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Title: Primary Sample Manual: Molecular Biology – Infectious Diseases

Changes made since previous version: Added a section for Clostridioides difficile PCR.

Note: *Please refer to the document record on IQM/Q-Pulse for the revision history of this document.*

CONTENTS:

INTRODUCTION

This is a list of the Molecular Infectious Disease tests performed at Eurofins Biomnis' Dublin Laboratory. For a searchable list of tests performed by Eurofins Biomnis in France, in our laboratories in Lyon and Paris, click here.

If you cannot find details of a test you require, please contact our Client Services department on Free Phone 1800-252-966 or 01 295 8545, or e-mail clientservices@ctie.eurofinseu.com.

For sample collection, please contact our Logistics department on Free Phone 1800-252-967, or e-mail logistics@ctie.eurofinseu.com.

TEST INFORMATION TEMPLATE	
Brief information on clinical background, indications for test and interpretation of test results.	
Preparation of Patient: any special preparation required, such as fasting. **Precautions:** any special circumstances, conditions etc. to be aware of.	
Accredited	Whether or not the test is accredited by INAB to ISO 15189. If the test is accredited (Yes), this section is colour-coded in green; if the test is not accredited (No), this section is colour-coded in orange.
Method	Test method. Standard Operating Procedure reference for this test.
Sample Requirements	Type of tube required and other information.
Turnaround Time	The maximum turnaround time in working days from receipt of the sample in the laboratory's Pre-Analytics department to the authorisation of the result. **Working days are Monday to Friday 08:00 to 18:00.**
Stability	Sample stability under various conditions.
Laboratory Sample Storage	Conditions under which samples are stored following testing, and the lenght of time for which the samples are stored.
Results and Source	Units and reference range(s) for the test. Source of the reference ranges: 1. Test manufacturer's Method Sheet.
Limitations of Test	The Limitations of the test as provided by the test manufacturer.
Notes	Any additional relevant information (e.g. notifiable diseases)

REFERENCES

1. Cobas® 4800 CT/NG Test Method Sheet Doc. Rev. 19.0.
2. Cobas® 4800 HPV Test Method Sheet Doc Rev. 24.0.
3. Hologic Aptima HCV Quant Dx Assay Kit Insert AW-13249-001 Rev. 005 2019-04
4. BD MAX Cdiff REF 442555 P0215(07) 2023-11

REASONS FOR REJECTION OF SAMPLES/NON-REPORTING OF TESTS

1. Samples received beyond the stability limits and/or not at the correct temperature indicated below for each test.
2. Samples received in the incorrect tube.
3. Samples received without the correct media.

CHLAMYDIA TRACHOMATIS & NEISSERIA GONORRHOEAE (CT/NG)

Chlamydia trachomatis (CT) is the second most leading cause of sexually transmitted diseases worldwide, with approximately 89.1 million cases occurring annually. CT is the causative infectious agent for a variety of diseases in men, including urethritis, proctitis, conjunctivitis and epididymitis. In women, the consequences of infection with CT are severe if left untreated. Infection can lead to endometriosis, salpingitis (with subsequent infertility and ectopic pregnancy) and perihepatitis. Additionally, infants from infected mothers can develop conjunctivitis, pharyngitis, and pneumonia.

Neisseria gonorrhoeae (NG) is the causative agent of gonorrhoeae. Acute urethritis is seen in the majority of men with gonorrhoeae, and acute epididymitis is the most common complication, particularly in young men. In women, the primary site of infection is the endocervix, there's a high prevalence of co-infections with CT, *Trichomonas vaginalis*, and bacterial vaginosis. Many women remain asymptomatic; when symptoms do occur, the most common are increased discharge, dysuria, and intermenstrual bleeding. Additionally, NG may cause pelvic inflammatory disease, endometriosis, tubo ovarian abscess, and pelvic peritonitis.

Preparation of patient: Prior to the collection of urine, the patient should not have urinated for at least one hour. For best results, female patients should not cleanse the labial area prior to collection. There is no physical preparation for the vaginal swab.

Precautions: None for patient. Media contains Guanidine Thiocyanate, adequate PPE for the person taking the sample.

Accredited	Yes
Method	The cobas® 4800 CT/NG Test is an in vitro nucleic acid amplification test for the qualitative detection of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) in patient specimens. The test allows for detection of CT/NG DNA in endocervical and vaginal swab specimens, and male and female urine in cobas® PCR Media. The intended targets for the cobas® 4800 CT/NG Test include all fifteen major *Chlamydia trachomatis* serovars, the Swedish *C. trachomatis* mutant (nvCT), and both wild-type and variant DR-9 sequences of *N. gonorrhoeae.* SOP: MB52
Sample Requirements	Sample Type: Urine or urine in cobas PCR Urine Media (cobas® PCR Urine Sample Kit). Endocervical or Vaginal swabs in cobas PCR Media (cobas® Media Dual Swab Kit).
Turnaround Time	4 working days from sample receipt
Stability	Urine specimens should be transferred into the cobas PCR media tube as soon as possible. If specimens cannot be transferred immediately, they can be stored at 2-30°C for up to 24 hours. Stabilised urine specimens are stable at 2-30°C for up to 12 months. Swabs collected with the cobas PCR Media Swab Kit may be stored at 2-30°C for up to 12 months.

Laboratory Sample Storage	30 days at 2°C-30°C.
Results and Source	CT and/or NG Detected Not Detected Invalid Cobas® 4800 CT/NG Test Method Sheet Doc. Rev. 19.0.

Limitations of Test	• The **cobas®** 4800 CT/NG Test has only been validated for use with female endocervical swab and clinician collected vaginal swab and clinician-instructed self-collected vaginal swab specimens collected in **cobas®** PCR Media (UT), female and male urine specimens stabilized in **cobas®** PCR Media (UUT).
	
	• Interfering substances include, but are not limited to the following:
	  • The presence of mucus in endocervical and cervical specimens may inhibit PCR and cause false negative test results. Mucus free specimens are required for optimal test performance. Use a sponge or an additional swab to remove cervical secretions and discharge before obtaining the specimen.
	  • Urine specimens stabilized in **cobas®** PCR Media containing greater than 0.35% (v/v) blood may give false negative results.
	  • Endocervical swab specimens, vaginal swab specimens and cervical specimens, each containing up to 5% (v/v) whole blood exhibited no interference effects. Whole blood levels above 5% (v/v) may give invalid or false negative results.
	  • Endocervical swab specimens, vaginal swab specimens and urine specimens, all stabilized in **cobas®** PCR Media and containing greater than 1 x 105 PBMC cells/mL may give invalid or false negative results.
	  • Urine specimens taken from patients who have used the over-the-counter product Replens® vaginal moisturizer may give invalid or false negative results.
	  • Urine specimens taken from patients who have used the over-the-counter product RepHreshTM Odor Eliminating Vaginal Gel and RepHreshTM Clean Balance may give invalid or false negative results.
	
	• Detection of *C. trachomatis* and *N. gonorrhoeae* is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, history of STD, presence of symptoms), stage of infection and/or infecting *C. trachomatis* and *N. gonorrhoeae* strains.
	
	• False negative results may occur due to polymerase inhibition. The CT/NG Internal Control is included in the **cobas®** 4800 CT/NG Test to help identify specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
	
	• Prevalence of infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or

	individuals with no risk of infection. Because the prevalence of *C. trachomatis* and *N. gonorrhoeae* may be low in some populations or patient groups, a false positive rate can exceed the true positive rate so that the predictive value of a positive test is very low. Since some patients that are truly infected will not be identified by testing a single specimen, the true rate of false positives cannot be determined or presumed from the clinical data. The rate of false positive test results may depend on training, operator ability, reagent and specimen handling or other such factors in each laboratory. • Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in the Package Insert. • The **cobas®** 4800 CT/NG Test is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications. • The **cobas®** 4800 CT/NG Test provides qualitative results. No correlation can be drawn between the Ct value reported for a positive **cobas®** 4800 CT/NG Test and the number of *C. trachomatis* and *N. gonorrhoeae* cells within the infected specimen. • The **cobas®** 4800 CT/NG Test for male and female urine testing is recommended to be performed on first catch urine specimens (defined as the first 10 to 50 mL of the urine stream). The effects of other variables such as first-catch vs. midstream, post-douching, etc. have not been evaluated. • Improperly collected endocervical swab specimens are likely to contain excess mucus, which may cause clots on the **cobas®** 4800 System. • The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated. • The **cobas®** 4800 CT/NG Test is not intended to replace cervical exam and endocervical sampling for diagnosis of urogenital infection. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents. • Though rare, mutations within the highly conserved regions of the cryptic plasmid or genomic DNA of *C. trachomatis* or the genomic DNA of *N. gonorrhoeae* covered by the **cobas®** 4800 CT/NG Test's primers and/or probes may result in failure to detect the presence of the bacterium. • The presence of PCR inhibitors may cause false negative or invalid results.
Notes	*Chlamydia trachomatis* and *Neisseria Gonorrhoeae* are notifiable diseases https://www.hpsc.ie/notifiablediseases/listofnotifiablediseases/

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HUMAN PAPILLOMAVIRUS (HPV) DNA

Persistent infection with human papillomavirus (HPV) is the cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN). The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide.

HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 118 different types of HPV, and approximately 40 different HPVs that can infect the human anogenital mucosa. However, only a subset of 13 to 18 of these types is considered high-risk for the development of cervical cancer and its precursor legions.

Although persistent infection with high-risk (HR) HPV is a necessary cause of cervical cancer and its precursor lesions, a very small percentage of infections progress to these disease states. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women experiencing HPV at some point. However, > 90% of infected women will mount an effective immune response and clear the infection in 6 to 24 months without any long-term health consequences.

Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV infection. The implementation of HPV DNA testing has increased the efficiency of cervical cancer screening programs by detecting high-risk lesions earlier in women 30 years and older with NILM cytology and by reducing the need for unnecessary colposcopy and treatment in patients 21 and older with ASC-US (abnormal) cytology.

Preparation of patient: Patient should avoid using any vaginal medications, lubricants or creams in the 2 days prior to the sample being taken.

Precautions: None for patient. Media is classified as hazardous, adequate PPE for the person taking the sample.

Accredited	Yes
Method	The cobas® 4800 HPV Test is a qualitative in vitro test for the detection of HPV in patient specimens, The test utilises amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridisation for the detection of 14 HR HPV types in a single analysis. The test specifically identifies HPV 16 and HPV 18 while concurrently detecting the other high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) at clinically relevant infection levels. SOP: MB51
Sample Requirements	Sample Type: Cervical smear in ThinPrep PreservCyt Solution. The total volume of sample in the tube should be at least 3ml.
Turnaround Time	10 working days from sample receipt for co-testing (all samples sent to Eurofins NMDL, The Netherlands for cytology following HPV testing).
Stability	Specimens collected in PreservCyt Solution can be transported at 2-30°C. Specimens may be stored at 2-30°C for up to 6 months after the date of collection.

Results and Source	HPV16 and/or HPV18 and/or Other HR HPV Detected Not Detected Invalid Cobas® 4800 HPV Test Method Sheet Doc Rev. 24.0.
Limitations of Test	• The **cobas®** 4800 HPV Test detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types. • The **cobas®** 4800 HPV Test for detection of human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is not recommended for evaluation of suspected sexual abuse. • The performance of the **cobas®** 4800 HPV Test has not been adequately established for HPV vaccinated individuals. • Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection. • Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer. • A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer. • Detection of high-risk HPV is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances. • Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in the Package Insert. • The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated. • Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the **cobas®** 4800 HPV Test's primers and/or probes may result in failure to detect the presence of the viral DNA. • The presence of PCR inhibitors may cause false negative or invalid results. • Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the **cobas®** 4800 System. If concentrations of whole blood exceed 2% (dark red or

	brown coloration) in PreservCyt® Solution, there is a likelihood of obtaining a false-negative result. • Use of the RepHresh® vaginal hygiene products has been associated with false-negative results in PreservCyt® Solution.

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HEPATITIS C VIRUS

Hepatitis C Virus (HCV) is a blood-borne pathogen and a worldwide public health burden with up to 170 million people infected globally and 350,000 annual deaths due to HCV related conditions, including cirrhosis and liver cancer. Transmission of HCV is through exposure to blood, blood products, or activities with potential for percutaneous exposure.

Clinically, there is a high prevalence of asymptomatic HCV infection, and, despite detectable antibody, chronic HCV infection occurs in up to 75% of patients. HCV laboratory testing algorithms require diagnosis of active HCV infections in antibody positive individuals through detection of HCV RNA in plasma or serum to allow appropriate link to care.

Genetically, HCV contains a positive-strand RNA genome of approximately 9500 nucleotides encoding structural proteins and non-structural proteins, the latter being key viral replicative proteins and targets of direct acting antivirals.

Sustained virological response, defined as undetected HCV RNA after successful therapy, is a key marker for an HCV cure.

Preparation of patient: None.

Precautions: None.

Accredited	No
Method	The Hologic® Aptima™ HCV Quant Dx assay is a nucleic acid amplification test that uses real-time transcription-mediated amplification (TMA) technology to detect and quantify HCV RNA before therapy for aiding diagnosis or to establish baseline viral load, as well as to measure on-treatment and post-treatment responses. This assay targets a conserved region of the HCV genome, detecting and quantitating genotypes 1, 2, 3, 4, 5, and 6. SOP: MB61
Sample Requirements	**<u>Whole Blood:</u>** ***Sample Type:*** • Whole Blood in Serum Tubes • Whole blood in Serum Separator Tubes (SSTs) ***Requirements:*** • Whole Blood samples **<u>must</u>** be centrifuged within 6 hours of specimen collection. ***Temperature:*** • 2°C - 30°C for up to 6 hours

	Serum: ***Sample Type:*** • Serum in Serum Tubes • Serum in Serum Separator Tubes (SSTs) ***Minimum Volume:*** Minimum volume for testing is 1200ul of Serum. ***Temperature:*** • 2°C - 30°C for up to 24 hours • 2°C - 8°C for up to 5 days
Turnaround Time	3 working days from sample receipt.
Stability	**Whole Blood:** 2°C - 30°C for up to 6 hours; must be centrifuged within 6 hours of sample collection. **Serum (Primary Tube):** 2°C - 25°C for up to 24 hours; 2°C - 8°C for up to 5 days. **Serum (Secondary Tube):** 2°C - 25°C for up to 24 hours; 2°C - 8°C for up to 5 days; -20°C for up to 60 days.
Laboratory Sample Storage	60 days at -20°C.
Results and Source	Non-Reactive for HCV RNA. Reactive for HCV RNA. Invalid * Quantitative result available on request. Hologic Aptima HCV Quant Dx Assay Kit Insert AW-13249-001 Rev. 005 2019-04
Limitations of Test	Reliable results are dependent on adequate specimen collection, transport, storage, and processing.
Notes	Hepatitis C Virus is a notifiable disease https://www.hpsc.ie/a-z/hepatitis/hepatitisc/casedefinitions/

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CLOSTRIDIOIDES DIFFICILE PCR

Clostridioides difficile is an anaerobic, gram-positive bacillus that is the leading cause of antibiotic associated diarrhoea and pseudomembranous colitis in health care facilities. Incidence of *Clostridioides difficile* infection has been increasing, and severe cases are becoming more common. Disease symptoms range from mild diarrhoea to sever colitis, and even bowel perforation and death. The most common risk factor is exposure to antibiotics.

The BD MAX Cdiff assay performed on the BD MAX System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridioides difficile* toxin B (*tcdB*) gene in human liquid or soft stool specimens from patients suspected of having *Clostridioides difficile* infection. The test, performed directly on the specimen, utilises real-time polymerase chain reaction (PCR) for the amplification of *Clostridioides difficile* toxin B DNA and fluorogenic target-specific hybridisation probes for the detection of the amplified DNA.

PCR methods for the detection of toxin B (or toxin A) have been developed with high sensitivity and specificity as compared to cell cytotoxicity and immunoassays. Additionally, the PCR test can be performed in less than 3 hours. The combination of these characteristics may allow for prompt targeted treatment of patients with *Clostridioides difficile* infection and thus potentially improve patient outcome, reduce recovery time, and improve infection control practices.

Preparation of patient: None.

Precautions: None.

Accredited	No
Method	The BD MAX Cdiff assay performed on the BD MAX System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridioides difficile* toxin B (*tcdB*) gene in human liquid or soft stool specimens from patients suspected of having *Clostridioides difficile* infection. The test, performed directly on the specimen, utilises real-time polymerase chain reaction (PCR) for the amplification of *Clostridioides difficile* toxin B DNA and fluorogenic target-specific hybridisation probes for the detection of the amplified DNA. SOP: MB66
Sample Requirements	Sample Type: Liquid or soft stool Minimum Volume: 10µL Temperature: • 2°C - 25°C for up to 48 hours (2 days) • 2°C - 8°C for up to 120 hours (5 days)
Turnaround Time	3 working days from sample receipt

Stability	2°C - 25°C for up to 48 hours (2 days) 2°C - 8°C for up to 120 hours (5 days)
Laboratory Sample Storage	Primary Sample: 7 days at 2°C - 8°C Sample Buffer Tubes: 5 days at 2°C - 8°C
Results and Source	Toxigenic Clostridioides difficile Detected Toxigenic Clostridioides difficile Not Detected Invalid Source: BD MAX Cdiff REF 442555 P0215(07) 2023-11
Limitations of Test	• The BD MAX Cdiff assay is intended for use only with unpreserved liquid or soft stools; performance characteristics of other clinical specimen types have not been established. • Erroneous test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. • A BD MAX™ Cdiff positive assay result does not necessarily indicate the presence of viable organisms. It does however indicate the presence of the *tcdB* gene and allows for presumptive detection of a *Clostridioides difficile* toxigenic organism. The BD MAX™ Cdiff assay cannot be used for species identification as it does not contain primers and probes specific to *Clostridioides difficile*. • As with all PCR-based in vitro diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible. • Mesalamine rectal suspension enema and Gynol II may cause slight inhibition in the BD MAX™ Cdiff assay (refer to the Interfering Substances section for further details). • Tums and Maalox liquid may inhibit the BD MAX™ Cdiff assay (refer to the Interfering Substances section for further details). • False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate bacterial cell lysis. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Sample Processing Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or whether bacterial cells have been adequately lysed. • BD MAX™ Cdiff assay results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to System failure, and require retesting that can lead to a delay in obtaining final results.

	• Mutations or polymorphisms in primer- or probe-binding regions may affect detection of *Clostridioides difficile tcdB* gene variants, resulting in a false negative result with the BD MAX™ Cdiff assay. • Variant toxigenic *Clostridioides difficile* without the *tcdB* gene or with a non-functional Toxin B protein are very rare.15-18 The BD MAX™ Cdiff assay targets the *tcdB* gene and it is unknown whether it would detect Toxin A+/Toxin B- variant strains. • As with all in vitro diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD MAX™ Cdiff assay performance may vary depending on the prevalence and population tested.
Notes	*Clostridioides difficile* is a notifiable disease https://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/clostridioidesdifficile/casedefinitions/

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