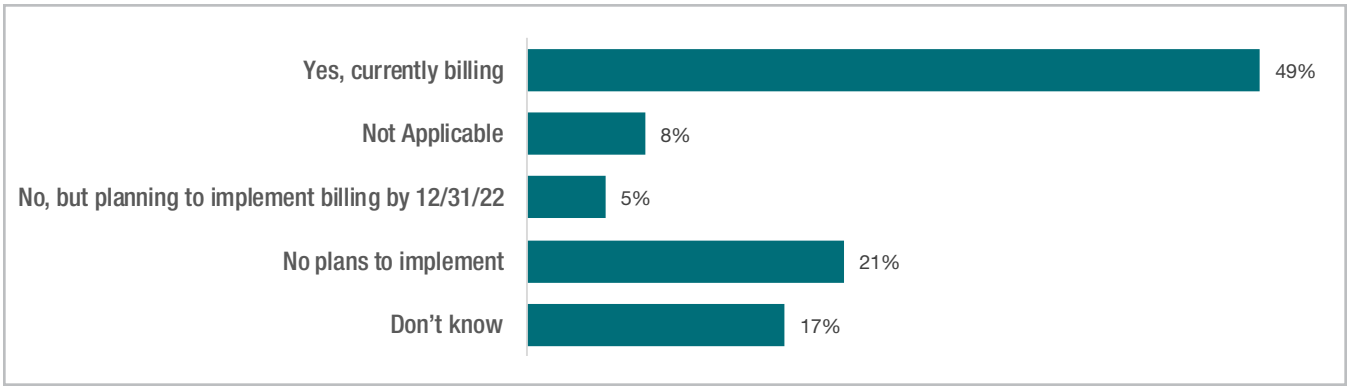


Figure 30. Third party billing at public health laboratories (Medicare, Medicaid, other health insurers) (n=76)



The types of payers that respondents reported billing are shown in **Figure 31**. Medicaid was the most common entity billed (n=34/37, 91.9%). Medicare, private insurance and submitting providers were billed by roughly half of public health laboratories.

Public health laboratories used a variety of mechanisms to seek reimbursement. About half (51.4%, n=19/37) billed insurers directly, while 21.6% billed providers. Other health department units handled billing for 10.8% of respondents, and another 8.1% utilized billing companies (**Figure 32**). Typically, revenue was deposited to the laboratory or health department accounts, but 10.8% of laboratories reported that revenue was deposited into state or city general funds (**Figure 33**).

Figure 31. **Payers that public health laboratories seek reimbursement from for STI testing (n=37)**

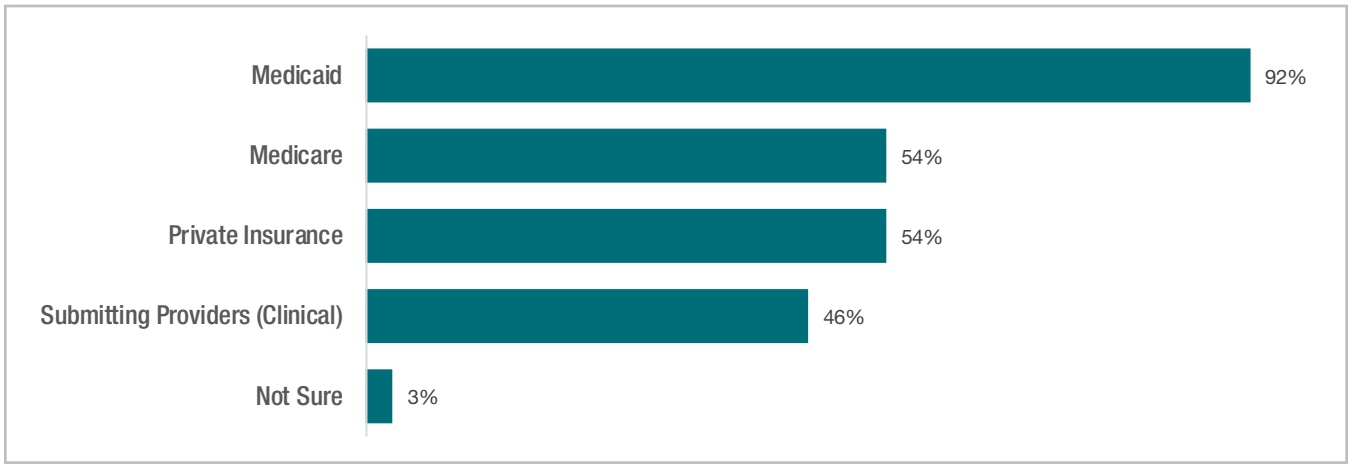


Figure 32. **Billing mechanisms used by public health laboratories (n=37)**

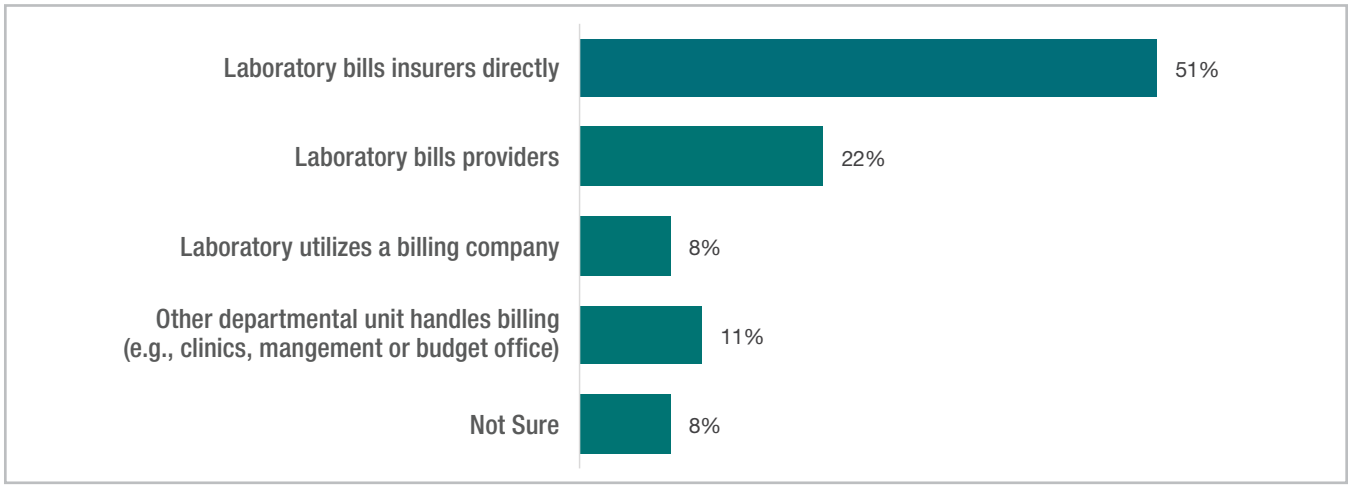
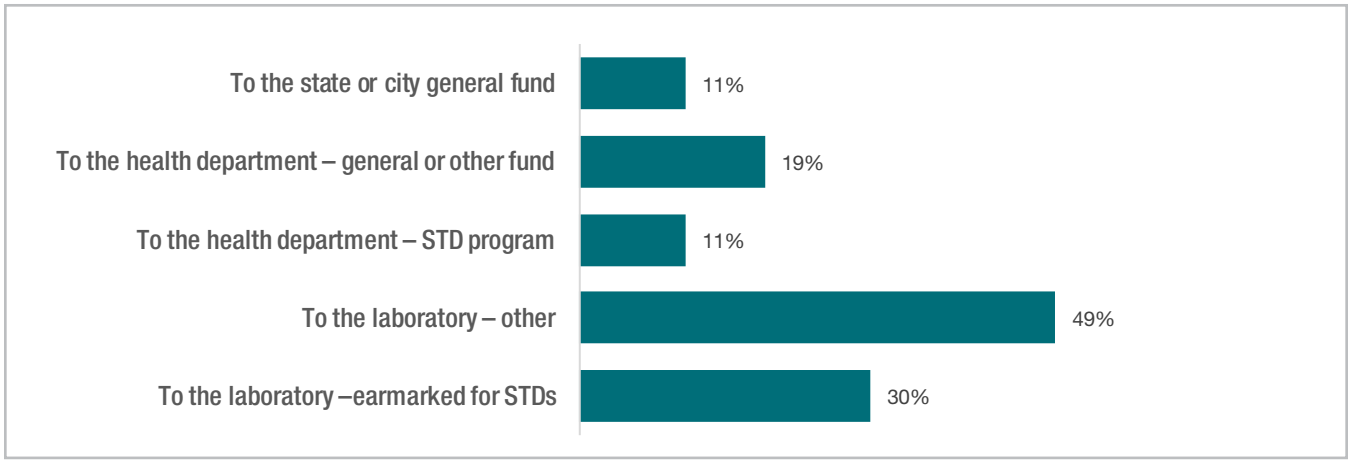


Figure 33. **Public health laboratory revenue distribution (n=37)**



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