TECHNICAL REPORT

STI laboratory diagnostics in Europe

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Marita van de Laar and Gianfranco Spiteri and produced by Public Health England in collaboration with Statens Serum Institut, Denmark and Örebro University Hospital, Sweden.

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We would like to thank all those who kindly participated in the survey.

Suggested citation: European Centre for Disease Prevention and Control. STI laboratory diagnostics in Europe. Stockholm: ECDC; 2013.

Stockholm, December 2013

ISBN 978-92-9193-505-5

doi 10.2900/91382

Catalogue number TQ-03-13-598-EN-N

Contents

Abbreviations

BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards Institute
ECDC	European Centre for Disease Prevention and Control
EIA	Enzyme Immunoassays
EQA	External quality assessment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
Euro-GASP	European Gonococcal Antimicrobial Surveillance Programme
LGV	Lymphogranuloma venereum
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
RPR	Rapid plasma reagin
STI	Sexually transmitted infections
TESSy	The European Surveillance System
TPHA	Treponema pallidum haemagglutination assay
TPPA	Treponema pallidum particle agglutination assay
WHO	World Health Organization

Executive summary

A survey was developed to assess STI diagnostic laboratory capacity and training needs throughout the EU/EEA. The survey was designed to collect information on the laboratory diagnosis of gonorrhoea, chlamydia and syphilis; clinical reporting; laboratory capacity for testing; laboratory accreditation and external quality assessment (EQA); training; reporting and laboratory systems. A major objective of the survey was to enable ECDC to support Member States in providing good quality laboratory surveillance data. The survey was performed in 2011 with the nominated contact points for STI surveillance, microbiology. In total 44 completed surveys from participating laboratories in 24 EU/EEA countries were received.

For the identification of *Neisseria gonorrhoeae* cultures; gram, oxidase and biochemical tests were the most common tests performed. Even though culture of *N. gonorrhoeae* is performed in many laboratories, the survey did highlight a lack of *N. gonorrhoeae* specimens being sent to the participating laboratories for diagnosis by culture from the clinics. Therefore the lack of participation in some surveillance programmes may not be due to lack of culture facilities, but rather lack of specimens for culture being sent to the laboratory from the clinics. It is encouraging that 32 laboratories perform *N. gonorrhoeae* susceptibility testing. The Etest susceptibility testing methodology and the CLSI guidelines are most frequently used across Europe. Capacity building could be focused on those laboratories that do not have skills in the culture and susceptibility testing of *N. gonorrhoeae*. All laboratories should be encouraged to participate in the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP). Further harmonisation of *N. gonorrhoeae* susceptibility testing methods and breakpoints is needed.

Molecular testing is performed for the detection of *N. gonorrhoeae* and *Chlamydia trachomatis* nucleic acid in 22 laboratories (14 countries) and 29 laboratories (19 countries) respectively. Specimens from non-genital sites are also tested. Retesting is important, particularly for *N. gonorrhoeae* nucleic acid detection because of cross reaction with commensal *Neisseria* species, and it is of some concern that repeat testing is not uniform.

Lymphogranuloma venereum (LGV) testing is performed in 14 laboratories (11 countries), which highlights a need for LGV testing to be made available in those countries that do not currently provide it.

A potential area for capacity building is the typing of specimens, in particular molecular typing, if there is a public health benefit and sufficiently robust methods can be established for each STI. Twelve laboratories perform *N. gonorrhoeae* typing, nine of which use molecular methods. Eight laboratories perform chlamydia typing (all molecular) and only two laboratories perform syphilis molecular typing.

With respect to the EU case definitions; all laboratories perform at least one of the recommended laboratory tests to define a case of gonorrhoea and all but one of the laboratories perform at least one of the recommended laboratory tests to define a case of *C. trachomatis*. It is very encouraging that the use of EIAs for the diagnosis of chlamydia is uncommon among participating laboratories. All laboratories performing LGV diagnosis comply with the EU laboratory criteria. The situation with syphilis case definitions is more complicated as only nine laboratories conform to the case definitions as regards serology testing. However, 29 laboratories perform a screening test (EIA, TPHA, TPPA) and an RPR/VDRL test, which highlights the need for the case definitions to be updated. In total, 18 laboratories detect *T. pallidum* via dark-field microscopy, direct fluorescence antibody test or using molecular methods. The situation for the laboratory confirmation of congenital syphilis is also quite variable. Six laboratories detect *T. pallidum* via dark-field microscopy or direct fluorescence antibody and 19 laboratories perform a specific IgM and a non-treponemal test. Eleven laboratories perform treponemal antibody testing that does not conform to the current case definitions, three perform PCR that is not listed and eight do not do any testing for congenital syphilis. This level of heterogeneity suggests some harmonisation is required.

There is a wide variation in the time taken for a positive and/or negative result to leave the laboratory, with some laboratories taking more than seven days to issue a report. The time taken for some laboratories to release congenital syphilis results and positive syphilis results is a matter of concern.

It is encouraging that 35 laboratories participate in EQA schemes. Twenty-eight laboratories are accredited, and it may be cause for concern that some laboratories are not accredited. Molecular testing stands out as the highest training need throughout the countries. For gonorrhoea there seems to be less requirement for training in culture and identification, and more requirement for susceptibility testing and typing. For chlamydia there is more need for training in molecular methods and typing than culture. The most common training need for syphilis is molecular typing, even though no reliable standard method is available.

The survey has enabled a certain level of laboratory capacity to be established and identified training needs across Europe. Even though a full representation of the EU/EEA was not achieved and there are differences in the laboratory functions of the respondents, the opportunity for capacity building has been identified in the following areas: algorithms for *N. gonorrhoeae* molecular testing; encouraging clinics to submit specimens for *N. gonorrhoeae* culture and non-genital specimens; facilities for LGV testing; updating of the EU syphilis case definitions and the development of an EU/EEA national reference network.

1. Introduction

The European Surveillance System (TESSy) at the European Centre for Disease Prevention and Control (ECDC) is designed to be the single point for Member States to submit and retrieve data on all communicable diseases under EU surveillance. The sexually transmitted infections (STIs) under EU surveillance are syphilis, congenital syphilis, gonorrhoea, chlamydia and lymphogranuloma venereum (LGV). The Commission Decision 28/IV/2008 lays down case definitions for reporting the STIs listed (Annex 5.1). The case definitions have the purpose of facilitating and harmonising the reporting on communicable diseases across EU/EEA Member States. According to the case definitions, confirmed cases should be laboratory confirmed and the laboratory methods are listed in the laboratory criteria section for each disease.

ECDC strives to ensure a high quality of standardised STI surveillance data, and one way to achieve this is to ensure the laboratory methods used to diagnose cases are of a suitable and recommended standard. However, it is not currently known what specific tests are performed by laboratories that report their results to a national surveillance centre for subsequent submission to TESSy. The laboratories may not have the capacity to perform the laboratory tests specified due to technical, resource-related and financial shortcomings. Identification of these gaps will enable ECDC to assist countries, where possible, to submit good quality and reliable data to TESSy and to improve the interpretation of the available STI surveillance data.

1.1 Objectives

The level of laboratory capacity and training needs throughout the EU/EEA was assessed by carrying out a survey across Member States. The survey will make it possible to gain more insight into:

- The microbiology laboratories that act as national contact points within the European STI surveillance network.
- Current laboratory capability to provide accurate surveillance data for submission to TESSy.
- The status of some of the main elements of laboratory capacity:
  - Laboratory diagnosis of gonorrhoea, chlamydia and syphilis
  - Clinical reporting
  - Laboratory capacity for testing
  - Laboratory accreditation and external quality assurance (EQA)
  - Training
  - Reporting and laboratory systems.

1.2 Methods and questionnaire

A survey on STI laboratory diagnostics for specialist and expert laboratories was launched (Annex 3) between late-2010 and mid-2011. The survey was designed to collect data on the following areas:

Laboratory function

This section was designed to establish the type of laboratory function (national, expert, regional and routine primary diagnostics) performed by the laboratory for the diagnosis of chlamydia, gonorrhoea and syphilis.

Laboratory diagnosis of gonorrhoea, chlamydia and syphilis

To obtain more information on laboratory diagnosis of gonorrhoea, chlamydia and syphilis, details were requested on the type of laboratory tests performed and the methods used for susceptibility testing of *N. gonorrhoeae*. For molecular testing questions, further information on the type of specimen was sought, along with details on molecular typing. To further assess capacity, laboratories were asked if they had to send specimens elsewhere, why and what further services they would require to be able to test themselves. Details on the number of isolates and specimens for the diagnosis of each disease were also requested, along with the number of laboratories that refer specimens to the respondents' laboratory. Information was also requested on laboratory capacity in terms of the number of tests performed in one year for each disease.

Clinical reporting

Details were requested on the mean time and range in days from the laboratory receiving specimens to the production of a positive and a negative report leaving the laboratory. This was to establish if any laboratories need assistance in improving their turnaround times.

Laboratory accreditation and external quality assessment (EQA)

Laboratory accreditation and regular participation in EQA schemes are important for a public health microbiology laboratory, and participation can assist in the generation of good quality laboratory data. Details were requested on laboratory accreditation and participation in EQA schemes, along with details on what other EQA services the laboratories require.

Training

Laboratories' training requirements in STI diagnostics were assessed so that ECDC can develop future training programmes to assist in capacity building.

Laboratory system

Laboratories were asked about their awareness of the EU case definitions and the number of public and private laboratories in the respondent country in order to facilitate the distribution of future surveys.

The competent bodies in EU/EEA countries have nominated national contact points for STI surveillance. The survey was emailed to the contact points for STI laboratory (hereafter referred to as 'STI microbiological contacts'). As there is often more than one laboratory expert contact point for each country, the contact points were asked to co-operate in deciding who was most suited to completing or coordinating the response to the questionnaire for each disease (syphilis, gonorrhoea and chlamydia). It was hoped that ideally up to a maximum of three completed surveys would be received from each country, one for each disease, or just one survey for all three.

1.3 Response

A total of 44 responses were received from 24 countries. No surveys were received from Finland, Iceland, Liechtenstein, Luxembourg, Norway or Poland.

The following countries submitted more than one completed survey:

- Cyprus (2)
- Greece (2)
- Ireland (10)
- Latvia (2)
- Lithuania (7)
- Slovakia (2)
- United Kingdom (2).

The reasons for multiple submissions include:

- National reference laboratories specialising in different bacterial STIs (Cyprus, Greece and Slovakia)
- One national reference centre and other regional laboratories having submitted (Ireland, Latvia, Lithuania)
- Having two national reference laboratories within the same country (United Kingdom – England and Scotland).

Not all completed surveys contained details on all STIs:

- Czech Republic - chlamydia and syphilis only
- Estonia - gonorrhoea and chlamydia only
- Germany and Romania - gonorrhoea and syphilis only
- Italy - gonorrhoea only
- Slovenia - chlamydia only.

The other ten countries (Austria, Belgium, Bulgaria, France, Hungary, Malta, the Netherlands, Portugal, Spain and Sweden) submitted one comprehensive survey dealing with all three infections.

1.4 Data analysis and presentation

Completed surveys were returned and results were transferred into a Microsoft Access database and all data was entered twice to avoid data entry errors.

The following analysis was performed:

- For each section of the survey, the data was combined from all the submitted surveys and an overall analysis carried out. The total number of laboratories is expressed along with the total number of countries, where applicable.
- The type of laboratory function (national, regional, expert and routine primary diagnostics) the laboratory performs for the diagnosis of chlamydia, gonorrhoea and syphilis was established. Where appropriate the analysis was further broken down by laboratory function. National and expert laboratories were kept separate, however the regional and primary diagnostic laboratories were combined due to low numbers in the regional category and because both types of laboratories do not perform any specialised expert function.
- If there was more than one response from each country, and there were differing responses within each country, this fact is highlighted in the text – i.e. if just one laboratory in Lithuania performs susceptibility testing then it is stated as: 'Lithuania (1 lab)'.
- The concordance of the laboratory tests performed and the EU case definitions were established (Annex 2).
- An STI-specific table containing data from each country was created (Annex 1) to present the overall capacity in each country. Where more than one response was received, the consensus and the number of laboratories in agreement are listed, and the national laboratory, where applicable is also highlighted.

It should be noted that the analysis is mainly descriptive and statements about laboratory capacity can only be made if a completed survey was received.

2 Results

2.1 Laboratory function

The STI microbiology contacts were asked to give details on the type of laboratory function (national, regional, expert and routine primary diagnostics) the laboratory performs for diagnosis of chlamydia, gonorrhoea and syphilis. Type of laboratory function refers to:

- national reference – laboratory receives specimens from the whole country and has a reference function;
- regional – laboratory receives specimens from a region in the country;
- expert – there is no national or regional focus, but rather the laboratory is an expert in diagnostics;
- routine primary diagnostics – laboratory either has one of the functions above or performs routine diagnostics only.

For each disease (gonorrhoea, syphilis and chlamydia), laboratories may have different laboratory functions, so the laboratory function for each disease has been identified (Tables 1–3). For those countries where more than one survey was received, individual survey identifiers have been included after the country names (Tables 1–3) to clarify the different country responses.

Acquiring details on the laboratory function enables the capacity level to be clearly assessed at each laboratory function level. For subsequent analysis based on laboratory function, regional and routine laboratories were merged together, as these have no specialist function for the relevant STIs. The national reference laboratories and the expert laboratories were kept separate. There were seven less complete surveys on syphilis diagnostics and six on chlamydia respectively, compared to gonorrhoea diagnostics.

The laboratory function for each STI is not always the same (Tables 1–3). With regard to national reference laboratories from the submitted surveys, there are 14, 11 and 13 national reference laboratories for gonorrhoea, chlamydia and syphilis respectively (Figures 1 a–c). Only Ireland, Latvia, Lithuania, Portugal and the UK have a national reference laboratory for all three diseases. Belgium, France, Germany, Spain and Sweden have a national reference laboratory for gonorrhoea only, Slovenia for chlamydia only and Hungary, Romania and Slovakia for syphilis only. Bulgaria has both a gonorrhoea and chlamydia reference laboratory, Denmark and Greece have both a gonorrhoea and syphilis reference laboratory, while Malta, Cyprus and the Czech Republic have a chlamydia and syphilis reference laboratory. There are no national reference laboratories in the Netherlands, Estonia, Austria and Italy.

Figure 1a. Countries with reference laboratories for gonorrhoea in EU/EEA

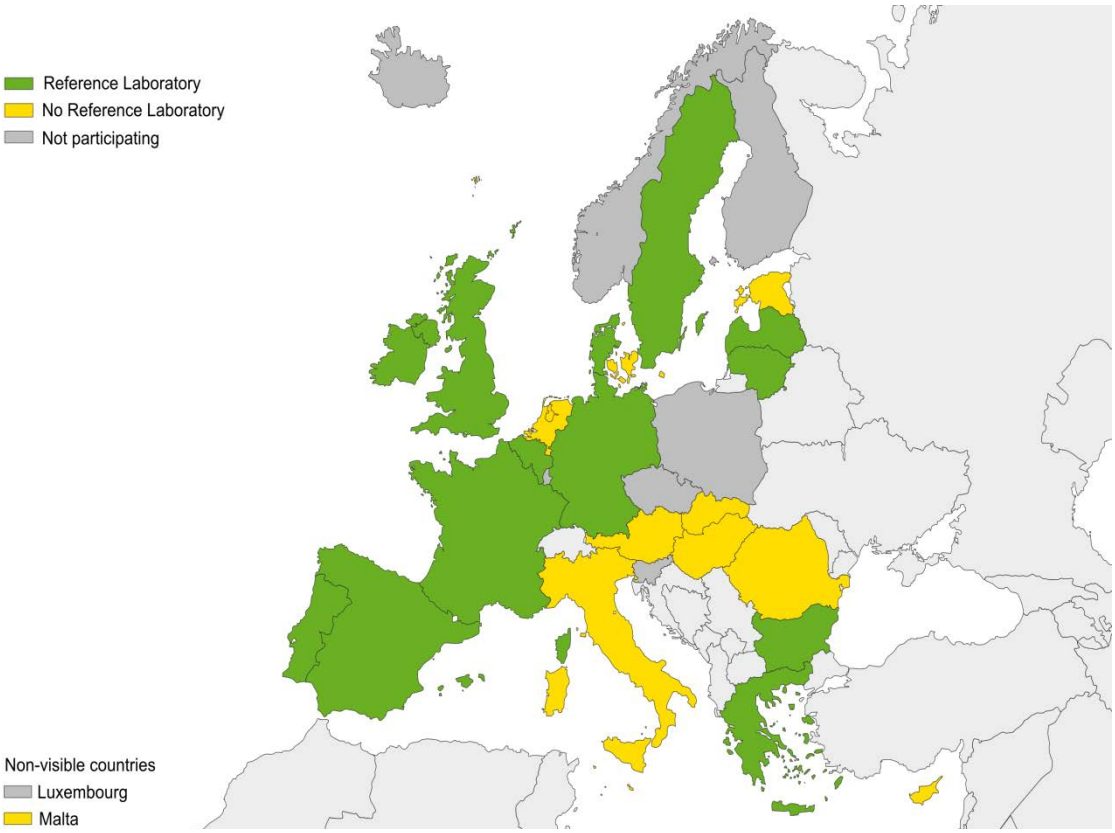


Figure 1b. Countries with reference laboratories for chlamydia in EU/EEA

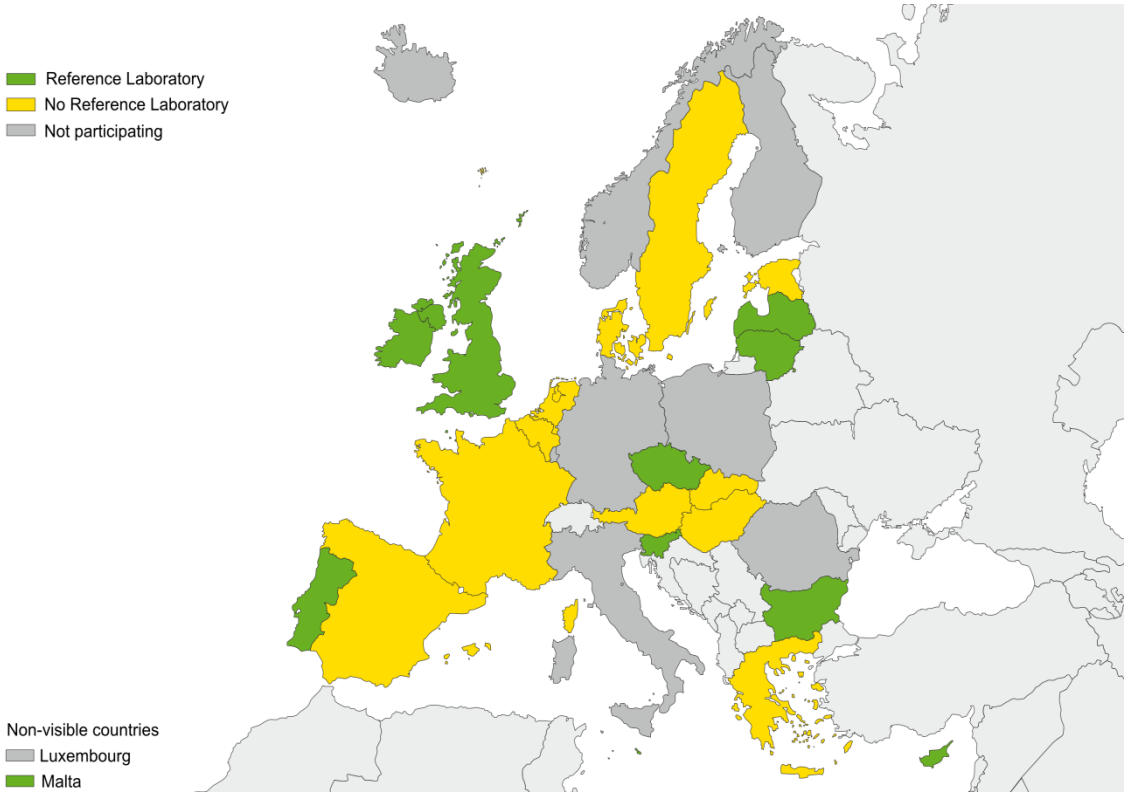
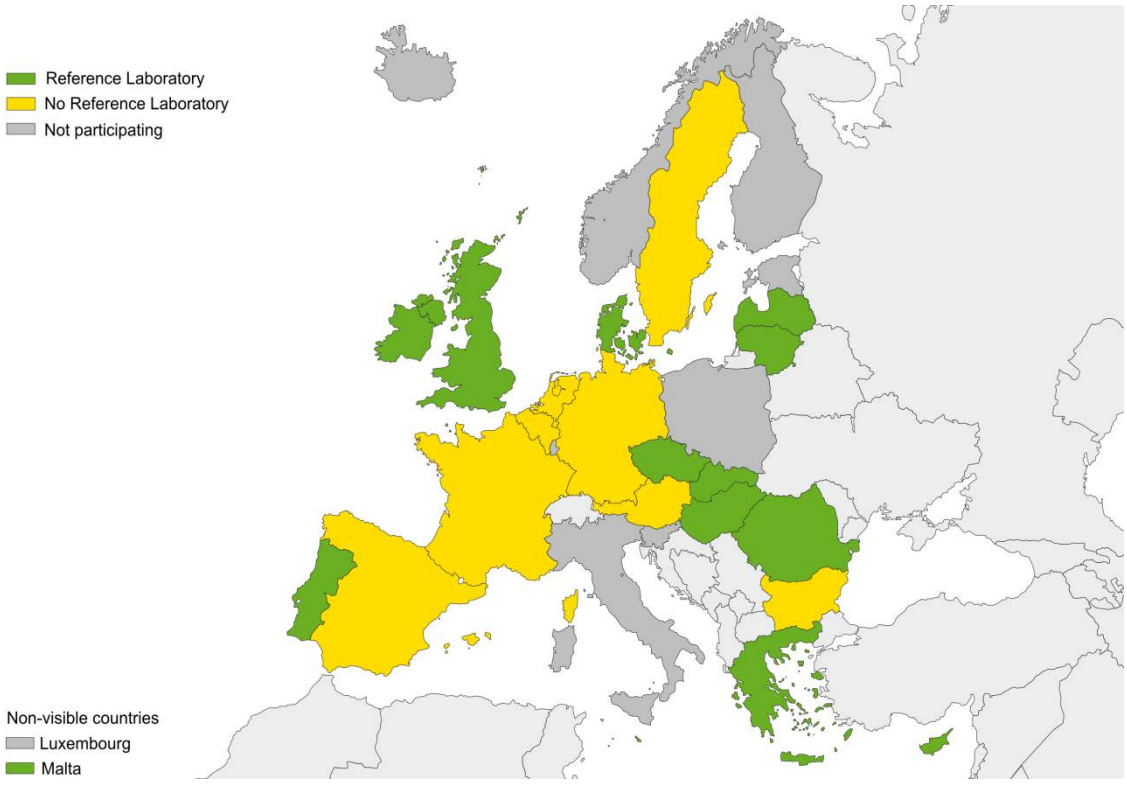


Figure 1c. Countries with reference laboratories for syphilis in EU/EEA



National reference laboratories specialise in specific STI pathogens and are generally developed and chosen for their high level of diagnostic excellence. A reference laboratory that performs high-quality diagnostics should in turn produce high-quality surveillance data. One advantage in having a national reference laboratory is the ability to have a national approach to the diagnostics of the STIs and specialist knowledge and expertise. The low number of national reference laboratories may reflect an area for capacity-building in some countries. However, the presence of a national reference laboratory could depend upon the national laboratory structure and on laboratory terminology. The lack of reference laboratories in some counties does not necessarily result in lower quality data from that country and it should, however, be noted that we may be missing completed surveys from national reference laboratories in some countries. It is, nevertheless, encouraging that even though some countries do not have a national reference laboratory, many laboratories perform an expert laboratory function, which will also ensure good quality diagnostic data, just not at a national level. Even though surveys were received from 19, 12 and 13 laboratories that perform a regional and/or primary routine diagnostic function for gonorrhoea, chlamydia and syphilis respectively, the subsequent inclusion of this data in the analysis is important. While identifying those countries that may lack national/expert laboratories, it also demonstrates that the diagnostic capacity and subsequent data is still of a high quality.

Table 1. Laboratory function for gonorrhoea diagnosis (n=40)

National reference laboratory (including expert) (n=14)	Expert laboratory (including regional) (n=7)	Regional (n=7)	Routine diagnostics only (n=12)
Belgium*	Austria*	Cyprus* (CY1)	Estonia
Bulgaria*	Greece* (GR2)	Ireland* (IE6/10)	Ireland (IE1-5/7)
Denmark*	Ireland* (IE8)	Slovakia* (SK1)	Latvia (LV1)
France*	Italy	The Netherlands*	Lithuania (3/6-7)
Germany*	Lithuania* (LT2/4/5)	Hungary	Malta
Greece* (GR1)		Romania	
Ireland* (IE9)			
Latvia* (LV2)			
Lithuania* (LT1)			
Portugal*			
Spain			
Sweden*			
UK (UK1/2)			

Note: *Also performs routine diagnostics. No data on the laboratory diagnosis of gonorrhoea from the following laboratories: Czech Republic, Cyprus (CY_2), Slovakia (SK_2) and Slovenia.

Table 2. Laboratory function for chlamydia diagnosis (n=33)

National reference laboratory (including expert) (n=11)	Expert laboratory (including regional) (n=10)	Regional and routine diagnostics (n=4)	Routine diagnostics only (n=8)
Bulgaria*	Austria*	Slovakia* (SK1)	Estonia
Cyprus* (CY1)	Denmark*	Ireland* (IE6/10)	France
Czech Republic*	Belgium*	Hungary	Ireland (IE1/5)
Ireland* (IE9)	Greece* (GR2)		Latvia (LV1)
Latvia* (LV2)	Ireland* (IE8)		Lithuania (LT3/7)
Lithuania* (LT1)	Lithuania (LT2/4/5)		Spain
Malta*	Sweden*		
Portugal*	The Netherlands		
Slovenia*			
UK (UK1/2)			

Note: *Also performs routine diagnostics. No data on the laboratory diagnosis of chlamydia from the following laboratories: Romania, Cyprus (CY_2), Slovakia (SK_2), Ireland (IE2-4/7), Greece (GR_1), Lithuania (LT_6), Germany and Italy.

Table 3. Laboratory function for syphilis diagnosis (n=34)

National reference laboratory (including expert) (n=13)	Expert laboratory (including regional) (n=8)	Regional and routine diagnostics (n=5)	Routine diagnostics only (n=8)
Cyprus* (CY2)	Austria*	The Netherlands*	France
Czech Republic*	Belgium	Bulgaria*	Germany
Denmark*	Ireland* (IE8)	Ireland* (IE6/10)	Ireland (IE1/7)
Greece* (GR2)	Lithuania (LT2/4/5)	UK (UK2)	Lithuania (LT3/6/7)
Hungary	Spain*		Latvia (LV1)
Ireland* (IE9)	Sweden*		
Latvia* (LV2)			
Lithuania* (LT1)			
Malta*			
Portugal*			
Romania*			
Slovakia (SK2)			
UK (UK1)			

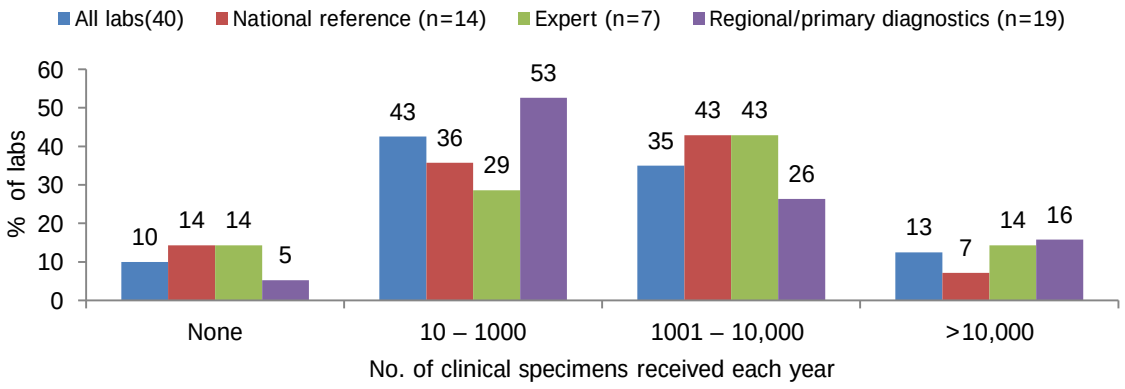
Note: *Performs routine diagnostics also. No data on the laboratory diagnosis of syphilis from the following laboratories: Cyprus (CY_1), Slovakia (SK_1), Ireland (IE2-5), Greece (GR_1), Estonia, Slovenia and Italy.

2.2 Gonorrhoea

Details were requested on the number of isolates and specimens for the diagnosis of gonorrhoea and the number of laboratories that refer specimens to the respondents' laboratory. This data was analysed by type of laboratory function. The individual countries and laboratories in each laboratory function type are described in Table 1.

There is a wide variation in the number of clinical specimens for the diagnosis of gonorrhoea (Figure 2). The majority of laboratories receive 10–1 000 (43%) and 1001–10 000 (35%) clinical specimens each year. When the results are broken down by laboratory function, the majority of the regional/primary diagnostics laboratories receive 10–1 000 (53%) specimens and the majority of the national/expert laboratories receive 1001–10 000 (43%) clinical specimens per year (Figure 5). In the highest category of >10 000 per year, the expert (14%) and the regional/primary diagnostic (16%) receive more clinical specimens than the national laboratories (7%).

Figure 2. Number of clinical specimens for gonorrhoea diagnosis received by participating laboratories (n=40)



Over half (53%) of the regional/primary diagnostic laboratories receive no *N. gonorrhoeae* cultures, and the national reference laboratories receive the largest number of cultures (86%), with 10–500 cultures being the most frequent number (Figure 3).

Figure 3. Number of *N. gonorrhoeae* cultures received by participating laboratories (n=40)

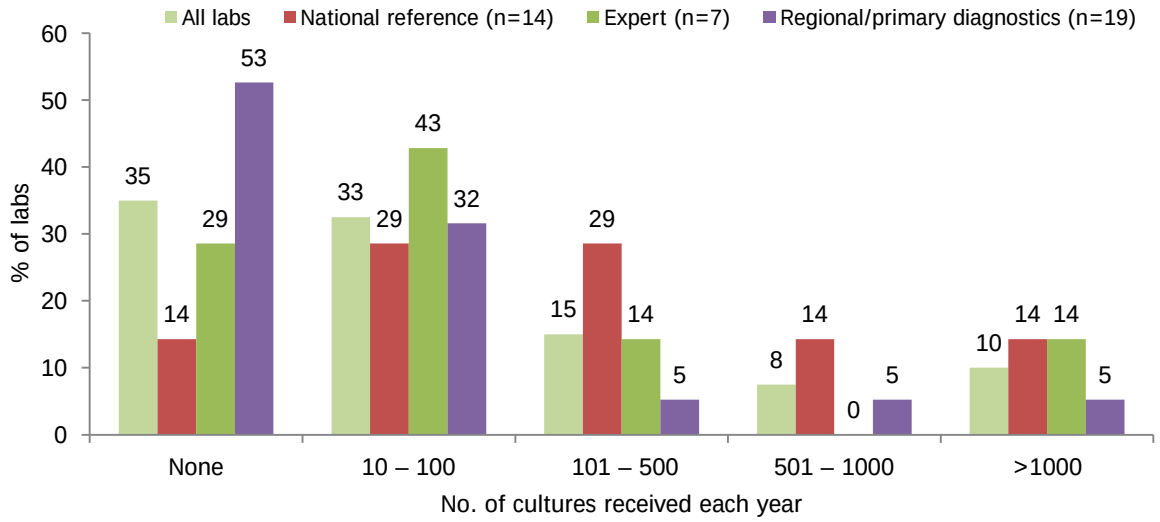
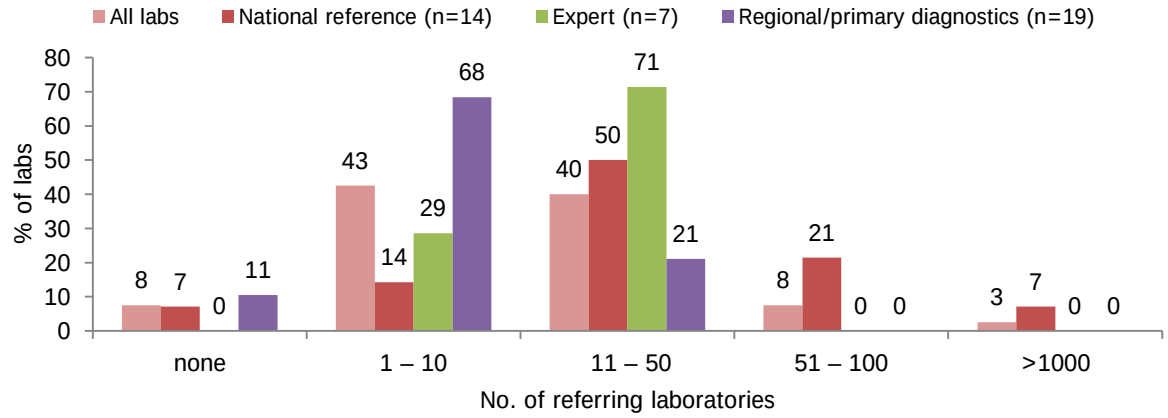


Figure 4. Number of laboratories that refer specimens/isolates to the respondents' laboratory (n=40)



The majority of regional/primary diagnostic laboratories (68%) have 1–10 other laboratories that refer specimens/isolates to the respondents' own laboratory (Figure 4). For the national and expert laboratories, the most frequent number of referring laboratories is 11–50. It is only the national reference laboratories that have over 51 laboratories referring specimens/isolates to them.

Laboratory diagnosis of gonorrhoea

Details on the laboratory diagnosis of gonorrhoea were established by requesting information on the type of laboratory tests performed. Three laboratories perform no gonorrhoea diagnostics (Czech Republic, and one laboratory in Slovakia and Cyprus) and the survey was not returned from the Slovenian microbiological contact.

Of the 36 laboratories that receive clinical gonorrhoea specimens:

- isolation of *N. gonorrhoeae* from a clinical specimen is performed by 29 laboratories/18 countries
- Detection of *N. gonorrhoeae* nucleic acid in a clinical specimen is performed by 22 laboratories/14 countries
- Demonstration of *Neisseria gonorrhoeae* by a non-amplified nucleic acid (NAAT) probe test in a clinical specimen is performed by four laboratories/three countries
- Microscopic detection of intracellular Gram negative diplococci in an urethral male specimen is performed by 26 laboratories/14 countries.

The relationship between these diagnostic tests and the EU 2009 case definition of gonorrhoea is described in Section 2.11.

Fifteen laboratories (13 countries) isolate *N. gonorrhoeae* from clinical specimens and also detect *N. gonorrhoeae* using nucleic acid amplification tests (NAATs). Fourteen laboratories (eight countries) perform isolation but no NAATs, while seven laboratories (four countries) perform NAAT testing and but do not isolate *N. gonorrhoeae* from clinical specimens.

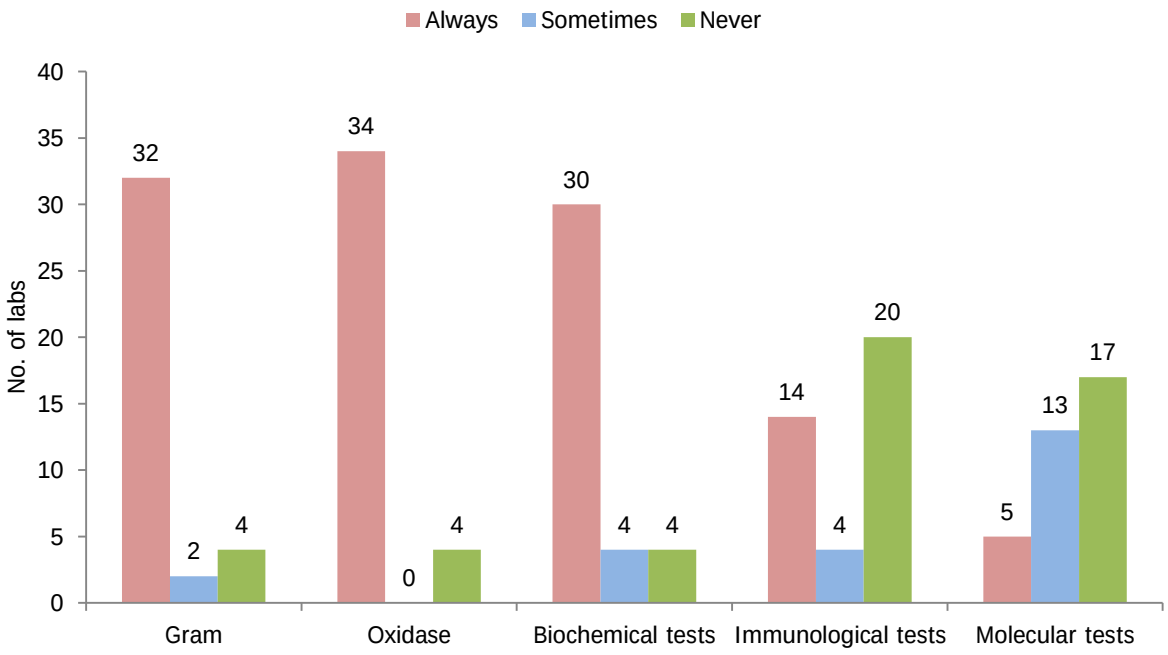
Confirmation of *N. gonorrhoeae* cultures

It is important that presumptive cultures of *N. gonorrhoeae* are correctly identified. This can be done by using a combination of the following tests:

- Gram staining – to distinguish between gram positive and gram negative gonococci and bacilli.
- Oxidase test – to detect the oxidase enzyme that *N. gonorrhoeae* produces for respiration.
- Biochemical tests – these tests utilise carbohydrates and enzymes to differentiate between the different species of *Neisseria*. For example, *N. gonorrhoeae* produce acid from glucose only, while *N. meningitidis* and *N. lactamica* can produce acid from glucose and maltose. Different enzymes are produced by different species of Neisseria, so enzymes can be used to differentiate.
- Immunological tests – require the detection of gonococcal antigens, such as the Por IA and IB antigens.
- Molecular tests – rely on the detection of gonococcal nucleic acid.

Thirty-four labs culture *N. gonorrhoeae* and therefore require further identification tests to confirm the culture identity. Along with gram straining and the oxidase test, biochemical tests are the most common tests used for the identification of *N. gonorrhoeae* cultures (Figure 5). All laboratories that culture (34) perform a gram stain and oxidase test in addition to a biochemical and/or immunological test. Eight laboratories perform a biochemical test in addition to a gram strain and oxidase test. The biochemical tests are more popular than the immunological tests; immunological tests were not performed in Cyprus, Denmark, Estonia, France, Hungary, Ireland (x 4 labs), Lithuania (x 4 labs), Latvia (one lab), the Netherlands, Portugal and Slovakia (one lab). This data suggests that the majority of the laboratories that responded from those Member States which joined the EU after 2004 use biochemical tests more than immunological tests.

Figure 5. Tests used for the identification of *N. gonorrhoeae* cultures



Molecular testing

A total of 22/36 laboratories (14 countries) detect *N. gonorrhoeae* nucleic acid by molecular test in a clinical sample. Five laboratories (four in Lithuania and one in Ireland) do not culture and only use molecular tests.

The type of clinical specimen (genital, rectal, pharyngeal) used for *N. gonorrhoeae* nucleic acid detection is described in Table 4. Genital specimens are the most common specimen type tested (21 labs). Sixteen laboratories perform testing on rectal specimens and fourteen on pharyngeal specimens. Of the 22 laboratories that test genital specimens, six of these laboratories do not test rectal or pharyngeal specimens; Estonia, Latvia (one lab) and Lithuania (four labs).

Due to the genetic homology between *N. gonorrhoeae* and other *Neisseria* spp., when there is a positive predictive value of less than 90%, it is recommended that laboratories repeat nucleic acid testing using a different target. For those laboratories that perform molecular testing on genital specimens, 50% use a different target for confirmation. The percentage of laboratories using a different target when rectal and pharyngeal specimens are used is over 69%.

Six laboratories do not perform any nucleic acid retesting on genital clinical specimens (either the same or a different target). Additionally, three and two laboratories respectively do not retest either rectal or pharyngeal specimens.

A number of different nucleic acid tests are used for the detection of *N. gonorrhoeae* nucleic acid and the Genprobe commercial assays and in-house PCRs are the most commonly used for the detection of *N. gonorrhoeae* nucleic acid in clinical specimens (Table 5).

Table 4. Clinical specimen used for *N. gonorrhoeae* nucleic acid detection

Clinical specimen tested	No. of laboratories
Genital	22
Repeat with same target	8
Repeat with different target	11
Rectal	16
Repeat with same target	5
Repeat with different target	11
Pharyngeal	14
Repeat with same target	5
Repeat with different target	11

Table 5. Molecular tests for the detection of *N. gonorrhoeae* nucleic acid

Platform	Target	Site of clinical assay specimen		
		Genital	Rectal	Pharyngeal
Abbott	*Opa* gene	4	3	3
Becton Dickinson	Pillin (Probetec ET test targets a different region)	2	1	1
Cobas Amplicor	Cytosine DNA methyltransferase	1	1	1
COBAS 4800	Direct repeat region (DR-9)	2	2	1
Genprobe Aptima Combo	16s rRNA	5	4	4
Genprobe Aptima GC	16s rRNA (different region to Combo)	2	1	1
Genprobe Pace 2	Ribosomal RNA	1		
PCR/RT-PCR (in-house)	-	8	5	5
Total		**25**	**17**	**16**

Referral of *N. gonorrhoeae* isolates to another laboratory

Five laboratories refer *N. gonorrhoeae* strains to another laboratory, three of which were primary diagnostic laboratories (Ireland (2 x labs) and Latvia (one lab), and two were national laboratories (France and one laboratory from Greece). It was not known if the laboratory to which the isolates or specimens were referred was in the same country. The reasons for isolate referral include:

- Identification (Ireland - IE4)
- Susceptibility testing (Ireland - IE4)
- Molecular typing (France)
- Medico-legal samples (Latvia LV1)
- Other studies, such as collaborative research projects (France, Ireland (IE1), Greece and Latvia (LV1)).

There are no requirements listed for further specialist services involving *N. gonorrhoeae*.

N. gonorrhoeae susceptibility testing

Thirty-two laboratories perform *N. gonorrhoeae* susceptibility testing (Table 6) covering 21 countries. Twelve laboratories (Bulgaria, Cyprus (one lab), Czech Republic, (Ireland (three labs), Lithuania (four labs), Slovenia and Slovakia (one lab)) do not perform susceptibility testing, and of these, three laboratories perform culture but no susceptibility testing (Bulgaria and Ireland (two labs). Only in Bulgaria and the Czech Republic is no susceptibility testing is performed at all (no information from Slovenia although Slovenia participates in Euro-GASP).

The Etest susceptibility testing methodology and the CLSI guidelines are the most frequently used across Europe. Fourteen laboratories participate in a national surveillance programme (Germany, Denmark, France, Greece (x 2 labs), Ireland (x 2 labs), Malta, the Netherlands, Portugal, Sweden, Spain and the UK (x 2 labs) and six in a regional programme (Austria, Ireland (one lab), Hungary, Sweden, Portugal and the UK (one lab). Nine laboratories in eight countries (Austria, Denmark, France, Greece (one lab), Hungary, Italy, Latvia (one lab) and the UK (2x lab) organise their own surveillance programmes to investigate the susceptibility of *N. gonorrhoeae* to antimicrobials.

Table 6. Susceptibility testing methods and guidelines

		No. of laboratories
Method	Disc	18
	Etest	24
	Agar Dilution	7
	Other	2
Guidelines or breakpoints	CLSI	23
	EUCAST	10
	BSAC	1
	WHO	3
	Other	2

Note: CLSI – Clinical and Laboratory Standards Institute; EUCAST - European Committee on Antimicrobial Susceptibility Testing; BSAC - British Society for Antimicrobial Chemotherapy; WHO – World Health Organization.

N. gonorrhoeae typing

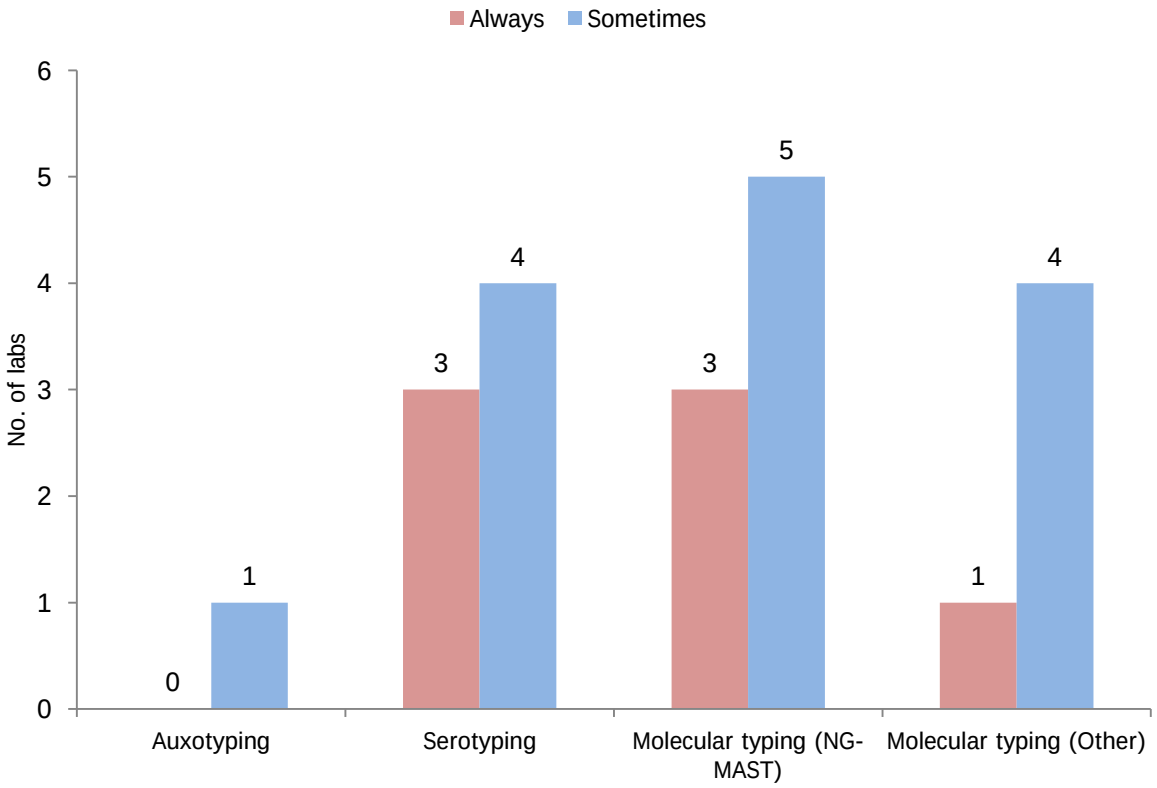
Typing of *N. gonorrhoeae* isolates is performed to discriminate the isolates based on their nutritional requirements (i.e. for vitamins, purines, pyrimidines and amino acids) and their serovars, which relates to antigenic differences (i.e. PorB) or their genotypes (DNA-based typing methods). The DNA-based typing methods are essentially split into two groups: those based on analysing DNA bands via electrophoresis such as PFGE (pulsed-field gel electrophoresis; Opa typing) and MLVA (multiple-locus variable-number tandem repeat analysis that looks at variation in the number of repeats sequences in different loci) and those based on DNA sequencing methods such as NG-MAST (*N. gonorrhoeae* multiantigen sequence typing which is based on sequence variation in two loci; porB and tbpb), MLST (multilocus sequence typing that looks for variation in seven housekeeping genes), and full length gene sequencing (i.e. porB).

Typing of *N. gonorrhoeae* isolates is performed by 12 laboratories (Figure 6) and molecular methods are used more than auxotyping (Greece, one lab) and serotyping (Belgium, France, Italy, Sweden, Spain and one lab from the UK and Greece). Molecular methods used for typing include NG-MAST (nine laboratories; Italy, Greece (one lab), UK (both labs), Portugal, Sweden, Denmark, Spain and France), PFGE (Sweden and Greece), the determination of resistance genes and polymorphisms, full-length porB gene sequencing, MLST, MLVA and Opa typing (Sweden).

Molecular typing is performed for:

- Antimicrobial resistance studies (n=8)
- Sexual network studies (n=8)
- Longitudinal studies (n=8)
- Contract tracing/medico-legal (n=7)
- Other studies (n=9), such as population and evolutionary studies and test-of-cure confirmation.

Figure 6. Laboratories performing typing of gonococcal isolates (n=12)



Summary

There is wide variation in the number of clinical specimens received by the laboratories. In general, the national and expert laboratories receive more specimens than the routine diagnostic laboratories. This is to be expected if the national and expert laboratories have a higher population coverage. However, information on the population covered by each laboratory was not requested. The national reference laboratories receive the most *N. gonorrhoeae* cultures, which could be due to their expertise in identification and susceptibility testing. It is not unexpected that isolates/specimens should be referred to national reference laboratories, making them part of a larger, more comprehensive network. A national network has advantages for obtaining representative samples for studies, such as Euro-GASP.

All laboratories perform a Gram strain, oxidase test and a biochemical or immunological test to identify *N. gonorrhoeae* cultures, however encouraging countries and more laboratories to culture, in particular for susceptibility testing, is a major area for potential capacity building. This will enable laboratories to participate in antimicrobial susceptibility surveillance programmes, which will in turn facilitate individual patient management and inform treatment guidelines. There are some countries (Bulgaria, Estonia and Lithuania) that have culture facilities, yet do not participate in Euro-GASP. This could be due to the associated clinics not taking specimens for culture from the patients.

The survey has shown that the comparability of susceptibility data generated by individual laboratories needs to be further assessed due to different methods and interpretation criteria. Recommended testing methodologies, along with evidence-based EU standard clinical breakpoints, will help in further standardising susceptibility testing for *N. gonorrhoeae* across Europe. This will further support the results of the Euro-GASP external quality assessment which have consistently shown high comparability among laboratories participating in the programme.

The use of NAAT for diagnosis of gonorrhoea is popular, given that 22/36 laboratories use this technology. However laboratories should be encouraged to perform repeat testing on different target specimens, in particular rectal and pharyngeal specimens. It is evident that even though commercial NAATs are not licensed to be used

with rectal and pharyngeal specimens the laboratories are using these tests to diagnose gonorrhoea at those sites. This is acceptable if the appropriate validation has been performed.

Based on this survey, molecular typing seems to be done only by the national or expert laboratories and depending on the added public health value, capacity building in this area may be required in future.

2.3 Chlamydia

Details were requested on the number of isolates and specimens for the diagnosis of chlamydia, along with the number of laboratories that refer specimens to the respondents' laboratory. This data was analysed by type of laboratory function. The individual countries and laboratories in each laboratory function type are described in Table 2.

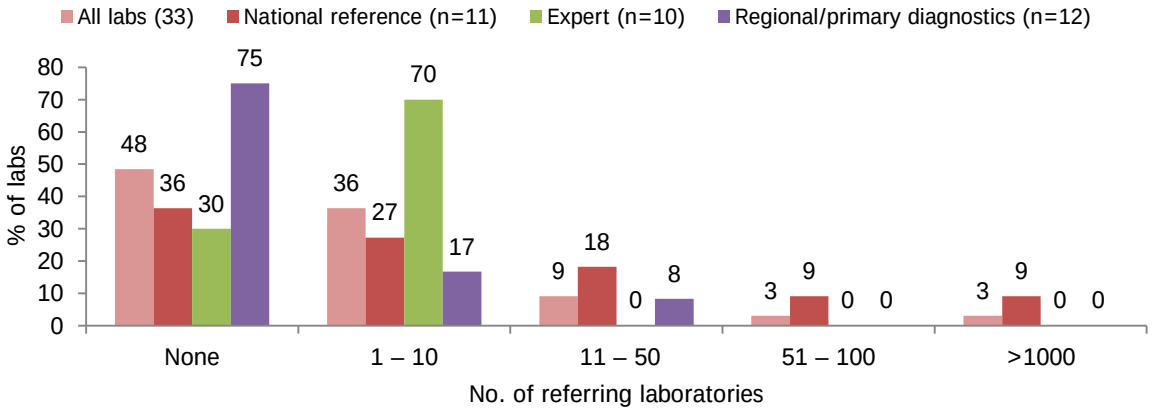
Of the thirty-three laboratories performing chlamydial diagnostics (Table 7), only four laboratories receive *C. trachomatis* cultures, all of which are national reference or expert laboratories (Table 7).

Table 7. Type of laboratory function receiving chlamydia specimens and cultures

Type of laboratory function	No. of laboratories (n=33)	
	Receive clinical specimens	Receive *C. trachomatis* cultures
National reference (n=11)	11	3 (Malta and 1 lab from Greece and the UK)
Expert (n=10)	10	1 (Sweden)
Regional/routine diagnostics (n=12)	12	0

There is a wide variation in the number of laboratories that send specimens or isolates to the participating laboratories (Figure 7). Expert laboratories are most likely to receive chlamydia specimens/isolates referred from another laboratory, however the national reference laboratories have the highest number of referring laboratories.

Figure 7. Number of laboratories referring specimens/isolates to respondents' laboratories



Laboratory diagnosis of chlamydia

Of the 33 laboratories that receive clinical chlamydia specimens and perform laboratory diagnosis for chlamydia:

- Isolation of *C. trachomatis* from a specimen of the ano-genital tract or from the conjunctiva is performed by 10 laboratories in eight countries.
- Demonstration of *C. trachomatis* by DFA test in a clinical specimen is performed by eight laboratories in seven countries.
- Detection of *C. trachomatis* nucleic acid in a clinical specimen is performed by 29 laboratories in 19 countries.
- Only one laboratory performed an EIA.

The relationship between these diagnostic tests and the EU 2009 case definition of chlamydia is described in Section 2.11.

Molecular testing

In total, 29/33 laboratories detect *C. trachomatis* nucleic acid by molecular test in a clinical sample. Four laboratories perform no molecular testing; Spain, Bulgaria, Latvia (one lab) and Lithuania (one lab).

The type of clinical specimen (genital, rectal, pharyngeal) used for *C. trachomatis* nucleic acid detection is described in Table 6. Genital specimens are the most common specimen type tested (29 labs), 20 laboratories perform testing on rectal specimens and 17 test pharyngeal specimens. Of the 29 laboratories that test genital specimens, eight of these laboratories do not test rectal or pharyngeal specimens; Cyprus (one lab), Estonia, Ireland (one lab), Latvia (one lab) and Lithuania (four labs). With the exception of Estonia and Ireland, these are the same laboratories that do not test pharyngeal or rectal specimens for *N. gonorrhoeae* nucleic acid.

There are a smaller number of laboratories repeating nucleic acid testing using the same or a different target than for *N. gonorrhoeae* (Tables 5 and 8).

A number of different nucleic acid tests are used for the detection of *C. trachomatis* nucleic acid and the Genprobe commercial assays and in-house PCRs or the Cobas and Genprobe commercial assays are the most commonly used for the detection of *C. trachomatis* nucleic acid in clinical specimens (Table 9).

Table 8. Clinical specimen used for *C. trachomatis* nucleic acid detection

Clinical specimen tested	No. of laboratories
Genital	29
Repeat with same target	10
Repeat with different target	7
Rectal	20
Repeat with same target	7
Repeat with different target	9
Pharyngeal	17
Repeat with same target	6
Repeat with different target	6

Table 9. Molecular tests for the detection of *C. trachomatis* nucleic acid

Platform	Target	Site of clinical assay specimen		
		Genital	Rectal	Pharyngeal
Abbott m2000	Cryptic plasmid (2 targets)	5	3	3
Becton Dickinson	Cryptic plasmid	5	1	1
COBAS Amplicor	Cryptic plasmid	2		
Cobas TAQMAN	Cryptic plasmid and MOMP	6	5	3
COBAS 4800	Cryptic plasmid and 2nd target on genome	1	1	2
Eppendorf		1		3
Genprobe Aptima Combo	Ribosomal RNA	4	3	3
Genprobe Aptima CT confirmation	Ribosomal RNA	2	1	
Genprobe pace 2	Ribosomal RNA	1		
PCR / RT PCR (in-house)	-	9	7	6
Total		**36**	**21**	**21**

Laboratory diagnosis of Lymphogranuloma venereum LGV

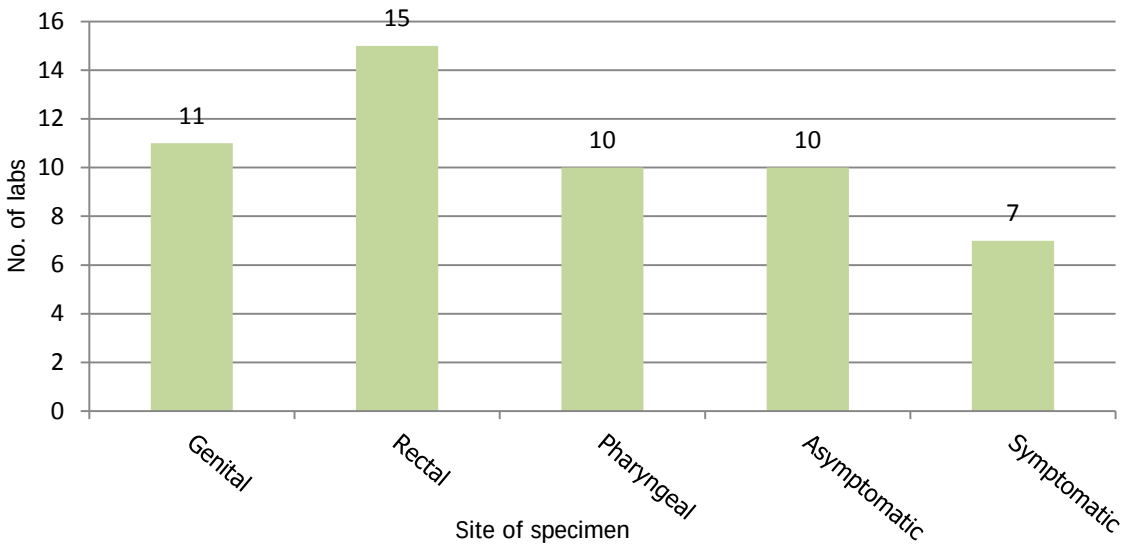
LGV diagnosis is performed in 14 laboratories (11 countries; Bulgaria, Hungary, Ireland (three labs), Sweden, UK (two labs), Belgium, France, Denmark, Portugal, Slovenia and the Netherlands). Below is a summary of the tests used for the diagnosis of LGV infection:

- Isolation of *C. trachomatis* from a specimen of the ano-genital tract or from the conjunctiva AND identification of serovar (genovar) L1, L2 or L3 is performed by five laboratories.
- Detection of *C. trachomatis* nucleic acid in a clinical specimen AND identification of serovar (genovar) L1, L2 or L3 is performed by 12 laboratories. Eight laboratories use a specific PCR for the detection of LGV genovar.

The relationship between these diagnostic tests and the EU 2009 case definition of LGV is described in Section 2.11.

All laboratories that perform testing for LGV do so with rectal specimens (Figure 8). Eleven and ten laboratories also test for LGV in genital and pharyngeal specimens respectively. Ten and seven laboratories test specimens from asymptomatic and symptomatic patients respectively. The Austrian laboratory was the only laboratory that tested both symptomatic and asymptomatic patients, however details on the laboratory tests used were not given. One laboratory in Ireland did no internal testing of LGV specimens, but sent specimens from symptomatic patients to another laboratory for LGV testing.

Figure 8. Site of specimen and presence of symptoms for LGV testing



Chlamydia trachomatis typing

Typing of *Chlamydia trachomatis* isolates is performed by eight laboratories. The most common method is omp1 (outer membrane protein 1) typing which is carried out in seven laboratories (Denmark, Greece (one lab), Hungary, Portugal, Sweden, Slovenia, and the UK (one lab)). Sweden performs MLST (multilocus sequence typing that looks for variation in housekeeping genes) and VNTR (variable-number tandem repeat analysis that looks at variation in the number of repeat sequences in loci) on chlamydia specimens. Other typing techniques performed include PCR-RFLP (restriction fragment length polymorphism that looks at different DNA bands on gel electrophoresis after restriction) (Hungary) and MLVA (multi-locus VNTR analysis) (the Netherlands). In addition, the Portuguese laboratory evaluates different loci for *C. trachomatis* typing. *C. trachomatis* typing is performed for:

- Sexual network studies =4
- Temporal studies =8
- Contract tracing/medico-legal =5
- Other =5, such as research, epidemiological surveys and to confirm LGV serovars.

Referral of *C. trachomatis* specimens to another laboratory

Ten laboratories (mainly in Ireland (six labs) and France and one lab from Lithuania) refer *Chlamydia trachomatis* specimens to another laboratory for:

- identification = 4
- molecular typing = 4
- LGV genotyping = 5
- other studies = 1.

It was not known if the laboratory to which the isolates or specimens were referred was in the same country. No laboratories reported requesting any other specialist services for *C. trachomatis* that were already available to them.

Summary

All but one of the laboratories performs appropriate tests for chlamydia diagnostics. The use of NAATs is by far the most popular test for chlamydia diagnostics. As with *N. gonorrhoeae* NAATs, it is evident that even though commercial molecular tests are not licensed to be used with rectal and pharyngeal specimens, some laboratories use these specimen types. This is acceptable if appropriate validation has been performed. Speciality in culture may be lacking but this may not necessarily be a problem as NAATs are more sensitive. However, care should be taken when using NAATs, in particular a single target, due to diagnostic escape strains such as the new variant chlamydia. Chlamydia culture work may become more necessary in the future if antibiotic resistance emerges and appropriate typing methods become available that need more chlamydial DNA than is contained in clinical specimens.

The results may highlight a need for LGV testing to be made available in those countries that do not currently test for LGV. Not all laboratories test specimens from asymptomatic patients which may mean that asymptomatic carriers may be missed.

Molecular typing is mainly performed by the national or expert laboratories and capacity building in this area may be required if a discriminatory molecular typing method for *C. trachomatis* is developed and shown to have a real public health benefit.

2.4 Dual gonorrhoea and chlamydia testing

Dual molecular testing for the detection of both *N. gonorrhoeae* and *C. trachomatis* nucleic acid in the same clinical specimen is performed by 15 laboratories (Austria, Belgium, France, Greece, Ireland (four labs), Lithuania (three labs), the Netherlands, Portugal and Slovakia (one lab).

As for the individual chlamydia and gonorrhoea molecular tests, the commercial assays are being used on rectal and pharyngeal specimens as well as genital specimens. Four laboratories performed dual molecular testing on genital specimens only (three labs from Lithuania and one Latvian lab). The same specificity issues regarding *N. gonorrhoeae* nucleic acid testing relate to dual testing (see Section 2.2 Gonorrhoea, Molecular testing). The Genprobe commercial assay is the most commonly used throughout the participating laboratories (Table 11).

Table 10. Clinical specimen used for dual *N. gonorrhoeae* and *C. trachomatis* nucleic acid detection

Clinical specimen tested	No. of laboratories
Genital	15
Repeat with same target	7
Repeat with different target	5
Rectal	11
Repeat with same target	5
Repeat with different target	5
Pharyngeal	8
Repeat with same target	4
Repeat with different target	6

Table 11. Molecular tests for the dual detection of *N. gonorrhoeae* and *C. trachomatis* nucleic acid

Platform	Site of clinical assay specimen		
	Genital	Rectal	Pharyngeal
Abbott m2000	5	3	3
Becton Dickinson	2	1	1
Cobas Amplicor	1	1	
COBAS 4800	1	1	1
COBAS	1	1	
Genprobe Aptima Combo	5	4	3
Genprobe CT confirmation	1		
Genprobe GC confirmation	1		
PCR	3	1	1
Total	**20**	**12**	**9**

Summary

Dual testing has obvious advantages, as the same clinical specimen can be used for both *N. gonorrhoeae* and *C. trachomatis* nucleic acid detection. Laboratories should be encouraged to perform repeat testing for the *N. gonorrhoeae* component of the tests. It should be considered that some laboratories and their associated clinics should test pharyngeal and rectal specimens so infections, particularly in men who have sex with men (MSM), are not missed and thereby morbidity and onward transmission can be reduced. Appropriate validation of these specimen types should be performed before testing non-genital specimens.

2.5 Syphilis

Details were requested on the number of isolates and specimens for the diagnosis of syphilis, along with the number of laboratories that refer specimens to the respondents' laboratory. This data was analysed by type of laboratory function. The individual countries and laboratories in each laboratory function type are described in Table 3.

Thirty three laboratories (20 countries) receive syphilis clinical samples, 32 of which 32 receive serology serum/plasma specimens and 15 receive also syphilitic lesion (ulcer swabs) or tissue specimens. One laboratory receives only syphilitic lesion samples and no serology specimens (Table 12). Variation can be observed in the number of laboratories that refer specimens or isolates to the laboratories that responded to the survey (Figure 9). Just over 80% of primary diagnostic laboratories have no other laboratories referring syphilis specimens or isolates to them (Figure 9). Most expert laboratories have 1–10 laboratories referring specimens to them and the national reference laboratories have the largest number of referring laboratories.

Syphilis laboratory diagnosis

Of the 33 laboratories that receive clinical syphilis specimens, the following laboratory techniques are performed to diagnose syphilis:

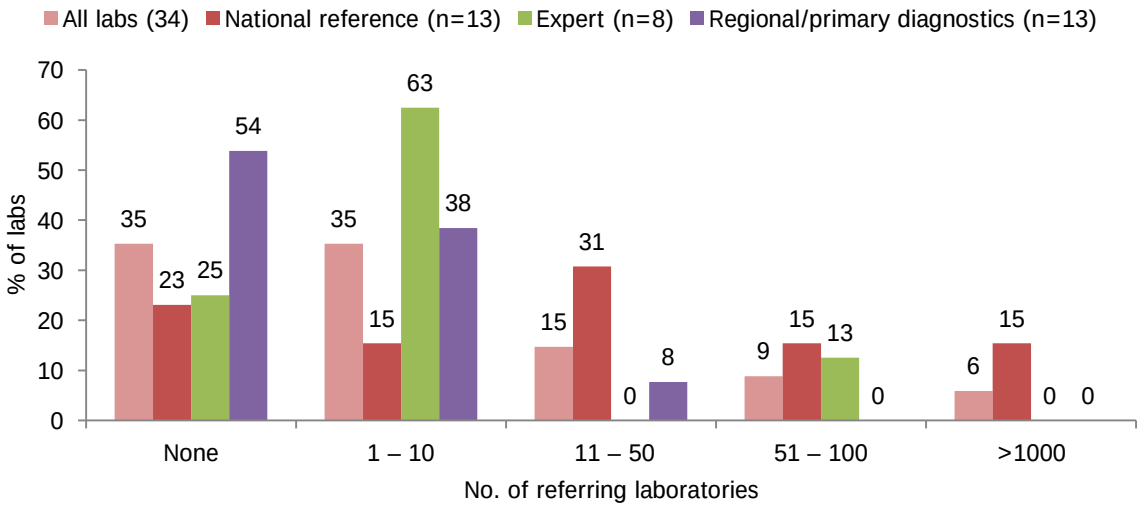
- Demonstration of *T. pallidum* in lesion exudates or tissues by dark-field microscopic examination is performed by 13 laboratories/12 countries.
- Demonstration of *T. pallidum* in lesion exudates or tissues by DFA test is performed by two laboratories/two countries.
- Demonstration of *Treponema* in lesion exudates or tissues by PCR is performed by 10 laboratories/9 countries.
- Of the 32 laboratories (19 countries) that perform a screening test (EIA, TPHA, TPPA) with the clinical serological specimens, 29 laboratories also use an RPR/VDRL test. Of the 29 laboratories that use a screening test and RPR, 19 laboratories additionally detect IgM treponemal antibodies, nine of which confirm with a second assay.
- One laboratory does not use a conventional screening test, but does use an RPR and IgM screening and confirmatory assay. This laboratory uses more specialised IgG tests instead of a screening test, such as EIA IgG, FTA-ABS IgG, Western blot IgG.

The relationship between these diagnostic tests and the EU 2009 case definition of syphilis is described in Section 2.11.

Table 12. Type of laboratory function that receives serology and lesion specimens

Type of laboratory function	No. of laboratories (n=33)	
	Clinical serology serum/plasma specimens received (n=32)	Syphilitic lesion (ulcer swabs) or tissue specimens received (n=16)
National reference	13	9
Expert	7	2
Regional/routine diagnostics	12	5

Figure 9. Number of laboratories referring specimens/isolates to the respondents' laboratory



Congenital syphilis laboratory diagnosis

A summary of the laboratory diagnosis for congenital syphilis follows:

- Demonstration of *T. pallidum* by dark field microscopy in the umbilical cord, the placenta, a nasal discharge or skin lesion material is performed by four laboratories/countries.
- Demonstration of *T. pallidum* by DFA-TP in the umbilical cord, the placenta, a nasal discharge or skin lesion material is performed by three laboratories/countries.
- Detection of *T. pallidum* - specific IgM (FTA-abs, EIA) AND a reactive non treponemal test (VDRL, RPR) in the child's serum is performed by 19 laboratories/16 countries (IgM and RPR).

In addition, there are five laboratories that use RPR, but no IgM test and two of those laboratories use a total antibody test instead of the IgM test (TPHA/TPPA/EIA).

Five laboratories use total antibody tests and three use PCRs.

There is one laboratory each that only performs western blot, or RPR or PCR for the diagnosis of congenital syphilis. (PCR laboratory only receives tissue/lesion specimens).

There are eight laboratories that receive clinical syphilis specimens yet do not perform any congenital syphilis diagnosis.

The relationship between these diagnostic tests and the EU 2009 case definition of congenital syphilis is described in Section 2.11.

Referral of syphilis specimens to another laboratory

Ten laboratories refer syphilis specimens to another laboratory, five of which are regional/primary diagnostic laboratories (Germany, Ireland (two labs), the Netherlands and one lab in Lithuania), four are expert laboratories (Ireland (one lab), Lithuania (two labs) and Sweden) and just one national reference laboratory, Malta. It was not known if the laboratory to which the isolates or specimens were referred was in the same country. The reasons for the referral of isolates to another laboratory are:

- additional testing = 6
- confirmation of positive results = 8
- confirmation of discrepant/atypical results = 9
- medico-legal = 2.

Syphilis molecular typing

The main typing system available for syphilis is based on the *tpr* and *arp* genes. This typing system has not been widely implemented and has mainly been used for research molecular epidemiological studies to date. For this reason, just two laboratories (Portugal and the UK (one lab)) perform syphilis molecular typing for:

- sexual network studies = 2
- longitudinal studies = 2
- contract tracing/medico-legal n = 2
- research = 1.

Summary

Serological treponemal and non-treponemal tests are widely used amongst the respondents and the most common tests are treponemal screening tests such as an EIA, TPPA or TPHA. It does not seem as though capacity building is required in syphilis diagnostics. However, not all laboratories perform analysis on syphilitic lesion specimens, which includes the detection of *T. pallidum* nucleic acid in chancres, even though this can aid in the diagnosis of early primary syphilis, before an antibody response is mounted. Capacity building in this area could be worth considering.

There seems to be wide variation in the methods used to diagnose congenital syphilis in the laboratory, and this may be due to the general difficulty in treponemal serology and also the very few cases of congenital syphilis in many countries, which may be due to low prevalence, or lack of reporting and/or diagnosis.

Molecular typing is only performed by two laboratories, so capacity building in this area may be required if an appropriately discriminatory molecular typing method for *T. pallidum* is developed and shown to have a real public health benefit.

2.6 Clinical reporting

This section describes the mean time and range in days from the laboratory receiving specimens to the production of a positive and a negative report leaving the laboratory. There is wide variation in the time taken for a positive result and a negative result to leave the laboratory (Table 13), with some laboratories taking more than seven days to issue a report. In the UK, it has been recommended that the overall turnaround time should be no more than seven working days[1]. It was unspecified in the survey which patient groups the specimens came from (e.g. ante-natal specimens).

Table 13. Time taken to produce clinical positive and negative reports

	Positive report (days)			Negative report (days)		
Clinical specimen	Mean time range	Overall mean	Range	Mean time range	Overall mean	Range
Gonorrhoea	0–7	3.2	0–10	1–7	2.7	0–10
Gonorrhoea (isolate)	2–5.8	3.2	1–10	1–5	2.5	1–7
Chlamydia (non-LGV)	1–10	3.6	0–16	0–7	2.9	0–54
LGV	0–16	5.5	0–21	0–7	3.1	0–14
Syphilis	0–7	2.8	0–34	0–5	1.9	0–7
Congenital syphilis	0–7	3.6	0–26	0–7	2.9	0–26

Summary

In terms of mean turnaround times, syphilis and then gonorrhoea positive/negative laboratory results are issued most quickly. Chlamydial non-LGV and congenital syphilis results take slightly longer to leave the laboratory and LGV results take the longest. This could be due to laboratories having to run extra tests to confirm LGV serovars or because laboratories have to send specimens for LGV testing elsewhere and this therefore increases the turnaround times.

It is of concern that the time taken for some laboratories to release results is well over seven days. In particular, the delay in releasing ante-natal or congenital syphilis results may have a clinical impact. Capacity building to improve turnaround times could therefore be useful. The production of European guidelines may help to achieve this aim.

2.7 Laboratory capacity

This section refers to laboratory capacity in terms of the number of tests performed in one year for each disease. The analysis was split into the three laboratory function types of national reference, expert and regional/routine primary diagnostics for each disease (Tables 1–3). Not all respondents supplied this information so the total number of laboratories displayed (Figures 9–14) may differ slightly than those described in Tables 1–3.

The majority of national reference and expert laboratories perform >1000 tests for gonorrhoea and chlamydia (Figures 9 and 10) every year, and the number of laboratory tests performed in the regional and primary diagnostic laboratories varies widely. The regional/primary diagnostic laboratories perform the highest number of dual chlamydia/gonorrhoea tests (Figure 12). The majority of laboratories perform 100 or less tests for LGV per year, with only one national reference and three expert laboratories performing >100 tests per year (Figure 13).

All the national reference laboratories and the majority of the expert and regional/primary diagnostic laboratories perform > 1000 syphilis tests per year (Figure 14). The number of tests performed for congenital syphilis is generally low, with most laboratories performing 100 or less tests per year (Figure 15). Only three national reference laboratories and one expert laboratory perform >500 tests per year for congenital syphilis (Czech Republic, one lab in the UK and two labs in Ireland).

1 Standards for the management of sexually transmitted infections (STIs). Medical Foundation for AIDS & Sexual Health (MEDFASH); 2010 (www.bashh.org/documents/2513)

Figure 10. **Number of *N. gonorrhoeae* laboratory tests performed each year**

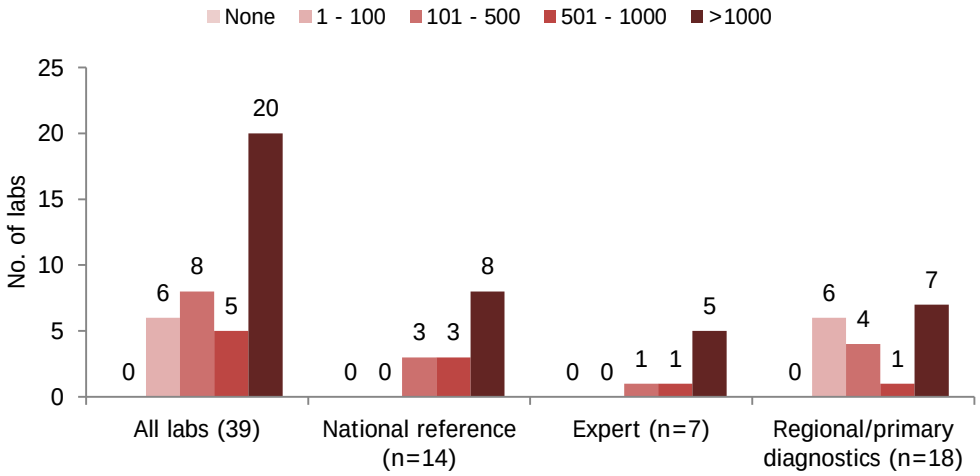


Figure 11. **Number of *C. trachomatis* laboratory tests performed each year**

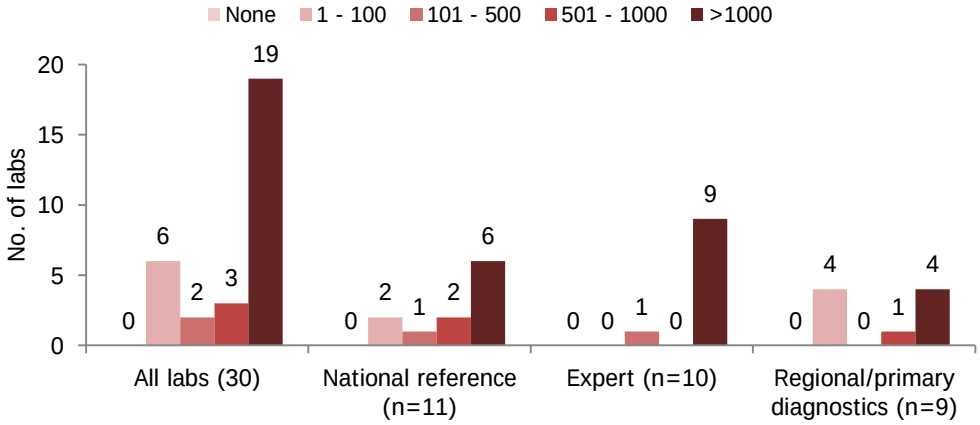


Figure 12. **Number of dual *C. trachomatis* and *N. gonorrhoeae* laboratory tests performed each year**

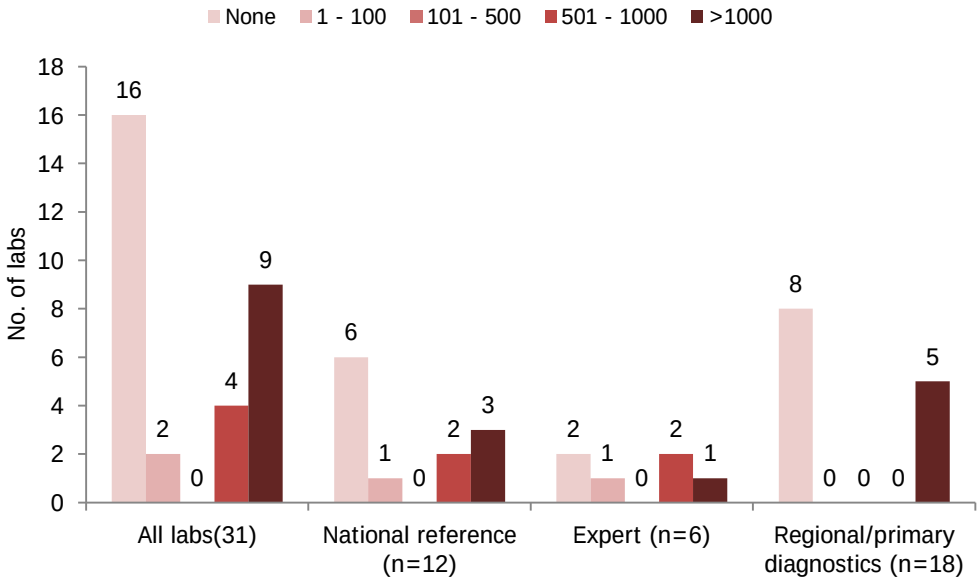


Figure 13. Number of LGV laboratory tests performed each year

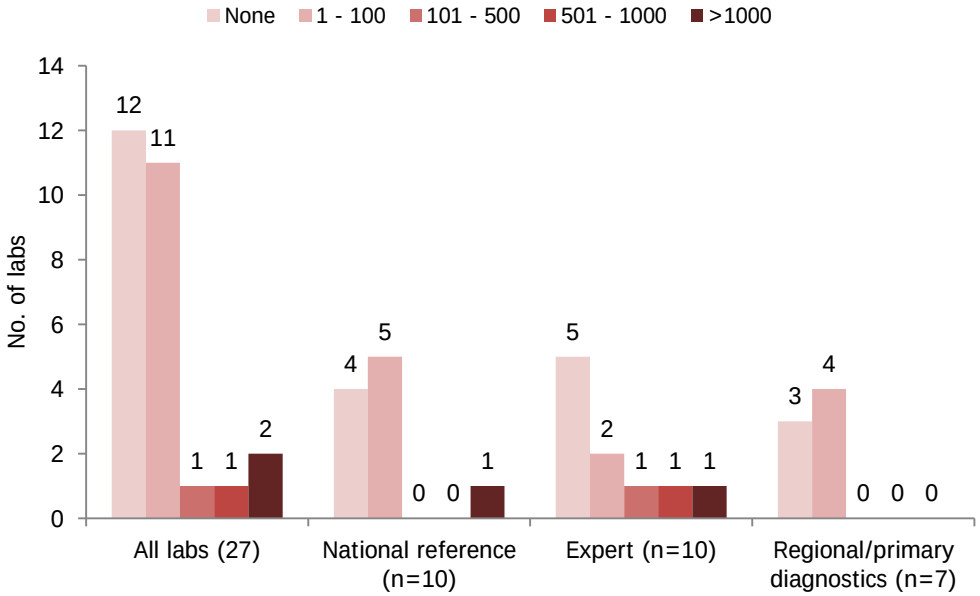


Figure 14. Number of syphilis laboratory tests performed each year

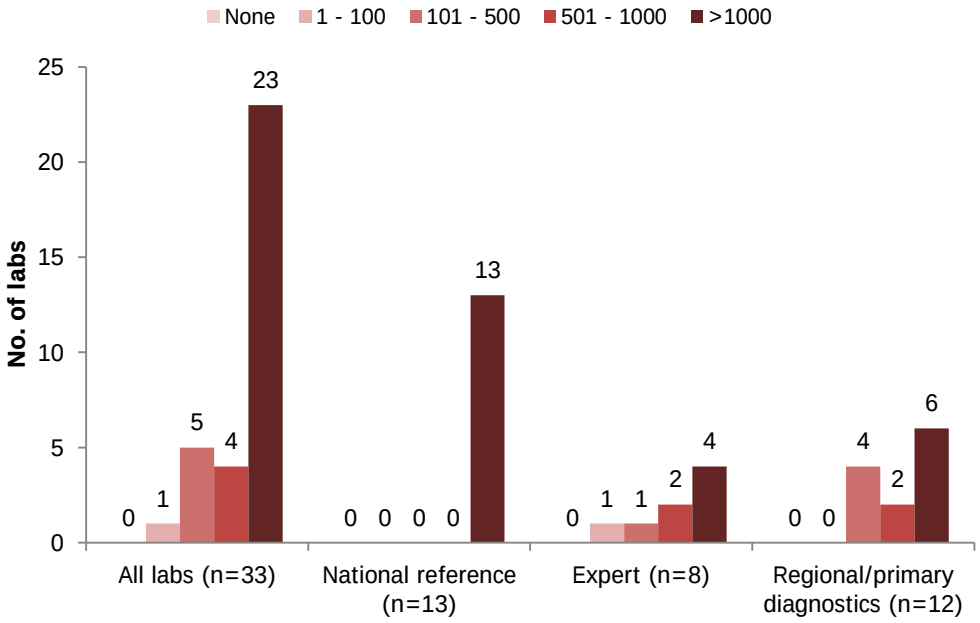
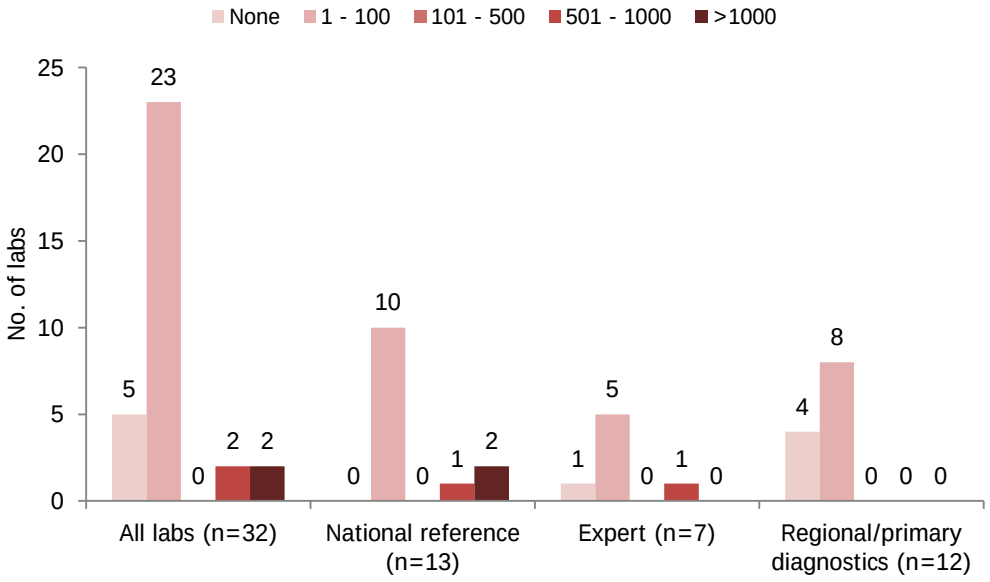


Figure 15. Number of congenital syphilis laboratory tests performed each year



Further analysis was performed on the number of positive chlamydia molecular tests performed in 2009 per patient group and gender (Table 14), as reported from 20 laboratories (13 countries). The largest number of tests was performed in females from the 20–24-year-old age group.

Table 14. Number of positive chlamydia molecular tests performed 2009, per patient group/gender

		No. of tests	Mean no. of tests	No. of labs
Age (years)	<15	0-37	4	16
	15-19	0-437	93	16
	20-24	0-876	196	16
	25-34	0-834	175	16
	35-44	0-127	31	16
	>45	0-39	12	16
	Unknown	0-4000	223	19
Gender	Male	0-810	186	20
	Female	0-1412	236	20
	Unknown	0-61	7	20

Summary

There is an obvious range in the number of tests performed for each disease and laboratory function. Without information on the area and the population covered by each laboratory, it is difficult to make inferences from this data. However the highest number of tests performed is for syphilis. Although the prevalence of syphilis is low, the high volume of testing could be due to screening programmes, such as ante-natal screening and blood donations. On the other hand, the number of tests performed for LGV and congenital syphilis are substantially lower, which is to be expected due to lower prevalence.

The breakdown of the number of positive chlamydia molecular tests performed in 2009 per patient group and gender is consistent with the epidemiological surveillance data, where the highest reported rates are in females in the 20–24-year-old age group (males and females).

2.8 Laboratory accreditation and external quality assessment

Thirty-five laboratories participate in EQA schemes (Table 15) and 27 of them are accredited. One laboratory stated that they are accredited but do not participate in an EQA scheme (Romania). Five laboratories are neither accredited or participate in an EQA scheme (Cyprus (one lab), Ireland (one lab), Greece (one lab), Latvia (one lab) and Lithuania (one lab)). A total of 12 laboratories are not accredited but participate in at least one EQA scheme (Table 15). It should be noted that it was not specified whether the accreditation was specifically for STI diagnostics and there may be some variation in how the respondents define accreditation. Three laboratories in two countries (Spain and the UK) stated that they would like the following EQA schemes that are not currently available:

- Chlamydia culture
- LGV molecular detection
- *T. pallidum* molecular detection.

Table 15. Participation in EQA schemes and accreditation

EQA scheme	No. of countries
GC NAATS	16
GC culture and identification	26
GC susceptibility testing	24
CT NAATs	23
CT culture	2
CT serology	6
Syphilis NAATs	2
Syphilis serology	25
Other	4
Accreditation	**Countries**
Laboratories not accredited	Cyprus* (both labs), Germany**, France*, Greece* (both labs), Hungary*, Ireland** (two labs), Italy*, Lithuania** (two labs), Latvia (one lab), Malta*, Portugal* and Spain*.

Note: *Country not accredited but participates in EQA; **one laboratory from that country participates in EQA.

Summary

EQA is an important feature of any diagnostic laboratory and 35 laboratories participate in EQA schemes. The one laboratory that stated it was not accredited and did not participate in an EQA actually participates in the Euro-GASP *N. gonorrhoeae* antimicrobial susceptibility testing EQA. This is an example of capacity building. Provision of the requested chlamydia culture, LGV and *T. pallidum* molecular detection EQAs could be considered at EU level. It may be cause for concern that some laboratories are not accredited and therefore accreditation and the subsequent quality assurance procedures associated with accreditation should be encouraged.

2.9 Training

Details of the requirements for training on STI laboratory techniques were requested. Tables 16 to 18 show a combined country response for the training requirements in relation to the different aspects of the bacterial STI diagnostics work. For *N. gonorrhoeae* laboratory techniques (Table 16) some laboratories identified training in culture and identification as a requirement, and there was more interest in susceptibility testing, molecular detection and typing training. For *C. trachomatis* laboratory techniques, molecular methods and typing in particular were identified as a training need but several countries also expressed an interest in culture. The most common training need for *T. pallidum* is molecular typing, however robust, discriminatory methods for *T. pallidum* typing are currently lacking.

Two laboratories stated that they had no laboratory experience with *N. gonorrhoeae* (Bulgaria and one laboratory in Lithuania). Ten laboratories (Bulgaria, Ireland (two labs), Latvia (two labs) Lithuania (three labs), Romania and Germany) stated on the surveys that they had no laboratory experience of chlamydia diagnostics, whereas eight laboratories (Bulgaria, Ireland (three labs), Lithuania (one lab), Slovakia (one lab), Spain and Germany) stated they had no syphilis diagnostics experience. This lack of experience relates to the function of the laboratory as not all laboratories perform all bacterial STI diagnostics (see Section 2.1).

Table 16. Required training in *N. gonorrhoeae* laboratory methods

Gonorrhoea				
Country (n=14)	**Culture and identification**	**Molecular detection**	**Susceptibility testing**	**Molecular typing**
Austria	√	√	√	
Belgium				√
Bulgaria		√		√
Germany	√	√	√	√
Estonia		√	√	
France	√	√	√	√
Greece		√		√
Hungary				
Ireland	√		√	√
Lithuania	√	√	√	√
Latvia	√	√	√	√
The Netherlands				√
Romania		√	√	√
Spain		√		
Total	**6**	**10**	**8**	**10**

Table 17. Required training in *C. trachomatis* laboratory methods

Chlamydia			
Country (n=15)	**Culture**	**Molecular detection**	**Molecular typing (inc. LGV)**
Austria	√	√	
Belgium			√
Bulgaria		√	
Estonia		√	
France		√	
Greece		√	√
Hungary	√		
Ireland		√	√
Lithuania	√	√	√
Latvia	√	√	√
Malta	√		√
Netherlands	√		
Romania	√	√	√
Slovenia	√	√	√
Spain		√	√
Total	**8**	**11**	**9**

Table 18. Required training in *T. pallidum* laboratory methods

Syphilis				
Country (n=16)	**Dark field microscopy**	**Serology**	**Molecular detection**	**Molecular typing**
Austria	√	√		
Belgium				√
Bulgaria			√	√
Cyprus		√		
Czech Republic				√
Germany	√	√	√	√
France	√	√		
Greece			√	√
Hungary				√
Ireland		√		√
Lithuania	√	√	√	√
Latvia	√		√	√
Malta	√		√	√
Netherlands	√			√
Romania			√	√
Sweden	√		√	√
Total	**8**	**6**	**8**	**13**

Summary

There appears to be a wide geographical distribution of training needs across Europe, with the biggest demand for molecular techniques. There seems to be consistency in training requirements for each of the three STIs, from eleven countries (Austria, Belgium, Bulgaria, France, Greece, Hungary, Ireland, Lithuania, Latvia, the Netherlands and Romania). Since 2010, ECDC has organised three STI laboratory training courses and with participants from all EU/EEA Member States except Austria, Belgium, Denmark, Finland and Iceland. In total 36 experts from 24 countries participated in the training courses.

2.10 Laboratory system

Information was requested on the public health laboratory system within the individual countries. This was to try to establish the plausibility of a future survey which could be sent to all laboratories within a country to establish each country's STI diagnostic capacity.

Fifteen laboratories (in 13 countries) were aware of how many public laboratories perform bacterial STI diagnostics in their country; the number of laboratories ranged from one (Estonia and Malta) to 4 200 (France).

Eleven laboratories (10 countries) were aware of how many private laboratories perform bacterial STI diagnostics in their country; the number of laboratories ranged from one (Ireland) to 3 800 (France), while Denmark stated that there are no private laboratories.

Twenty-six laboratories were aware of how many expert/reference laboratories perform bacterial STI diagnostics in their country; the number of laboratories ranged from 0–20. Additionally, it could be interesting to ascertain how many laboratories report to the bacterial STI surveillance system within each country. These laboratories could then be targeted for future surveys.

Seventeen laboratories (16 countries) were aware of how many laboratories report to the bacterial STI surveillance system in their country, and the numbers ranged from 0 (Portugal) to 355 (France). However, only ten countries would have been able to list these laboratories for future surveys.

Summary

The large amount of heterogeneity in the laboratory structure around Europe and with respect to public and private laboratories poses challenges to European public health, in particular with regard to achieving a comparable level of reporting across Europe.

2.11 Case definitions

Countries are encouraged to apply the 2009 EU case definitions for syphilis, congenital syphilis, gonorrhoea and chlamydia to improve harmonisation and comparability across EU/EEA. Confirmed cases for reporting purposes should be laboratory confirmed. The laboratory methods are clearly listed in the laboratory criteria section for each disease (Annex 1). It is recognised however that the case definitions currently used in a number of countries for STI may differ from the new EU case definitions so data is accepted even if it does not conform with the EU case definitions. This section compares the laboratory tests used for the laboratory diagnosis of the reportable bacterial STIs in relation to the current EU case definitions.

Thirty five laboratories are aware of the EU case definitions for bacterial STIs and congenital syphilis, but seven (Ireland (three labs), Lithuania (four labs) and Slovenia) are not, and Estonia did not respond to the question.

Laboratory criteria for EU 2009 case definition of gonorrhoea

Of the 36 laboratories that receive clinical gonorrhoea specimens:

- Isolation of *N. gonorrhoeae* from a clinical specimen is performed by 29 laboratories
- Detection of *N. gonorrhoeae* nucleic acid in a clinical specimen is performed by 22 laboratories
- Demonstration of *N. gonorrhoeae* by a non-amplified nucleic acid probe test in a clinical specimen is performed by four laboratories
- Microscopic detection of intracellular Gram negative diplococcic in an urethral male specimen is performed by 26 laboratories.

All laboratories perform at least one of the recommended laboratory tests in the EU case definitions to define a case of gonorrhoea.

Laboratory criteria for EU 2009 case definition of chlamydial infection non-LGV

Of the 33 laboratories that receive clinical chlamydia specimens:

- Isolation of *C. trachomatis* from a specimen of the ano-genital tract or from the conjunctiva is performed by 10 laboratories
- Demonstration of *C. trachomatis* by DFA test in a clinical specimen is performed by eight laboratories
- Detection of *C. trachomatis* nucleic acid in a clinical specimen is performed by 29 laboratories.

All but one of the laboratories performs at least one of the recommended laboratory tests to define a case of chlamydia.

NAATs are the most common methods used for chlamydia laboratory diagnosis.

One laboratory performed an EIA which is not a recommended laboratory test.

Laboratory criteria for EU 2009 case definition of chlamydial infection LGV

Of the 33 laboratories that receive clinical chlamydia specimens, 14 test for LGV:

- Isolation of *C. trachomatis* from a specimen of the ano-genital tract or from the conjunctiva AND Identification of serovar (genovar) L1, L2 or L3 is performed by five laboratories.
- Detection of *C. trachomatis* nucleic acid in a clinical specimen AND Identification of serovar (genovar) L1, L2 or L3 is performed by 12 laboratories.

All laboratories performing LGV diagnosis comply with the EU case definition laboratory criteria. Of the 15 laboratories, eight use a specific PCR for the detection of LGV genovar.

Laboratory criteria for EU 2009 case definition of syphilis

At least one of the following laboratory tests:

- Demonstration of *T. pallidum* in lesion exudates or tissues by dark-field microscopic examination is performed by 13 laboratories
- Demonstration of *T. pallidum* in lesion exudates or tissues by DFA test is performed by two laboratories
- Demonstration of *Treponema* in lesion exudates or tissues by PCR is performed by 10 laboratories
- Detection of *T. pallidum* antibodies by screening test (TPHA, TPPA or EIA) AND additionally detection of Tp-IgM antibodies (by IgM-ELISA, IgM immunoblot or 19S-IgM-FTA-abs) confirmed by a second IgM assay;
  - Of the 32 labs that perform a screening test (EIA, TPHA, TPPA) with the clinical serological specimens, 29 also use an RPR/VDRL test. This is not in line with the ECDC case definitions, but it demonstrates an argument for updating the case definitions.
  - Of the 29 labs that use a screening test and RPR, 19 additionally detect IgM treponemal antibodies, only 9 of which confirm with a second assay. This suggests that only nine labs conform correctly to the case definitions when detecting treponemal antibody.
  - One laboratory that does not use a screening test, but used an RPR and IgM screening and confirmatory assay, uses more specialised IgG tests in place of a screening test, such as EIA IgG, FTA-ABS IgG, Western blot IgG.

Laboratory criteria for EU 2009 case definition of congenital syphilis

At least one of the following three:

- Demonstration of *Treponema pallidum* by dark field microscopy in the umbilical cord, the placenta, a nasal discharge or skin lesion material – performed by four laboratories
- Demonstration of *Treponema pallidum* by DFA-TP in the umbilical cord, the placenta, a nasal discharge or skin lesion material – performed by three laboratories
- Detection of *Treponema pallidum* - specific IgM (FTA-abs, EIA) AND a reactive non treponemal test (VDRL, RPR) in the child's serum – performed by 19 laboratories (IgM and RPR).

In addition, there are five labs that use RPR, but no IgM test, as required by the EU laboratory criteria case definitions. Two of those laboratories use a total antibody test instead of the IgM test (TPHA/TPPA/EIA).

Five laboratories use total antibody tests and three use PCRs which are not listed in the case definitions.

There is one laboratory that performs only western blot, or RPR or PCR for the diagnosis of congenital syphilis respectively. (PCR lab only receives tissue/lesion specimens only).

There are eight labs that receive clinical syphilis specimens yet do not perform any congenital syphilis diagnosis.

There seems to be wide variation in the methods used to diagnose congenital syphilis in the laboratory and this may be due to a lack of uniform guidelines. Information on what would constitute a 'probable' case of syphilis was not determined in this survey.

Summary

From the responses submitted, there appears to be a good level of conformity between the laboratory tests performed and those listed in the EU case definitions for gonorrhoea, chlamydia and LGV. This is encouraging with respect to confidence in the surveillance data submitted to TESSy. With regard to syphilis serology however, there seems to be less concordance. This could demonstrate a need to update the case definitions to include one treponemal test and one non-treponemal test for a confirmed laboratory case using serum/plasma samples. The use of two different IgM tests, although scientifically justified, is technically too demanding and may not be required. The variation in the methods used to diagnose congenital syphilis in the laboratory, may be due to the general difficulty in treponemal serology.

3 Conclusions

This survey demonstrates the large amount of laboratory diversity across the EU/EEA. It is difficult to make any clear conclusions at country or European level as we do not have full representation of the EU/EEA. Moreover, the fact that different types of laboratories respond across Europe, and sometimes within each country, makes the analysis and the interpretation of the results difficult. Even though the survey was designed to be completed by national reference or expert laboratories only, the amount of responses received from regional and routine primary diagnostic laboratories suggests that a structure involving national reference laboratories does not exist in a number of countries.

The low number of national reference laboratories may reflect an area for capacity building in the individual countries, as having a national reference laboratory in each country provides a national centre of expertise and also ensures access to a national collection of specimens and cultures, thereby improving representativeness for surveillance programmes such as Euro-GASP. However the lack of a reference laboratory may be due to the public health laboratory structure in that country and the quality of laboratory data may be high, irrespective of the laboratories' national reference function. Countries where no information was received at all, or for just one or two STIs, should be approached to determine whether the lack of response was because the survey did not reach the most suitable recipient, or whether there was a real lack of STI diagnostics, giving rise to a real need for capacity building in that country.

Despite the difficulties in representativeness and the lack of validation of the data, certain conclusions can be drawn and subsequent recommendations made.

Isolation of *N. gonorrhoeae* from a clinical specimen is the most common test for defining a case of gonorrhoea, followed by microscopic detection of intracellular gram negative diplococcic in an urethral male specimen and then the detection of *N. gonorrhoeae* nucleic acid. All laboratories perform a gram strain, oxidase test and a biochemical or immunological test to identify *N. gonorrhoeae* cultures. Even though *N. gonorrhoeae* diagnostics seem appropriate, encouraging countries to culture, in particular for susceptibility testing, is a major area for potential capacity building. This will enable laboratories to participate in antimicrobial susceptibility surveillance programmes, which in turn will help with the management of individual patients and the improvement of treatment guidelines. The variety of susceptibility testing methodologies and breakpoints identified in the survey suggests that some harmonisation is desirable. The use of NAATs is by far the most popular test for chlamydia diagnostics. Speciality in culture may be lacking but this may not necessarily be a problem if NAATs are more sensitive. However, chlamydia culture work may become more necessary in the future if antibiotic resistance emerges and appropriate typing methods requiring large amounts of chlamydia DNA become available.

Serological treponemal and non-treponemal tests are widely used amongst the respondents, the most common tests being treponemal screening tests such as an EIA, TPPA or TPHA. However, not all laboratories perform analysis on syphilitic lesion specimens, which includes the detection of T. pallidum nucleic acid in chancres, even though this can aid in the diagnosis of early primary syphilis before an antibody response is mounted.

For all three diseases, molecular typing is performed by the national or expert laboratories and capacity building in this area may be required if molecular typing for bacterial STIs is shown to have a real public health benefit. An ECDC study has recently been performed to address this question for *N. gonorrhoea*[2]. The study showed a potential public health benefit in performing molecular surveillance of gonorrhoea.

Validation of molecular techniques when using non-genital specimens and guidelines on when to repeat nucleic acid testing may be required at a European level, however some guidelines from the UK[3,4] are available and these could be interpreted locally. Testing of pharyngeal and rectal samples should be encouraged in some laboratories and their associated clinics to ensure infections are not missed, particularly in MSM, in order to reduce morbidity and onward transmission.

Individual countries should consider issuing guidelines on turnaround times for the results from a public health diagnostic laboratory, as in some countries the turnaround times need to be improved.

The provision of the requested EQAs (chlamydia culture, LGV molecular detection and *T. pallidum* molecular detection) could be made available to an EQA provider and ECDC should forward this information. Training requirements seem to be focused on molecular methods.

At the laboratory diagnostic test level, there does not seem to be a major need for capacity building as nearly all laboratories, irrelevant of laboratory function type, perform suitable laboratory tests. This is further validated by the high level of concordance between the laboratory tests performed and those listed in the EU case definitions.

2 ECDC (2012). Molecular typing of *Neisseria gonorrhoeae*. Pilot study 2010-2011

3 Heath Protection Agency (2010). Detection of *Neisseria gonorrhoeae* using molecular methods. National Standard Method QSOP 62 Issue 1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp

4 Heath Protection Agency (2008). Commercial and in-house diagnostic tests: evaluations and validations. National Standard Method QSOP 23 Issue 4.1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp

This additionally gives some assurances as to the quality of the laboratory data that is eventually reported to TESSy. However, further consultation is required for the syphilis serology case definitions, where the lower concordance may be due to inappropriate laboratory techniques being specified rather than those actually performed. Additionally there seems to be wide variation in the methods used to diagnose congenital syphilis in the laboratory, and this may be due to the general difficulty in treponemal serology and also the very few cases of congenital syphilis in many countries. There may be a need for clearer guidelines for the diagnosis of congenital syphilis.

Possible future work to develop laboratory capacity for the diagnosis of STIs across Europe could include:

- Updating the syphilis serology EU laboratory case definitions.
- Use of *N. gonorrhoeae* culture to be encouraged for susceptibility testing, even if only for sentinel sites or sentinel populations.
- Collaboration with EUCAST to establish recommended testing methodologies, along with evidence-based EU standard clinical breakpoints to help standardise susceptibility testing for *N. gonorrhoeae* across Europe.
- Ensuring that the facility for LGV testing is available in all countries by producing appropriate laboratory guidelines.
- Development of clear guidance on the public health benefit of molecular typing.
- Production of guidance on the validation of non-genital specimens in nucleic acid tests.
- Production of guidance on when to perform retesting of specimens, particularly in low prevalence areas and on non-genital specimens.
- Encouraging clinics to obtain and laboratories to test rectal and pharyngeal specimens so infections are not missed.
- Developing training in molecular methods and susceptibility testing.

The interpretation of the data at country and EU/EEA level is challenging because the representativeness and completeness in this survey are unknown; a number of countries have not submitted the survey and we must acknowledge that EU-wide mapping of STI laboratory capability cannot be done on the basis of these results. The heterogeneity in EU/EEA with respect to public health and private laboratories and the massive number of laboratories across Europe make it impossible to draw major conclusions based on this survey. However, for certain individual countries the information obtained is useful for obtaining a better picture of the existing STI laboratory diagnostics in respective countries.

Annex 1. Diagnostic procedures per country

	Country (no. of responses if more than one)																								
GONORRHOEA	**Austria**	**Belgium**	**Bulgaria**	*** Cyprus (2)**	**Czech Republic**	**Denmark**	**Estonia**	**France**	**Germany**	*** Greece (2)**	**Hungary**	**** Ireland (10)**	**National lab only**	**Italy**	**** Latvia (2)**	**National lab only**	**** Lithuania (7)**	**National lab only**	**Malta**	**Portugal**	**Romania**	*** Slovakia (2)**	**Slovenia**	**Spain**	**Sweden**	**The Netherlands**	**† UK (2)**
National lab		√	√			√		√	√	√[1]		√[1]	√		√[1]	√	√[1]	√		√				√	√		√[2]
Regional lab	√			√[1]		√				√[1]	√	√[4]	√	√			√[1]				√	√[1]				√	
Expert lab	√					√				√[1]		√[2]	√	√			√[2]										
Routine diagnostics	√	√	√	√[1]		√	√	√	√	√[2]		√[10]	√		√[2]		√[7]		√	√		√[1]			√	√	
Clinical specimens	√	√	√	1/2		√	√	√	√	1/2	√	√	√		√	√	√	√	√	√		1/2	Survey from laboratory performing gonorrhoea diagnostics not received.		√	√	√
Culture	√	√	√			√		√	√	√	√	5/10		√	1/2	√	3/7		√	√	√			√	√		√
Gram		√	√	1/2		√	√	√	√	√√	√	9/10			√	√	3/7		√	√	√	1/2		√	√	√	√
Oxidase	√	√	√	1/2		√	√	√	√	√	√	9/10			√	√	3/7		√	√	√	1/2		√	√	√	√
Biochemical tests	√	√	√	1/2		√	√	√	√	√√	√	9/10			1/2	√	3/7		√	√	√	1/2		√	√	√	√
Immunological tests	√	√	√						√	√		5/10			1/2				√		√			√	√		√
Molecular tests/NAATs	√					√		√	√	1/2	√	2/10	√		1/2	√	2/7	√		√		1/2		√	√	√	√ √
Refer NG isolates to another laboratory								√		1/2		2/10			1/2												
Perform susceptibility testing	√	√		1/2		√	√	√	√	√	√	7/10		√	√	√	3/7		√	√	√	1/2		√	√	√	√

	Country (no. of responses if more than one)																								
GONORRHOEA	**Austria**	**Belgium**	**Bulgaria**	*** Cyprus (2)**	**Czech Republic**	**Denmark**	**Estonia**	**France**	**Germany**	*** Greece (2)**	**Hungary**	**** Ireland (10)**	**National lab only**	**Italy**	**** Latvia (2)**	**National lab only**	**** Lithuania (7)**	**National lab only**	**Malta**	**Portugal**	**Romania**	*** Slovakia (2)**	**Slovenia**	**Spain**	**Sweden**	**The Netherlands**	**† UK (2)**
Perform GC typing		√				√		√		1/2							2/7			√				√	√	√	√
Meets case definition	√	√	√	√		√	√	√	√	√	√	√	√		√	√	√	√	√	√	√	√			√	√	√
Case definitions based on laboratories diagnosing gonorrhoea																											

Key

$√^{n}$	Number of labs
√	Yes or always
√	Sometimes
√√	One lab answering 'Yes or always' and the other lab answering 'Sometimes'
n/n	e.g. 1/2 is the number of labs answering 'Yes or always' out of the total number of responses for that country
n/n	e.g. 1/2 is the number of labs answering 'Sometimes' out of the total number of responses for that country
*	National reference laboratories specialising in different bacterial STIs: Cyprus, Greece, Slovakia
**	One national reference centre, along with other different regions/laboratories: Ireland, Latvia, Lithuania
†	Two national reference laboratories within the same country: UK (England and Scotland)
	Note: case definitions only based on laboratories performing diagnostics for that disease

	Country (no. of responses if more than one)																								
CHLAMYDIA	**Austria**	**Belgium**	**Bulgaria**	*** Cyprus (2)**	**Czech Republic**	**Denmark**	**Estonia**	**France**	**Germany**	*** Greece (2)**	**Hungary**	**** Ireland (10)**	**National lab only**	**Italy**	**** Latvia (2)**	**National lab only**	**** Lithuania (7)**	**National lab only**	**Malta**	**Portugal**	**Romania**	*** Slovakia (2)**	**Slovenia**	**Spain**	**Sweden**	**The Netherlands**	**†UK (2)**
National lab			√	√[1]	√							√[1]	√		√[1]	√	√[1]	√	√	√			√				√[2]
Regional lab	√									√[1]	√	√[4]	√				√[1]		√			√[1]	√		√	√	
Expert lab	√	√				√				√[1]		√[1]	√				√[2]								√	√	
Routine diagnostics	√	√	√	√[1]	√	√	√	√		√[1]		√[6]	√		√[2]	√	√[6]	√	√	√		√[1]	√	√	√		
Clinical specimens	√	√	√	1/2	√	√	√	√		1/2	√	6/10	√		√	√	6/7	√	√	√		1/2	√	√	√	√	√
Culture																			√				√		√		1/2
DFA test			√		√	√					√				√	√	1/7	√					√	√			
Detection of nucleic acid		√		1/2	√	√	√	√		1/2	√	6/10	√		1/2	√	5/7	√	√	√		1/2	√		√	√	√
Identification of serovar (L1, L2 or L3)		√	√			√		√			√	2/10	√							√			√		√	√	√
Refer CT isolates to another laboratory								√	√			7/10	√				1/7										
Perform CT typing										1/2	√									√			√		√	√	1/2
Meets case definition CT	√	√	√	√	√	√	√	√		√	√	√	√		√	√	5/6	√	√	√		√	√	√	√	√	√
Meets case definition LGV		√	√			√		√			√	√	√							√			√		√		√
Case definitions based on laboratories diagnosing chlamydia or LGV																											

Key

√[n]	Number of labs
√	Yes or always
√	Sometimes
√√	One lab answering 'Yes or always' and the other lab answering 'Sometimes'
n/n	e.g. 1/2 is the number of labs answering 'Yes or always' out of the total number of responses for that country
n/n	e.g. 1/2 is the number of labs answering 'Sometimes' out of the total number of responses for that country
*	National reference laboratories specialising in different bacterial STIs: Cyprus, Greece, Slovakia
**	One national reference centre, along with other different regions/laboratories: Ireland, Latvia, Lithuania
†	Two national reference laboratories within the same country: UK (England and Scotland)
	Note: Case definitions only based on laboratories performing diagnostics for that disease

	Country (no. of responses if more than one)																								
SYPHILIS	**Austria**	**Belgium**	**Bulgaria**	*** Cyprus (2)**	**Czech Republic**	**Denmark**	**Estonia**	**France**	**Germany**	*** Greece (2)**	**Hungary**	**** Ireland (10)**	**National lab only**	**Italy**	**** Latvia (2)**	**National lab only**	**** Lithuania (7)**	**National lab only**	**Malta**	**Portugal**	**Romania**	*** Slovakia (2)**	**Slovenia**	**Spain**	**Sweden**	**The Netherlands**	**† UK (2)**
National lab				√[1]	√	√				√[1]	√	√[1]	√		√[1]	√	√[1]	√	√	√	√	√[1]					√[1]
Regional lab	√	√	√									√[4]	√		√[1]				√						√	√	√[2]
Expert Lab	√	√										√[2]	√				√[3]					√[1]		√	√		
Routine diagnostics	√		√	√[1]	√	√		√	√	√[1]		√[6]	√		√[2]		√[7]		√	√	√			√	√	√	
Clinical serology serum/plasma specimens	√		√	1/2	√	√		√	√	1/2	√	7/10	√		√	√	√	√	√	√	√	1/2		√	√	√	1/2
Syphilitic lesion or tissue specimens	√	√			√	√		√	√		√				1/2	√	2/7	√		√	√			√		√	√
Detection of *T.pallidum* antibodies	√	√	√	1/2	√	√		√	√	1/2	√		√		√	√	√	√	√	√	√			√	√	√	1/2
RPR	√	√	√	1/2	√	√		√	√	1/2	√	5/10	√		√	√	√	√	√	√	√	1/2		√	√	√	1/2
Detection of IgM antibodies	√	√		1/2	√	√		√		1/2	√	3/10	√		√	√	2/7	√		√	√	1/2		√	√		1/2
Dark field microscopy	√	√			√	√		√	√			1/10			1/2	√	2/7	√		√	√					√	
DFA test		√			√			√												√							
Molecular detection		√			√	√		√			√	1/10					1/7	√		√				√		√	√
Confirm case of congenital syphilis	√	√		1/2	√	√		√		1/2	√	5/10	√		√	√	2/7	√	√	√		1/2		√	√	√	
Refer syphilis specimens to another laboratory									√			4/10					3/7		√						√	√	
Perform syphilis typing																				√							1/2
Syphilis ECDC case definition needs updating, so compliance not defined at this time.																											

Key

√[n]	Number of labs
√	Yes or always
√	Sometimes
√√	One lab answering 'Yes or always' and the other lab answering 'Sometimes'
n/n	e.g. 1/2 is the number of labs answering 'Yes or always' out of the total number of responses for that country
n/n	e.g. 1/2 is the number of labs answering 'Sometimes' out of the total number of responses for that country
*	National reference laboratories specialising in different bacterial STIs: Cyprus, Greece, Slovakia
**	One national reference centre, along with other different regions/laboratories: Ireland, Latvia, Lithuania
†	Two national reference laboratories within the same country: UK (England and Scotland)
	Note: case definitions only based on laboratories performing diagnostics for that disease

	Country (no. of responses if more than one)																								
Accreditation and EQA	**Austria**	**Belgium**	**Bulgaria**	*** Cyprus (2)**	**Czech Republic**	**Denmark**	**Estonia**	**France**	**Germany**	*** Greece (2)**	**Hungary**	**** Ireland (10)**	**National lab only**	**Italy**	**** Latvia (2)**	**National lab only**	**** Lithuania (7)**	**National lab only**	**Malta**	**Portugal**	**Romania**	*** Slovakia (2)**	**Slovenia**	**Spain**	**Sweden**	**The Netherlands**	**† UK (2)**
Laboratory accredited or registered	√	√	√		√	√	√					8/10	√		1/2	√	5/7	√			√	√	√		√	√	√
Participates in EQA schemes	√	√	√	√	√	√	√	√	√	1/2	√	9/10	√	√	1/2	√	6/7	√	√	√	√	√	√	√	√	√	√

Key

$√^{n}$	Number of labs
√	Yes or always
√	Sometimes
√√	One lab answering 'Yes or always' and the other lab is answering 'Sometimes'
n/n	e.g. 1/2 is the number of labs answering Yes or always' out of the total number of responses for that country
n/n	e.g. 1/2 is the number of labs answering 'Sometimes' out of the total number of responses for that country
*	National reference laboratories specialising in different bacterial STIs: Cyprus, Greece, Slovakia
**	One national reference centre, along with other different regions/laboratories: Ireland, Latvia, Lithuania
†	Two national reference laboratories within the same country: UK (England and Scotland)
	Note: case definitions only based on laboratories performing diagnostics for that disease

Annex 2 – EU case definitions

Source: Commission Decision of 28/IV/2008 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council.

Chlamydial infection

(*Chlamydia trachomatis* including *lymphogranuloma venereum* (LGV))

Clinical criteria

Any person with at least one of the following clinical forms:

Chlamydial infection (non-LGV)

At least one of the following six:

- Urethritis
- Epididymitis
- Acute salpingitis
- Acute endometritis
- Cervicitis
- Proctitis

In new-born children at least one of the following two:

- Conjunctivitis
- Pneumonia

Lymphogranuloma venereum (LGV)

At least one of the following five:

- Urethritis
- Genital ulcer
- Inguinal lymphadenopathy
- Cervicitis
- Proctitis

Laboratory criteria

Chlamydial infection (non-LGV)

At least one of the following three:

Isolation of *Chlamydia trachomatis* from a specimen of the ano-genital tract or from the conjunctiva
Demonstration of *Chlamydia trachomatis* by DFA test in a clinical specimen
Detection of *Chlamydia trachomatis* nucleic acid in a clinical specimen

LGV

At least one of the following two:

- Isolation of *Chlamydia trachomatis* from a specimen of the ano-genital tract or from the conjunctiva
- Detection of *Chlamydia trachomatis* nucleic acid in a clinical specimen AND
- Identification of serovar (genovar) L1, L2 or L3

Epidemiological criteria

An epidemiological link by human to human transmission (sexual contact or vertical transmission).

Case classification

- Possible case: N/A
- Probable case: Any person meeting the clinical criteria and with an epidemiological link
- Confirmed case: Any person meeting the laboratory criteria.

Gonorrhoea

(*Neisseria gonorrhoeae*)

Clinical criteria

Any person with at least one of the following eight:

- Urethritis
- Acute salpingitis
- Pelvic inflammatory disease
- Cervicitis
- Epididymitis
- Proctitis
- Pharyngitis
- Arthritis

OR

Any new-born child with conjunctivitis.

Laboratory criteria

At least one of the following four:

- Isolation of *Neisseria gonorrhoeae* from a clinical specimen
- Detection of *Neisseria gonorrhoeae* nucleic acid in a clinical specimen
- Demonstration of *Neisseria gonorrhoeae* by a non-amplified nucleic acid probe test in a clinical specimen
- Microscopic detection of intracellular gram negative diplococci in an urethral male specimen.

Epidemiological criteria

An epidemiological link by human to human transmission (sexual contact or vertical transmission)

Case classification

- Possible case: N/A
- Probable case: Any person meeting the clinical criteria and with an epidemiological link
- Confirmed case: Any person meeting the laboratory criteria.

Syphilis

(*Treponema pallidum*)

Clinical criteria

Primary syphilis

Any person with one or several (usually painless) chancres in the genital, perineal, anal area or mouth or pharyngeal mucosa or elsewhere extragenitally

Secondary syphilis

Any person with at least one of the following three:

Early latent syphilis (< 1 year)

A history of symptoms compatible with those of the earlier stages of syphilis within the previous 12 months

Late latent syphilis (> 1 year)

Any person meeting laboratory criteria (specific serological tests)

Laboratory criteria

At least one of the following four laboratory tests:

- Demonstration of *Treponema pallidum* in lesion exudates or tissues by dark-field microscopic examination
- Demonstration of *Treponema pallidum* in lesion exudates or tissues by DFA test
- Demonstration of *Treponema* in lesion exudates or tissues by PCR
- Detection of *Treponema pallidum* antibodies by screening test (TPHA, TPPA or EIA)

AND

additionally detection of Tp-IgM antibodies (by IgM-ELISA, IgM immunoblot or 19S-IgM-FTA-abs) – confirmed by a second IgM assay

Epidemiological criteria

Primary/secondary syphilis: An epidemiological link by human to human (sexual contact)
Early latent syphilis (< 1 year): An epidemiological link by human to human (sexual contact) within the 12 previous months

Case classification

- Possible case: N/A
- Probable case: Any person meeting the clinical criteria and with an epidemiological link
- Confirmed case: Any person meeting the laboratory criteria for case confirmation

Syphilis, congenital and neonatal

(*Treponema pallidum*)

Clinical criteria

Any infant < 2 years of age with at least one of the following ten:

- Hepatospenomegaly
- Mucocutaneous lesions
- Condyloma lata
- Persistent rhinitis
- Jaundice
- Pseudoparalysis (due to periostitis and osteochondritis)
- Central nervous involvement
- Anaemia
- Nephrotic syndrome
- Malnutrition.

Laboratory criteria

Laboratory criteria for case confirmation

At least one of the following three:

- Demonstration of *Treponema pallidum* by dark field microscopy in the umbilical cord, the placenta, a nasal discharge or skin lesion material
- Demonstration of *Treponema pallidum* by DFA-TP in the umbilical cord, the placenta, a nasal discharge or skin lesion material
- Detection of *Treponema pallidum* - specific IgM (FTA-abs, EIA) AND
- a reactive non-treponemal test (VDRL, RPR) in the child's serum.

Laboratory criteria for a probable case

At least one of the following three:

- Reactive VDRL-CSF test result
- Reactive non-treponemal and treponemal serologic tests in the mother's serum
- Infant's non-treponemal antibody titre is fourfold or greater than the antibody titre in the mother's serum.

Epidemiological criteria

Any infant with an epidemiological link by human to human transmission (vertical transmission)

Case classification

- Possible case: N/A
- Probable case: Any infant or child meeting the clinical criteria and with an epidemiological link and/or meeting the laboratory criteria for a probable case.
- Confirmed case: Any infant meeting the laboratory criteria for case confirmation.

Annex 3 - STI laboratory diagnostics survey for expert and specialist laboratories

CONTACT INFORMATION

Name:
Address:

Country:
Email address:
Telephone number:
Date survey completed:

LABORATORY FUNCTION AND STAFF

1. Please indicate the type of expert/specialist function your laboratory performs for gonorrhoea, chlamydia and syphilis

1.1 National (Receives isolates or specimens from the whole country/acts as a reference centre)

1.1.1 Gonorrhoea: Yes ☐ No ☐

1.1.2 Chlamydia: Yes ☐ No ☐

1.1.3 Syphilis: Yes ☐ No ☐

1.2 Regional (Receives isolates or specimens from an area within the country)

1.2.1 Gonorrhoea: Yes ☐ No ☐ If yes, please specify area/s:

1.2.2 Chlamydia: Yes ☐ No ☐ If yes, please specify area/s:

1.2.3 Syphilis: Yes ☐ No ☐ If yes, please specify area/s:

1.3 Expert (is not designated as a reference centre but receives isolates or specimens from other laboratories)

1.3.1 Gonorrhoea: Yes ☐ No ☐ If yes please answer 1.3.4 and 1.3.5

1.3.2 Chlamydia: Yes ☐ No ☐ If yes please answer 1.3.4 and 1.3.5

1.3.3 Syphilis: Yes ☐ No ☐ If yes please answer 1.3.4 and 1.3.5

1.3.4 Receive isolates or specimens from the whole country

1.3.4.A Gonorrhoea: Yes ☐ No ☐

1.3.4.B Chlamydia: Yes ☐ No ☐

1.3.4.C Syphilis: Yes ☐ No ☐

1.3.5 Receive isolates or specimens from a region

1.3.5.A Gonorrhoea: Yes ☐ No ☐ If yes, please specify region/s:

1.3.5.A Chlamydia: Yes ☐ No ☐ If yes, please specify region/s:

1.3.5.A Syphilis: Yes ☐ No ☐ If yes, please specify region/s:

1.4 Laboratory performs routine diagnostics for STIs and does not have an expert/specialist function

1.4.1 Gonorrhoea: Yes ☐ No ☐

1.4.2 Chlamydia: Yes ☐ No ☐

1.4.3 Syphilis: Yes ☐ No ☐

1.5 Laboratory also performs routine diagnostics for STIs, as well as serving as a national, regional or expert laboratory

1.5.1 Gonorrhoea: Yes ☐ No ☐

1.5.2 Chlamydia: Yes ☐ No ☐

1.5.3 Syphilis: Yes ☐ No ☐

2. Please give information regarding the staffing structure in your laboratory (staff employed to work either full time or part time on bacterial STIs; gonorrhoea, chlamydia and syphilis)

2.1 Medical staff

2.1.1 Number employed:

2.1.2 Ideal number required to fulfil laboratory service:

2.2 Technical staff

2.2.1 Number employed:

2.2.2 Ideal number required to fulfil laboratory service:

2.3 Research staff

2.3.1 Number employed:

2.3.2 Ideal number required to fulfil laboratory service:

2.4 Administrative

2.4.1 Number employed:

2.4.2 Ideal number required to fulfil laboratory service:

GONORRHOEA

3. Please give information on the laboratory diagnosis of *N. gonorrhoeae*

3.1 Clinical specimens received Yes ☐ No ☐

If yes, approximately how many per year: 10 – 1000 ☐ 1001 – 10,000 ☐ >10,000 ☐

3.2 Cultures of *N. gonorrhoeae* received Yes ☐ No ☐

If yes, approximately how many per year: 10 – 100 ☐ 101 – 500 ☐ 501 – 1000 ☐ >1000 ☐

3.3 Approximately how many laboratories refer specimens or isolates to your laboratory

None ☐ 1 – 10 ☐ 11 – 50 ☐ 51 – 100 ☐ >100 ☐

3.4 Please indicate which laboratory methods are used to confirm a case of gonorrhoea

3.4.1 Isolation and confirmation of *Neisseria gonorrhoeae* from a clinical specimen Yes ☐ No ☐

3.4.2 Identification of culture is performed by

3.4.2.A Gram: Always ☐ Sometimes ☐ Never ☐

3.4.2.B Oxidase: Always ☐ Sometimes ☐ Never ☐

3.4.2.C Biochemical tests: Always ☐ Sometimes ☐ Never ☐

3.4.2.D Immunological tests: Always ☐ Sometimes ☐ Never ☐

3.4.2.E Molecular tests: Always ☐ Sometimes ☐ Never ☐

3.4.3 Detection of *Neisseria gonorrhoeae* nucleic acid in a clinical specimen Yes ☐ No ☐

3.4.3.A Do you test genital samples Yes ☐ No ☐

3.4.3.A.i Molecular platform used:

3.4.3.A.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

3.4.3.A.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

3.4.3.B Do you test rectal samples Yes ☐ No ☐

3.4.3.B.i Molecular platform used:

3.4.3.B.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

3.4.3.B.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

3.4.3.C Do you test pharyngeal samples Yes ☐ No ☐

3.4.3.C.i Molecular platform used:

3.4.3.C.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

3.4.3.C.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

3.4.4 Demonstration of *Neisseria gonorrhoeae* by a non-amplified nucleic acid probe test in a clinical specimen Yes ☐ No ☐

3.4.5 Microscopic detection of intracellular gram negative diplococci in an urethral male specimen Yes ☐ No ☐

3.4.6 Other laboratory methods used to confirm a case of gonorrhoea

Yes ☐ No ☐ If yes, please specify:

4. Do you refer *N. gonorrhoeae* strains to another laboratory Yes ☐ No ☐ If "no" proceed to question 5

4.1 Reasons for sending strains

4.1.1 Identification Yes ☐ No ☐

4.1.2 Susceptibility testing Yes ☐ No ☐

4.1.3 Molecular typing Yes ☐ No ☐

4.1.4 Medico-legal samples Yes ☐ No ☐

4.1.5 Strains have been requested for other studies Yes ☐ No ☐

4.2 **Please list other specialist services you require for *N. gonorrhoeae***

5. Do you perform *N. gonorrhoeae* susceptibility testing Yes ☐ No ☐ If "no" proceed to question 6

5.1 Methods used for susceptibility testing

5.1.1 Disc Yes ☐ No ☐

5.1.2 Etest Yes ☐ No ☐

5.1.3 Agar dilution Yes ☐ No ☐

5.1.4 Other Yes ☐ No ☐ If yes, please specify:

5.2 Recommended method used

5.2.1 CLSI Yes ☐ No ☐

5.2.2 EUCAST Yes ☐ No ☐

5.2.3 BSAC Yes ☐ No ☐

5.2.4 WHO Yes ☐ No ☐

5.2.5 Other Yes ☐ No ☐ If yes, please specify:

5.3 Do you participate in any *N. gonorrhoeae* susceptibility surveillance programme

5.3.1 National surveillance Yes ☐ No ☐ If yes, please specify:

5.3.2 Regional surveillance Yes ☐ No ☐ If yes, please specify:

5.4 Do you organise a *N. gonorrhoeae* susceptibility surveillance programme

Yes ☐ No ☐ If yes, please specify:

6. Do you perform typing of gonococcal isolates Yes ☐ No ☐ If "no" proceed to question 7

6.1 Auxotyping Always ☐ Sometimes ☐ Never ☐

6.2 Serotyping Always ☐ Sometimes ☐ Never ☐

6.3 Molecular typing

6.3.1 NG-MAST Always ☐ Sometimes ☐ Never ☐

6.3.2 Other Always ☐ Sometimes ☐ Never ☐ If yes, please specify:

6.4 Why is molecular typing performed

6.4.1 Antimicrobial resistance studies Yes ☐ No ☐

6.4.2 Sexual network studies Yes ☐ No ☐

6.4.3 Temporal studies Yes ☐ No ☐

6.4.4 Contact tracing / medico-legal Yes ☐ No ☐

6.4.5 Other (please specify) Yes ☐ No ☐ If yes, please specify:

CHLAMYDIA

7. Please give information on the laboratory diagnosis of *Chlamydia trachomatis*

7.1 Clinical specimens received Yes ☐ No ☐

7.2 *Chlamydia trachomatis* cultures received Yes ☐ No ☐

7.3 Approximately how many laboratories refer specimens / isolates to your laboratory

None ☐ 1 – 10 ☐ 11 – 50 ☐ 51 – 100 ☐ >100 ☐

7.4 Please indicate which laboratory methods are used to confirm a case of chlamydial infection (all serovars, including L serovars)

7.4.1 Isolation of *Chlamydia trachomatis* from a specimen of the ano-genital tract or from the conjunctiva Yes ☐ No ☐

7.4.2 Demonstration of *Chlamydia trachomatis* by DFA test in a clinical specimen Yes ☐ No ☐

7.4.3 Detection of *Chlamydia trachomatis* nucleic acid in a clinical specimen Yes ☐ No ☐

7.4.3.A Do you test genital samples Yes ☐ No ☐

7.4.3.A.i Molecular platform used:

7.4.3.A.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

7.4.3.A.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

7.4.3.B Do you test rectal samples Yes ☐ No ☐

7.4.3.B.i Molecular platform used:

7.4.3.B.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

7.4.3.B.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

7.4.3.C Do you test pharyngeal samples Yes ☐ No ☐

7.4.3.C.i Molecular platform used:

7.4.3.C.ii Do you repeat positive results with the same molecular target Yes ☐ No ☐

7.4.3.C.iii Do you confirm positive results with a different molecular target Yes ☐ No ☐

7.4.4 Other laboratory methods used to confirm a case of chlamydial infection

Yes ☐ No ☐ If yes, please specify:

7.5 Please indicate which laboratory methods are used to confirm a case of lymphogranuloma venereum (LGV) caused by

L serovars (L1, L2 or L3)

7.5.1 Isolation of *Chlamydia trachomatis,* followed by identification of serovar (genovar) L1, L2 or L3

Yes ☐ No ☐

7.5.2 Molecular detection of *Chlamydia trachomatis* nucleic acid in a clinical specimen followed by identification of serovar (genovar) L1, L2 or L3 Yes ☐ No ☐

7.5.3 Molecular detection of serovar (genovar) L1, L2 or L3 using a specific PCR for L serovars

Yes ☐ No ☐

7.5.4 Please describe the method used to identify serovars L1, L2 or L3:

7.5.5 Other laboratory methods used to confirm a case of LGV

Yes ☐ No ☐ If yes, please specify:

7.6 Do you test genital samples for LGV Yes ☐ No ☐

7.7 Do you test rectal samples for LGV Yes ☐ No ☐

7.8 Do you test pharyngeal samples for LGV Yes ☐ No ☐

7.9 Do you test asymptomatic patients for LGV Yes ☐ No ☐

7.10 Do you test only symptomatic patients for LGV Yes ☐ No ☐

8. Do you refer *Chlamydia trachomatis* specimens to a another laboratory Yes ☐ No ☐ If "no" proceed to question 9

8.1 Reasons for sending specimens

8.1.1 Identification Yes ☐ No ☐

8.1.2 Molecular typing Yes ☐ No ☐

8.1.3 Medico-legal samples Yes ☐ No ☐

8.1.4 Specimens have been requested by the reference laboratories for other studies Yes ☐ No ☐

8.1.5 LGV genotyping Yes ☐ No ☐

8.2 **Please list other specialist services you require for *Chlamydia trachomatis***

9. Do you perform *Chlamydia trachomatis* typing Yes ☐ No ☐ If "no" proceed to question 10

9.1 Serotyping/genotyping (omp1) Yes ☐ No ☐

9.2 Molecular typing Yes ☐ No ☐

9.2.1 MLST yes/no Yes ☐ No ☐

9.2.2 VNTR yes/no Yes ☐ No ☐

9.2.3 Other Yes ☐ No ☐ If yes, please specify:

9.3 Why is molecular typing performed

9.3.1 Sexual network studies Yes ☐ No ☐

9.3.2 Temporal studies Yes ☐ No ☐

9.3.3 Contact tracing/medico-legal Yes ☐ No ☐

9.3.4 Other Yes ☐ No ☐ If yes, please specify:

DUAL GONORRHOEA & CHLAMYDIA TESTING

10. Do you perform dual molecular testing for gonorrhoea and chlamydia Yes ☐ No ☐ If "no" proceed to question 11

10.1 Do you test genital samples Yes ☐ No ☐

10.1.1. Molecular platform used:

10.1.2. Do you repeat positive results with the same molecular target Yes ☐ No ☐

10.1.3. Do you confirm positive results with a different molecular target Yes ☐ No ☐

10.2. Do you test rectal samples Yes ☐ No ☐

10.2.1. Molecular platform used:

10.2.2. Do you repeat positive results with the same molecular target Yes ☐ No ☐

10.2.3. Do you confirm positive results with a different molecular target Yes ☐ No ☐

10.3. Do you test pharyngeal samples Yes ☐ No ☐

10.3.1. Molecular platform used :

10.3.2. Do you repeat positive results with the same molecular target Yes ☐ No ☐

10.3.3. Do you confirm positive results with a different molecular target Yes ☐ No ☐

SYPHILIS

11. Please give information on the laboratory diagnosis of syphilis

11.1 Clinical serology serum/plasma specimens received Yes ☐ No ☐

11.2 Syphilitic lesion (ulcer swabs) or tissue specimens received Yes ☐ No ☐

11.3 Approximately how many laboratories refer specimens/isolates to your laboratory

None ☐ 1 – 10 ☐ 11 – 50 ☐ 51 – 100 ☐ >100 ☐

11.4 Please indicate which laboratory methods are used to confirm a case of syphilis

11.4.1 Detection of *T. pallidum* antibodies by screening test (TPHA, TPPA or EIA) (if yes, please specify which test) Yes ☐ No ☐ If yes, please specify:

AND additionally

11.4.1.A RPR (VDRL) Yes ☐ No ☐

11.4.1.B Detection of IgM antibodies to *Treponema pallidum*

Yes ☐ No ☐ If yes, please specify:

11.4.1.B.i Do you confirm IgM positive results by a second IgM assay?

Yes ☐ No ☐ If yes, please specify:

11.4.2 Demonstration of *T. pallidum* in lesion exudates or tissues by dark-field microscopic examination to indicate infectious syphilis Yes ☐ No ☐

11.4.3 Demonstration of *T. pallidum* in lesion exudates or tissues by DFA test to indicate infectious syphilis Yes ☐ No ☐

11.4.4 Molecular detection of *T. pallidum* in lesion exudates or tissues to indicate infectious syphilis

Yes ☐ No ☐

11.4.5 Other laboratory methods used to confirm a case of syphilis

Yes ☐ No ☐ If yes, please specify:

11.5 Please indicate which laboratory methods are used to confirm a case of congenital syphilis

11.5.1 Demonstration of *T. pallidum* by dark field microscopy in the umbilical cord, the placenta, a nasal discharge or skin lesion material Yes ☐ No ☐

11.5.2 Demonstration of *T. pallidum* by DFA-TP in the umbilical cord, the placenta, a nasal discharge or skin lesion material Yes ☐ No ☐

11.5.3 Detection of *T. pallidum* - specific IgM Yes ☐ No ☐ If yes, please specify:

11.5.4 Reactive non treponemal test (VDRL, RPR) in the baby/child's serum Yes ☐ No ☐

11.5.5 Other laboratory methods used to confirm a case of congenital syphilis

Yes ☐ No ☐ If yes, please specify:

12. Do you refer syphilis (serology and/or lesion/tissue) specimens to another laboratory Yes ☐ No ☐ If "no" proceed to question 13

12.1 Reasons for sending samples:

12.1.1 Additional testing Yes ☐ No ☐

12.1.2 Confirmatory testing of positive results Yes ☐ No ☐

12.1.3 Confirmatory testing of atypical or discrepant serological profile Yes ☐ No ☐

12.1.4 Medico legal samples Yes ☐ No ☐

12.1.5 Specimens that have been requested by the reference laboratories for other studies

Yes ☐ No ☐

12.2 Please list other specialist services you require for syphilis:

13. Do you perform syphilis molecular typing Yes ☐ No ☐ If "no" proceed to question 14

13.1 Why is molecular typing performed

13.1.1 Sexual network studies Yes ☐ No ☐

13.1.2 Temporal studies Yes ☐ No ☐

13.1.3 Contact tracing/medico-legal Yes ☐ No ☐

13.1.4 Other Yes ☐ No ☐ If yes, please specify:

CLINICAL REPORTING

14. Please indicate the MEAN TIME and/or RANGE from receiving the specimen to a POSITIVE report leaving the laboratory (in days)

14.1 Gonorrhoea clinical specimen	**14.4 LGV clinical specimen**
14.1.a Mean time (days):	**14.4.a Mean time (days):**
14.1.b Range (days):	**14.4.b Range(days):**
14.2 Gonorrhoea isolate	**14.5 Syphilis clinical specimen**
14.2.a Mean time (days):	**14.5.a Mean time (days):**
14.2.b Range (days):	**14.5.b Range (days):**
14.3 Chlamydia non-LGV clinical specimen	**14.6 Congenital syphilis clinical specimen**
14.3.a Mean time (days):	**14.6.a Mean time (days):**
14.3.b Range (days):	**14.6.b Range (days):**

15. Please indicate the MEAN TIME and/or RANGE from receiving the specimen to a NEGATIVE report leaving the laboratory (in days)

15.1 Gonorrhoea clinical specimen	**15.4 LGV clinical specimen**
15.1.a Mean time (days):	**15.4.a Mean time (days):**
15.1.b Range (days):	**15.4.b Range(days):**
15.2 Gonorrhoea isolate	**15.5 Syphilis clinical specimen**
15.2.a Mean time (days):	**15.5.a Mean time (days):**
15.2.b Range (days):	**15.5.b Range (days):**
15.3 Chlamydia non-LGV clinical specimen	**15.6 Congenital syphilis clinical specimen**
15.3.a Mean time (days):	**15.6.a Mean time (days):**
15.3.b Range (days):	**15.6.b Range (days):**

LABORATORY CAPACITY

16. Please indicate the number of laboratory tests performed in one year (any 12 month period; please use most recent available data). If possible, please consider multiple tests on one specimen, *i.e.* an IgM and RPR test on one syphilis specimen are two laboratory tests.

16.1 *N. gonorrhoeae*	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐
16.2 *C. trachomatis*	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐
16.3 Dual GC/CT	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐
16.4 LGV	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐
16.5 Syphilis	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐
16.6 Congenital syphilis	None ☐	<10 ☐	11 – 100 ☐	101 – 500 ☐	501 – 1000 ☐	>1000 ☐

17. For *C. trachomatis* testing only please give more details on the number of positive molecular tests performed in 2009 for each patient age group and patient gender group.

17.1 Age in years (please enter no. of CT positive molecular tests performed each year)	**17.2 Gender (please enter no. of CT positive molecular tests performed each year)**
17.1.a <15	**17.2.a Male**
17.1.b 15-19	**17.2.b Female**
17.1.c 20-24	**17.2.c Unknown**
17.1.c 25-34	
17.1.c 35-44	
17.1.c >45	
17.1.c Unknown	

LABORATORY ACCREDITATION AND EXTERNAL QUALITY ASSURANCE (EQA)

18. Is your laboratory accredited/registered Yes ☐ No ☐ If "no" proceed to question 20

18.1 Name of accrediting body:
18.2 Date of accreditation:
18.3 Length that the accreditation is valid for:

19. Does your laboratory participate in an EQA scheme Yes ☐ No ☐ if "no" proceed to question 20

19.1 Please indicate which EQA schemes your laboratory participates in:

19.1.1 *N. gonorrhoeae* detection (GC-NAATs) Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.2 *N. gonorrhoeae* culture and identification Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.3 *N. gonorrhoeae* susceptibility testing Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.4 *C. trachomatis* detection (NAATS) Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.5 *C. trachomatis* culture Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.6 *C. trachomatis* serology Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.7 Syphilis molecular detection Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.8 Syphilis serology Yes ☐ No ☐
If yes, please specify the EQA scheme provider:

19.1.9 Other Yes ☐ No ☐ If yes, please specify EQA scheme and provider:

19.2 Please list any other EQA schemes that are not currently available that are required:

TRAINING

20. Please indicate if training is required in your laboratory, particularly for full competence to fulfil the EU laboratory criteria (28/IV/2008*) and to participate in the ECDC *N. gonorrhoeae* AMR surveillance programme

* http://ec.europa.eu/health/ph_threats/com/docs/1589_2008_en.pdf

20.1 *N. gonorrhoeae*

20.1.1 *N. gonorrhoeae* culture & identification Yes ☐ No ☐

20.1.2 *N. gonorrhoeae* molecular detection Yes ☐ No ☐

20.1.3 *N. gonorrhoeae* susceptibility testing Yes ☐ No ☐

20.1.4 *N. gonorrhoeae* molecular typing Yes ☐ No ☐

20.1.5 Does your laboratory have any experience in any of the above? Yes ☐ No ☐

20.2 Chlamydia

20.2.1 *C. trachomatis* culture Yes ☐ No ☐

20.2.2 *C. trachomatis* molecular detection Yes ☐ No ☐

20.2.3 *C. trachomatis* molecular typing (inc. LGV) Yes ☐ No ☐

20.2.4 Does your laboratory have any experience in any of the above? Yes ☐ No ☐

20.3 Syphilis

20.3.1 Dark field microscopy Yes ☐ No ☐

20.3.2 Syphilis serology Yes ☐ No ☐

20.3.3 Syphilis molecular detection Yes ☐ No ☐

20.3.4 Syphilis molecular typing Yes ☐ No ☐

20.3.5 Does your laboratory have any experience in any of the above? Yes ☐ No ☐

REPORTING AND LABORATORY SYSTEM

21. Please provide information on the provision of data to surveillance systems

21.1 Do you send confirmed results to a surveillance centre:

21.1.1 Gonorrhoea: Yes ☐ No ☐ If yes, please specify:

21.1.2 Chlamydia: Yes ☐ No ☐ If yes, please specify:

21.1.3 Syphilis: Yes ☐ No ☐ If yes, please specify:

21.2 Is the reporting mandatory or voluntary:

21.2.1 Gonorrhoea: Mandatory ☐ Voluntary ☐

21.2.2 Chlamydia: Mandatory ☐ Voluntary ☐

21.2.3 Syphilis: Mandatory ☐ Voluntary ☐

21.3 For gonorrhoea reporting, how often is the data transferred:

21.3.1 Daily: Yes ☐ No ☐

21.3.2 Weekly: Yes ☐ No ☐

21.3.3 Monthly: Yes ☐ No ☐

21.3.4 Quarterly: Yes ☐ No ☐

21.3.5 Annually: Yes ☐ No ☐

21.4 For chlamydia reporting, how often is the data transferred:

21.4.1 Daily: Yes ☐ No ☐

21.4.2 Weekly: Yes ☐ No ☐

21.4.3 Monthly: Yes ☐ No ☐

21.4.4 Quarterly: Yes ☐ No ☐

21.4.5 Annually: Yes ☐ No ☐

21.5 For syphilis reporting, how often is the data transferred:

21.5.1 Daily: Yes ☐ No ☐

21.5.2 Weekly: Yes ☐ No ☐

21.5.3 Monthly: Yes ☐ No ☐

21.5.4 Quarterly: Yes ☐ No ☐

21.5.5 Annually: Yes ☐ No ☐

21.6 Is your laboratory aware of the EU case definitions* for bacterial STIs and congenital syphilis Yes ☐ No ☐

* http://ec.europa.eu/health/ph_threats/com/docs/1589_2008_en.pdf

22. Please provide information on the public health laboratory system within your country

22.1 Are you aware of how many laboratories perform bacterial STI diagnostics in your country (please give numbers if known, and please feel free to comment)

22.1.1 Number of public laboratories: Information unknown ☐

Any comments:

22.1.2 Number of private laboratories: Information unknown ☐

Any comments:

22.1.2 Number of expert/reference laboratories for STI diagnostics: Information unknown ☐

Any comments:

22.2 Are you aware of how many laboratories in your country report to the bacterial STI surveillance system in your country?

Yes ☐ No ☐ If yes, please specify number:

22.3 Would you be able to provide ECDC with a list of these laboratories for future surveys? Yes ☐ No ☐

22.4 Please provide any other comments you feel are relevant to this survey:

Any comments: