

## Note on STI diagnosis

Prof Ch. MARTIN, May 13<sup>th</sup> 2024

General note: The terms "male" and "female" do not presuppose the patient's gender, but refer to the sex at birth.

### Summary table of STI diagnostic tests

	<b>Test Gold standard</b>	<b>Test 2</b>	<b>commentaires</b>	<b>Diagnostic window</b>
<b><i>Treponema pallidum</i></b>	TPPA+ and VDRL/RPR $\geq 1$		With or without sympt	3-6 wks
<b><i>Chlamydia trachomatis</i></b>	PCR + any site		With or without sympt	5-14 d
<b><i>Neisseria gonorrhoeae</i></b>	PCR + any site	Culture +	With or without sympt	3-14 d
<b>HIV</b>	<ul style="list-style-type: none"> <li>• Elisa + and (WB+ or VL +)</li> <li>• VL+</li> </ul>		With or without sympt	10 à 60 d
<b>HPV</b>	Abnormal cytology	+/- expert advice in some cases	With or without sympt	More rapid progression if immunodepression
<b><i>Trichomonas vaginalis</i></b>	PCR + any site	Vaginal pH vaginal + microscopy +, liquid phase cytology	With or without sympt	4 à 28 d
<b>Hepatitis B</b>	AgHBs+ during $\geq 6$ mo		With or without sympt !Incl Hepatitis D	4-12 wks

## 1. *Treponema pallidum* (Syphilis)

For most patients, syphilis is diagnosed by serological testing of blood samples. There are no methods for direct detection of the micro-organism.

There are both non-treponemal and treponemal diagnostic tests. A single test is not sufficient for diagnosis, as serological tests (especially non-treponemal tests) can be associated with false-positive results.

-Treponemal tests: The latest versions of these tests are automated, making them simpler and easier to use. As a result, they are increasingly used as an initial screening test for syphilis.

Specific treponemal tests include:

- Fluorescent treponemal antibody absorption (FTA-ABS)
- Microhemagglutination test for antibodies to *T. pallidum* (MHA-TP)
- *T. pallidum* particle agglutination assay (TPPA)
- *T. pallidum* enzyme immunoassay (TP-EIA)
- Chemiluminescence immunoassay (CIA)

As a group, these tests are based on the detection of antibodies to specific treponemal antigens and therefore tend to be more specific than non-treponemal tests. Treponemal tests are qualitative and are reported as either "reactive" or "non-reactive". Treponemal tests generally remain positive for life.

Non-treponemal tests include :

- Rapid plasma reagin (RPR)
- Venereal Disease Research Laboratory (VDRL)
- Tolidine Red Unheated Serum Test (TRUST)

These tests generally become positive about 10 to 15 days after the onset of the primary chancre (i.e. about 6 weeks after infection). These tests are semi-quantitative in that the quantity of antibodies present (IgM and IgG) generally reflects the activity of the infection. Positive non-treponemal tests are reported as antibody titer. The titer peaks between 1 and 2 years after infection, and remains positive with low titers at a very advanced stage of the disease, but effective treatment accelerates antibody decline. The evolution of the titer is monitored after treatment to assess the therapeutic response.

Sources :

1. DOI: 10.1111/jdv.16946
2. DOI: [10.15585/mmwr.rr7301a1](https://doi.org/10.15585/mmwr.rr7301a1)
3. DOI: [10.1038/nrdp.2017.73](https://doi.org/10.1038/nrdp.2017.73)

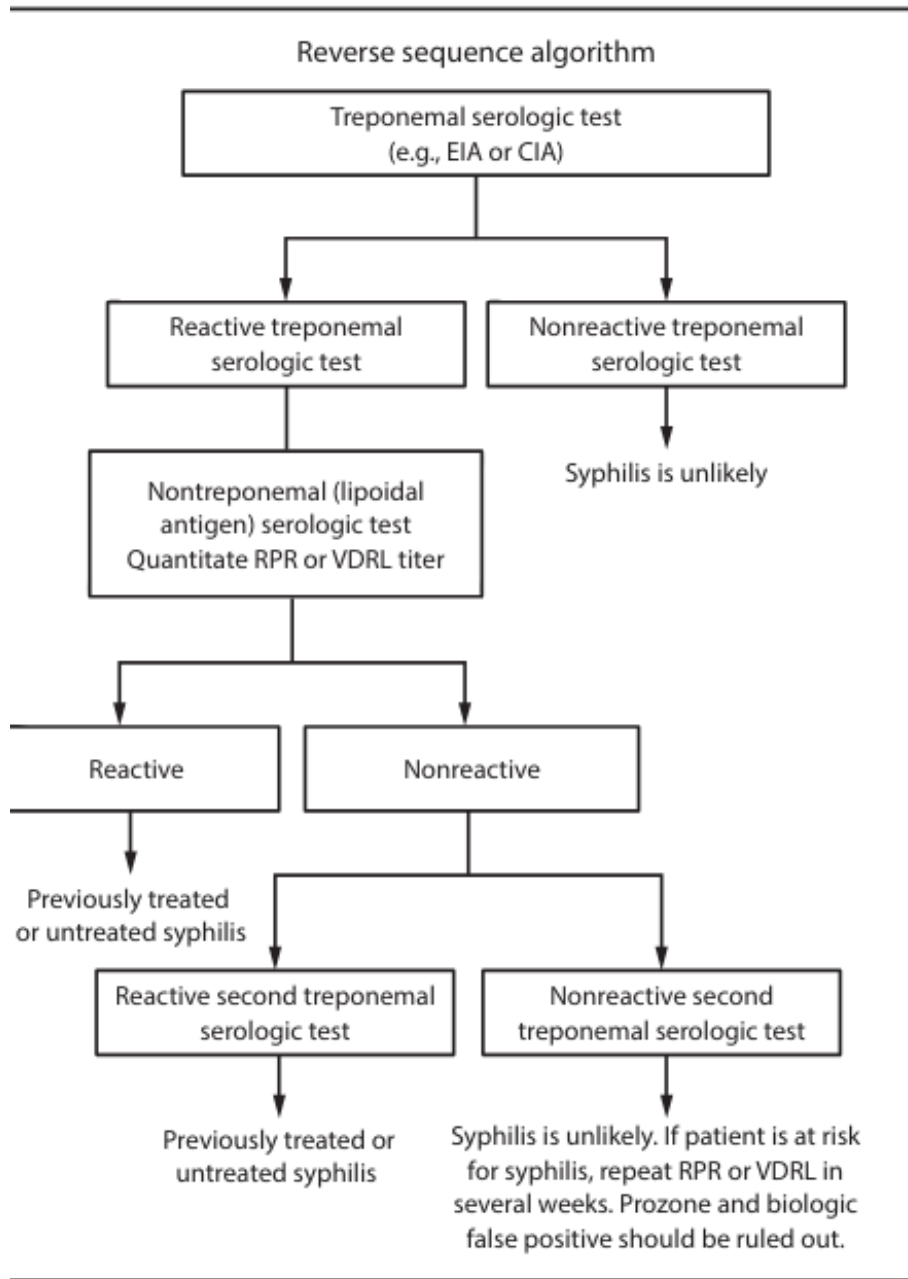
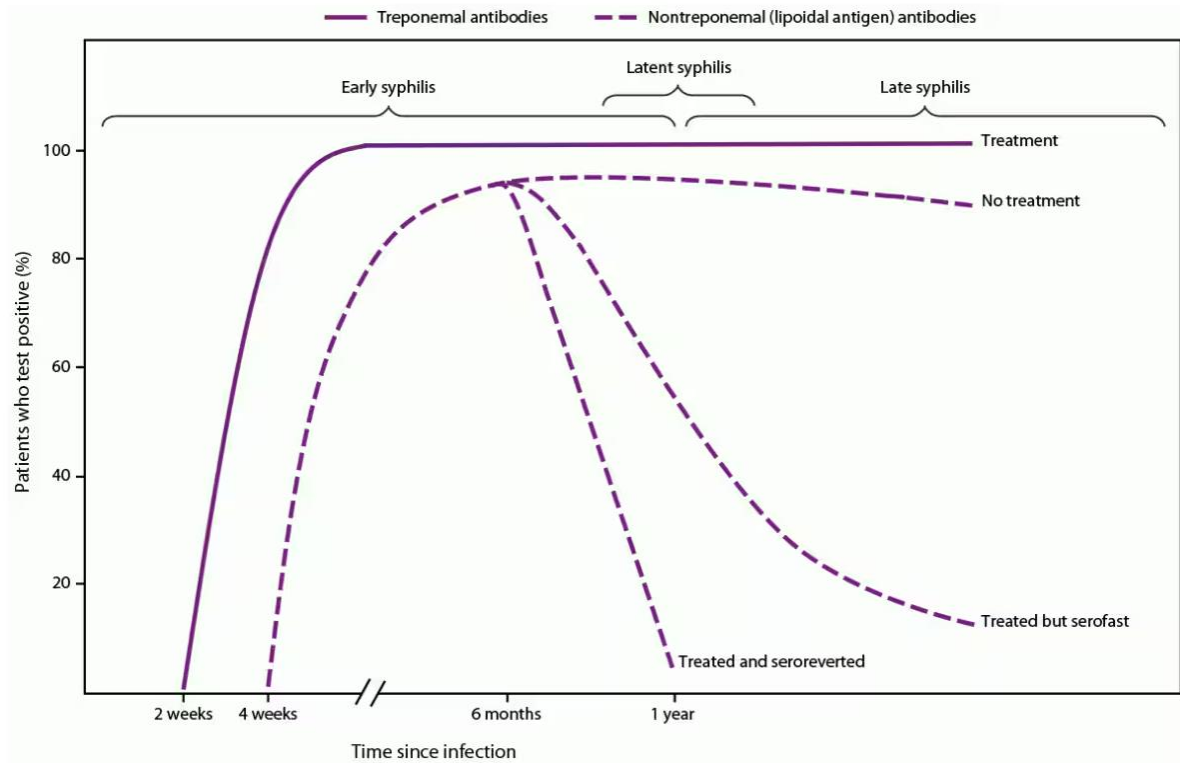


Figure 1: Syphilis diagnostic algorithm



Source: Adapted from Peeling RW, Mabey D, Kamb ML, Chen X-S, Radolf JD, Benzaken AS. Syphilis. Nat Rev Dis Primers 2017;3:17073. Used with permission

Figure 2: Evolution of treponemal and non-treponemal tests over time

## 2. *Chlamydia trachomatis*

*Chlamydia trachomatis* infections can be symptomatic or asymptomatic. Despite the possibility of spontaneous resolution, all people diagnosed with *Chlamydia trachomatis* must be treated to prevent complications and transmission.

Nucleic acid amplification tests (PCR as a rule) are the most widely used. These sensitive and specific tests have become the gold standard and are the preferred method of diagnosis, where available. They are performed on the following specimens:

- (Self-)vaginal smear (women)
- Endocervical smear (women)
- First-stream urine (men or women)
- Urethral smear (men)
- Rectal smear (men or women)
- Conjunctival smear (men or women)
- Pharyngeal smear (men or women)

### 3. *Neisseria gonorrhoeae*

*Neisseria gonorrhoeae* infections can be symptomatic or asymptomatic. Despite the possibility of spontaneous resolution, all people diagnosed with *Neisseria gonorrhoeae* must be treated to prevent complications and transmission.

The use of nucleic acid amplification tests (PCR as a rule) is recommended as the diagnostic method of choice for genital and extragenital infections caused by *N. gonorrhoeae* in symptomatic and nonsymptomatic individuals, although culture remains an important tool in cases of suspected antibiotic resistance.

These tests are performed on the following samples:

- First-stream urine (men or women)
- Urethral smear (men)
- Rectal smear (men or women)
- Pharyngeal smear (men or women)
- (Self-)vaginal smear (women)
- Endocervical smear (women)
- Conjunctival smear (men or women)

#### 4. HIV (human immunodeficiency virus)

Primary and chronic HIV infection may be symptomatic or asymptomatic.

The diagnostic tests of choice include 4th generation serology (IgM and IgG antibodies, including p24 antigen), HIV Western/Immuno blot and HIV viral load tests. Figure 3 shows the incubation windows for these tests.

Figure 3

#### Time to positivity of HIV diagnostic tests

Test	Target of detection	Approximate time to positivity (days)
<b>Enzyme-linked immunoassay</b>		
First generation	IgG antibody	35 to 45
Second generation	IgG antibody	25 to 35
Third generation	IgM and IgG antibody	20 to 30
Fourth generation	IgM and IgG antibody and p24 antigen	15 to 20
<b>Western blot</b>		
	IgM and IgG antibody	35 to 50 (indeterminate) 45 to 60 (positive)
<b>HIV viral load test</b>		
Sensitivity cutoff 50 copies/mL	RNA	10 to 15
Ultrasensitive cutoff 1 to 5 copies/mL	RNA	5

This table demonstrates the approximate time to positivity following infection for various diagnostic tests for HIV.

IgG: immunoglobulin G; IgM: immunoglobulin M.

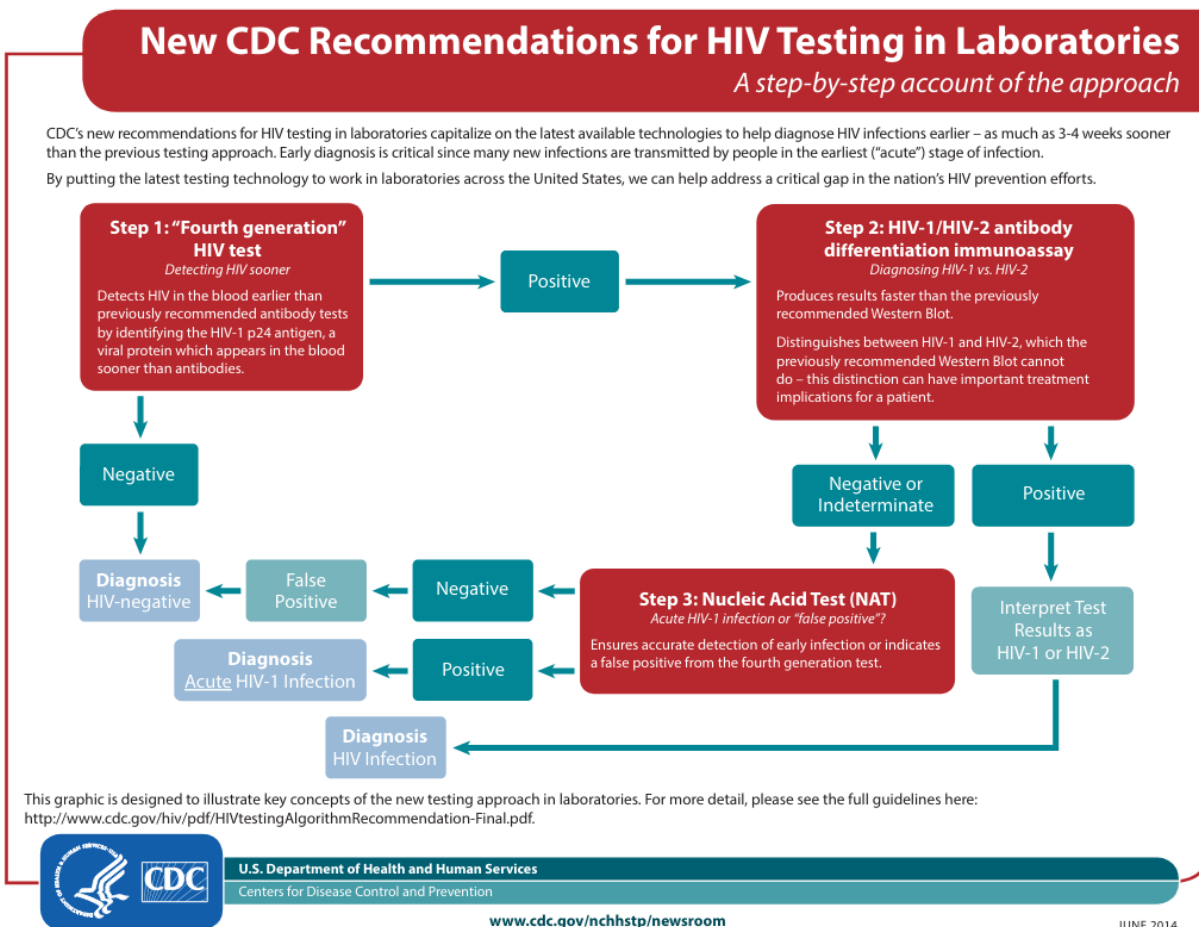
#### References:

1. Branson BM, Stekler JD. Detection of acute HIV infection: We can't close the window. *J Infect Dis* 2012; 205:521.
2. Owen SM. Testing for acute HIV infection: implications for treatment as prevention. *Curr Opin HIV AIDS* 2012; 7:125.
3. Cohen MS, Gay CL, Busch MP, et al. The detection of acute HIV infection. *J Infect Dis* 2010; 202:S270.

For routine HIV screening and diagnosis, a fourth-generation HIV-1/2 combination serology test should be used, which detects HIV p24 antigen and HIV antibodies; in the event of a positive

result, a confirmatory HIV-1/HIV-2 antibody differentiation immunoassay should be performed (Western or ImmunoBlot). Combined antigen/antibody tests, such as those used in 4th generation serologies, are better able to identify acute/early infection than antibody-only tests, as they can detect the HIV p24 antigen at a time when the antibody is not yet present. If there is a risk of acute HIV infection, HIV RNA tests (HIV viral load) should be used. Indeterminate results are those when the initial screening test is positive (i.e. third-generation antibody assay or combined fourth-generation assay) and the confirmatory test (HIV-1/HIV-2 differentiation assay or Western/Immuno blot) is indeterminate or negative. An indeterminate test may result from recently acquired HIV infection or a false-positive screening test. Plasma HIV RNA assays (HIV viral load) should then be performed in these patients. Figure 4 shows the diagnostic algorithm for HIV infection.

Figure 4



## 5. HPV (*human papilloma virus*)

There are over 200 types of papillomavirus. Their association with condylomas, malignant tumors and other pathologies is well established (see Figure 5), in particular with cancers of the anogenital tract (cervical, vaginal, vulvar, penile, anal) and those of the head and neck.

Primary and, more frequently, secondary immune deficiency disorders (e.g., HIV infection) can predispose patients to HPV infections and the development of malignant tumors in affected tissues.

Vulvar and vaginal cancers - Vulvar and vaginal cancers are uncommon. Unlike cervical cancer, not all cancers of the external genitalia are associated with HPV infection. The fraction attributable to HPV infection has been estimated at between 29% and 43% for vulvar cancer, 87% for vulvar intraepithelial neoplasia, 70% for vaginal cancer and 69% to 100% for vaginal intraepithelial neoplasia.

Unlike non-HPV-associated cancers of the external genitalia, HPV-associated vulvar cancers occur at a younger age, have basaloid rather than keratinizing pathology, lack p53 mutations and are associated with sexual risk factors. HPV-associated vaginal cancers have similar characteristics, but overall, vaginal cancer is more likely to be HPV-associated.

Different HPV types have a propensity to infect different anatomical sites, and are therefore associated with different diseases (Figure 5).

- Cutaneous cells
- Anogenital epithelium
- Other mucosal surfaces

Cervical and anal: all cellular dysplasias and neoplasias are HPV-related. Diagnosis is therefore cytological, and the presence of HPV should not be used as a diagnostic criterion.

- Cervical: dysplasia grade 1-2-3: CIN 1-2-3/ neoplasia
- Anal: atypical squamous cells of undetermined significance (ASC-US), low-grade SIL (LSIL), atypical squamous cells that cannot exclude high-grade SIL (ASC-H), and high-grade SIL (HSIL)/ neoplasia

Warts, condylomata acuminata and papillomatoses are always HPV-related. It is therefore also a cytological/anatomopathological diagnosis.

Vaginal, vulvar, and head and neck (intraepithelial) neoplasia: first cytological diagnosis of dysplasia/ neoplasia, then expert opinion on the link with HPV.

Figure 5

### Disease associations with selected human papillomavirus types

Disease	HPV type frequently associated
Cutaneous warts	
Common and plantar warts	1, 2, and 4
Flat wart	3, 10
Butcher's wart	7, 2
Bowen's disease	
Genital	16
Extragenital	2, 3, 4, 16
Epidermodysplasia verruciformis	2, 3, 5, 8, 9, 10, 12, 14, 15, 17
Condylomata acuminata	6, 11
Squamous intraepithelial lesions*	
Low grade	16, 31, 6, 11
High grade	16, 31, 52, 18
Oropharyngeal cancer	16
Anal cancer	16
Respiratory papillomatosis	6, 11

This table lists the more commonly reported human papillomavirus types associated with various conditions. However, the most prevalent HPV types associated with particular lesions can vary by geography and demographics of the population studied.

\* These include squamous intraepithelial lesions and cancers of the cervix, vagina, vulva, anus, and penis. Other high-risk types associated with squamous intraepithelial lesions include 33, 45, and 58.

Data from:

1. Hariri S, Unger ER, Powell SE, et al. Human papillomavirus genotypes in high-grade cervical lesions in the United States. *J Infect Dis* 2012; 206:1878.
2. Insinga RP, Liaw KL, Johnson LG, et al. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev* 2008; 17:1611.

## 6. *Trichomonas vaginalis*

*Trichomonas vaginalis* infections can be symptomatic or asymptomatic. Despite the possibility of spontaneous resolution, all people diagnosed with *Trichomonas vaginalis* must be treated to prevent complications and transmission.

The diagnostic tests of choice are nucleic acid amplification tests (PCR as a rule) or vaginal pH analysis + microscopy. The choice of approach is based on test availability and provider training.

Liquid-based cervical cytology is not very sensitive but highly specific, and can also be used as a diagnostic basis for initiating treatment.

Compared with PCR, the sensitivity of microscopy ranges from 26% to 68%.

These tests are performed on the following samples:

- (Auto-)Vaginal smear (women) : PCR, vaginal pH analysis + microscopy
- Urethral smear (women or men): PCR only
- Rectal smear (women or men) : PCR only
- Endocervical smear (women) : PCR, liquid-phase cervical cytology

Sources :

1. DOI: [10.1007/s11908-015-0484-7](https://doi.org/10.1007/s11908-015-0484-7)
2. DOI: [10.1002/dc.20256](https://doi.org/10.1002/dc.20256)

## 7. Hepatitis B virus (HBV)

Most patients with chronic hepatitis B infection have no history or symptoms of liver disease: the infection may be symptomatic or asymptomatic.

Infection with the hepatitis B virus is associated with changes in serum levels of hepatitis B antigens and antibodies. These markers are used to define different clinical states (Figure 6).

Based on the results of this test, patients may have :

- No evidence of previous infection or immunity (negative for all three serological markers).
- Evidence of immunity due to previous infection (anti-HBs positive, anti-HBc total positive, HBsAg negative).
- Evidence of immunity following vaccination (anti-HBs positive, anti-HBc total negative, HBsAg negative).
- HBV infection (HBsAg positive and total anti-HBc positive).

Acute hepatitis B - The diagnosis of acute hepatitis B is based on the detection of hepatitis B surface antigen (HBsAg) and hepatitis B core IgM antibody (anti-HBc). During the initial phase of infection, markers of HBV replication, hepatitis B e antigen (HBeAg) and HBV DNA, are also present.

Chronic hepatitis B - The diagnosis of chronic HBV infection is based on the persistence of HBsAg for more than six months.

Occult hepatitis B - There is a subgroup of patients with occult HBV infection, defined as the presence of PCR-detectable HBV DNA in HBsAg-negative but anti-HBc-positive patients.

Note on hepatitis Delta or D: The infection with the hepatitis D virus (HDV) is caused by a defective virus: the hepatitis D virus, which cannot persist in the human body unless the hepatitis B virus is also present. People with hepatitis D are always both infected with both HDV and hepatitis B virus (HBV).

Figure 6

