UNIVERSITY OF VERONA

DEPARTMENT OF
DIAGNOSTICS AND PUBLIC HEALTH
INFECTIOUS DISEASES SECTION

PHD SCHOOL
LIFE AND HEALTH SCIENCES

PHD IN
APPLIED LIFE AND HEALTH SCIENCES

XXXII° Cycle, Year 2016

PHD THESIS

COMPARING STIs POCTs WITH LABORATORY TESTS IN A MSM POPULATION ATTENDING A HIV/STIs CLINIC IN THE FRAMEWORK OF THE WHO ProSPeRo INITIATIVE

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PhD thesis

Verona, 1st Dec 2019

Abstract

Despite the concerted efforts in promoting prevention and testing campaigns and the availability of highly effective treatments, HIV and other sexually transmitted infections (STIs) continue to represent a pressing public health issue worldwide. These infections disproportionally affect the so-called vulnerable populations. In Europe, the majority of HIV/STIs new diagnosis are amongst Men who have Sex with Men.

Notwithstanding the rapid development of diagnostic technologies for HIV and other STIs, laboratory-based tests are not always suitable for being used both in resource-limited settings, where diagnostic access and delivery are difficult, and in high-income countries to reach the vulnerable groups. To overcome this issue, WHO endorsed the development of STIs Point-Of-Care Tests (POCTs) to tackle HIV/STIs worldwide.

So far, little is known either about the STIs POCTs performance in real life setting or about the potential impact of the replacement of standard laboratory methods with the POCTs approach among different high-risk target populations. To collect robust and reliable data on those issues, in 2017 the Reproductive Health and Research (RHR) Department of WHO launched an independent evaluation on the performance of STIs POCTs among target populations called Project on Sexually Transmitted Infections Point-of-care testing established by the Reproductive Health and Research Department of WHO (ProSPeRo initiative).

This thesis is to report the methodological structure and the main finding of the two studies carried out at the HIV/STIs clinic of Verona, Italy between 2017 and 2018. Considered that in the European context, and in Italy as well, the most at risk population for HIV, syphilis and gonorrhoeae is that of Men who have Sex with Men, we decided to implement the two clinical-based studies amongst them: the evaluation of the dual HIV/syphilis POCTs and that of the NG/CT POCT in genital and extragenital sites.

Between May 2018 and February 2019, 492 individuals were enrolled in the HIV/Syphilis study and 300 in the NG/CT one. In the HIV/Syphilis POCT evaluation, the rapid tests yielded an almost perfect performance as far as the HIV component is concerned. As for the treponemal component, despite specificity was

very high, sensitivity was found to range between 75,8 and 81,7%. In the NG/CT POCT study, the performance varied according to the anatomical site and the considered pathogen. Indeed, although specificity was found to be always above 98%, when the NG component of the POCT is taken into account, the sensitivity ranged from 75% at pharyngeal site to 87,5% at rectal and urethral site. As for the CT component of the POCT, sensitivity was 100% with pharyngeal samples and 90%, 83,3% with rectal and urethral samples, respectively.

STIs POCTs are valuable tools to tackle HIV/STIs worldwide although their routinary use have to be considered in close relation with the epidemiological characteristics of the population itself, beyond the analytical characteristics of the test. The findings of this thesis support the need for independent evaluation of new diagnostic technology before being integrated in the clinical practice as well as importance of alignment of such an evaluation with the best international standards.

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List of acronyms

ANC	AnteNatal Care
CDC	Centers for Diseases Control and Prevention (CDC - Atlanta, USA)
CLIA	ChemiLuminescence ImmunoAssay
CT	*Chlamydia trachomatis*
DNA	DeoxyriboNucleic Acid
DPP	Dual Path Platform
DTS	Dried Tube Specimens
ECDC	European Centre for Disease Control and prevention
ERC	WHO research Ethics Review Committee
EU/EEA	European Union/European Economic Area
EQA	External Quality Assurance
HIV	Human Immunodeficiency Virus
HPV	Human PapillomaVirus
I	Invalid
ICT(s)	ImmunoChromatographic Test(s)
IQC	Internal Quality Control
LR	Likelihood Ratio
MR	Micro Reader
MSM	Men who have Sex with Men
NAA	Nucleic Acid Amplification
NAAT(s)	Nucleic Acid Amplification Test(s)
NG	*Neisseria gonorrhoeae*
NPV(s)	Negative Predictive Value(s)
NR	Non Reactive
OIAs	Optical ImmunoAssay(s)
PCR	Polymerase Chain Reaction
POC	Point-Of-Care
POCT(s)	Point-Of-Care Test(s)
PPV(s)	Positive Predictive Value(s)

ProSPeRo	Project on Sexually transmitted infections Point-of-care testing Established by the Reproductive health and research department of the WHO
QA	Quality Assurance
QC	Quality Control
R	Reactive
R1	Reader 1
R2	Reader 2
RHR	Reproductive Health and Research (Department)
RP2	WHO Research Proposal Review Panel
RPR	Rapid Plasma Reagin
STI(s)	Sexually Transmitted Infection(s)
SW(s)	Sex Worker(s)
TDR	special programme for Research and training in Tropical Diseases
TGW	TransGender Women
TPP	Target Product Profiles
TPPA	*Treponema pallidum* Particle Agglutination (Assay)
WHO	World Health Organization

General introduction

Background and rationale

The available data on HIV/STIs at European level confirm that these diverse and multifaceted epidemics continue to represent a pressing public health issue. Notwithstanding the rapid development of testing technologies and therapeutic tools in recent years, and despite the concerted efforts in promoting prevention and testing campaigns, the increasing trends for HIV and other STIs remain a reality.

In addition, there is a need for harmonised collection of reliable and comparable bio-behavioural data across European countries within the framework of a Second-Generation Surveillance System approach [1]. There is indeed urgency in complementing such estimates and better understanding the epidemiological patterns, including associated risk factors for HIV and other STIs particularly amongst key and vulnerable populations worldwide, such as Men who have Sex with Men (MSM) [2 3].

Recent reports from the European Centre for Disease Control and Prevention (ECDC) highlight that in the European Union/European Economic Area (EU/EEA) countries, the highest proportion of new HIV diagnoses is reported in MSM and in more than half of these countries, HIV prevalence amongst MSM is estimated to be approximately 5%, or possibly even higher [4-6]. In the same geographical area, in terms of new HIV positive diagnosis, MSM accounted for roughly 40% of cases [4]. Considering the data prospectively, whilst the overall number of new cases has been relatively stable over the last decade, the number of new diagnoses amongst MSM aged 20–24 years doubled in the period 2004-2013 [7]. These reports and recent scientific publications underline how strongly MSM are disproportionally affected by HIV in the current European epidemic scenario, compared to non-MSM populations such as heterosexuals [2 3 8].

The high burden of infections within the MSM population is not limited to HIV, but it also includes all the other most frequent STIs, such as syphilis and gonorrhoeae. As described in the most recent report published by ECDC in 2019, both the epidemiological curves of syphilis and gonorrhoeae are increasing, and this is particularly evident when the MSM population is taken into account. Indeed, in

2017, they accounted for 67% and 47% of new syphilis and gonorrhoeae cases respectively [9] [10].

As for *Chlamydia trachomatis* (CT) infection, MSM seems to be at lower risk than heterosexuals, accounting for only 10% of new cases during 2017 [11]. It has to be noticed that, the distribution of chlamydia notifications by gender and the excess of diagnoses reported among females is most likely due to the fact that women are prioritised by testing policies across the EU/EEA to reduce the incidence of pelvic inflammatory disease and other long-term complications [11]. In addition, the screening for Chlamydia infection is recommended only at genital level. This could possibly explain why MSM accounted for a low number of infections. Indeed, in the literature, there is increasing evidence that MSM are frequently diagnosed with *C. trachomatis* infection in extra-genital sites, such as pharynx and rectum [12-15], which are not considered in the Chlamydia surveillance systems in EU/EEA.

Evolution of POCTs

The existing diagnostics for the most frequent STIs worldwide, namely syphilis, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* are laboratory-based platforms, which typically require a consolidated laboratory infrastructure and well-trained laboratory technicians [16]. In addition, test turnaround time is often long, requiring patients to return for test results on a subsequent clinic visit. This diagnostic approach has two main consequences:

i) A lack of treatment specificity due to syndromic management as primary approach. Although it continues to be the strategy recommended by public health professionals for STI treatment in many resource limited countries [17] [18], it is suitable only for symptomatic patients and it lacks specificity as symptoms may reflect non-sexually transmitted conditions [19],

ii) A significant loss in the continuum of care and services. If laboratory test results are not collected, there is a missed opportunity in increasing the number of people aware of being infected with an STI who can benefit from treatment [20] [21]. Indeed, worldwide, limitations of accessibility to health facilities due to several reasons, which range from

geographical distance from nearest medical facility to stigma and discrimination in approaching health care staff, increase the probability that if treatment is not provided during the first visit, loss to follow-up is highly likely [21].

While the laboratory-based diagnostics for STIs are effective, they may not always be suitable for use both in resource-limited settings, where diagnostic access and delivery are difficult, and in high-income countries to reach the vulnerable groups. In response to this challenge, there has been a significant effort in the last decade, to develop new diagnostic tools, such as Point-Of-Care Tests (POCTs). Although there is no univocal definition of POCTs, they are usually defined as diagnostic tools that allow patient diagnoses outside of a typical clinical laboratory (i.e.: physician's office, hospital bedside, patient's home, in the field) [21] and/or treatment administration within the same medical encounter [22]. Owing to these characteristics, Point-Of-Care (POC) diagnostics represent a strategic tool to facilitate achievement of the vision set forth in the 2030 Agenda for Sustainable Development goals [21]. To contribute to the attainment of the said goals, in 2016 the WHO published a new 5-year long (2016-2021) global health sector strategy on STIs [20] outlining three overarching programmes, namely (i) universal health coverage, (ii) the continuum services related to STIs, and (iii) the public health approach. Each programme lays out the vision, goal, targets, guiding principles and strategic direction for the elimination of STIs as public health threats, and priority actions to be taken by stakeholders (countries, international public health institutions, partners, etc.) for tackling STIs [20]. In this context, the WHO reinforced the growing importance of POCTs as promising tools to advancing STIs control and prevention.

The role of STIs POCTs in controlling STIs was acknowledged by the WHO since mid-2000s when the WHO set the trajectory of STIs POCT further research and development. In 2006 a group of experts published a paper in which the required characteristics a POCT were listed [23]. These characteristics were summed up in the acronym ASSURED: **A**ffordable (i.e. low cost), **S**ensitive and **S**pecific (i.e. Accuracy >95%), **U**ser-friendly (i.e. easy to use, no or minimal training is required), **R**apid and robust (i.e. rapid turnaround time in order to have the patient still accessible for treatment), **E**quipment-free, **D**eliverable to end-users [21 23 24]. This

ASSURED approach expanded the role of HIV/STIs testing from rapid to POC or near-patient testing [23]. This development led not only to improved access to testing but also to a prompt referral for HIV/STIs treatment, decreasing the chances of further transmission in the community and development of long-term complications and sequelae for individual health. Thanks to this ASSURED approach, the so called "Test and Treat Strategy" was born [25 26].

Background description of the ProSPeRo studies

Despite the fact that in the last decade many POCTs for HIV/STIs have been produced and put on the market [27], there is no WHO guidance and recommendation available regarding the placement of these new tools in the diagnostic pattern of HIV/STIs. In addition, in the 2016-2021 STIs health sector strategy, the WHO included the validation and standardisation of innovative diagnostic technologies and approaches as one of the key priorities and acknowledged the lack of reliable, low-cost POCTs as a major barrier to advancing STIs control and prevention [20]. Little is known either about the POCTs performance in real life setting or about the potential impact of the replacing standard laboratory methods with the POCTs approach among different high-risk target populations, the placement of POCTs in the diagnostic algorithm for HIV/STIs represents a challenge for researchers, clinicians and policy makers. A first issue relates to the clinical performance of the rapid tests when adopted in specific settings (i.e. the possibility of false positives and false negatives). As an example, positive predictive values (PPV) and negative predictive values (NPV) are related to the specific characteristics of the population (prevalence of the infection): therefore, the same POC test, when used to test different populations, might result in different performance. This might be related to the epidemiological characteristics of the population in itself, rather than to the performance of the test. This implies that specific validations are required to better understand the performance of the tests, considering the epidemiological patterns that characterised that specific target population. An additional challenge is represented by the assessment of the acceptability of the POCT approach when included in the current clinical practice. This implies the clarification of the acceptability from the viewpoint of the users (i.e. health care staff) and from the

perspective of the clients (i.e. individuals attending clinics and/or community testing facilities where POCT is adopted). In the case of the latter, different target populations (i.e. MSM, sex workers, pregnant women, etc.) might experience different levels of acceptability, considering also environmental and broad social factors [28].

Finally, and most importantly, a comprehensive assessment of the potential impact of the human component (i.e. role of the health care staff performing and/or reading POCT results) when adopting POCTs should be carefully taken into consideration [29].

In the context of the bio-behavioural survey among MSM (Sialon II project) conducted between 2013-2014 in 13 European cities [3 30], an ancillary study for the assessment of the actual impact of POCTs in the clinical routine was conducted [31]. Even if the theoretical background of the use of POCTs is well developed and promising practices are in place, the Sialon II study concretely assessed the clinical performances of POCTs (dual test for syphilis) targeting MSM when adopted in specific settings [31]. The uniqueness of this piloting study was related to the use of weighted estimates for syphilis (gathered through the Sialon II project), allowing a precise assessment of the performance (PPV and PPN) of the POCTs. In addition, a comprehensive assessment of the potential impact of the human component (i.e. role of the health care staff in performing and/or reading the POCT results) when using POCTs was made. This was the first time such an assessment has been carried out in the context of a POCTs study targeting MSM.

Building on the results of the Sialon II study and with a view to collecting further evidence in real-life settings and to filling the gap of knowledge in the use of different POCTs amongst different high-risk target populations [29], in 2017 the Reproductive Health and Research (RHR) Department of the WHO launched an independent evaluation on the performance of STIs POCTs among target populations called Project on Sexually Transmitted Infections Point-of-care testing established by the Reproductive Health and Research Department of the WHO (ProSPeRo initiative https://www.who.int/reproductivehealth/topics/rtis/pocts/en/). This global study is currently ongoing [29].

The final aim of ProSPeRo is to provide robust data on the POCTs analytical characteristics, feasibility, acceptability and utility. On these findings international recommendations on the use of STIs POCTs to advance the control over HIV/STIs will be based. This multi-country, multifaceted study was built on the findings of the previous research phase which set the trajectory to be followed during the implementation of the project.

The research phase of ProSPeRo took place between 2014 and 2015 and addressed the main research questions on HIV/STIs. In short, the pillars of this preliminary phase were:

i. **The WHO expert technical consultations**

In 2014 and 2015 the WHO called for two technical consultations in which a selected group of experts indicated the STIs for which POCTs were most urgent and the populations in greatest need of them.

On the basis of the WHO definitions of key and vulnerable populations [32] and of the epidemic trends of HIV/STIs amongst them [4 9 10 33], the group of experts settled on engaging MSM and transgender women (TGW), male and female sex workers (SWs) and women at risk for STIs, with a particular focus on those pregnant attending antenatal care clinics (ANC).

In consideration of the main challenging areas in the fight against STIs, the STI group of experts also established the research priorities as follows: the implementation of screening among key and vulnerable populations (MSM and TGW, SWs, women at higher risk for STIs or attending ANC during pregnancy) and the improvement of STIs case management among symptomatic individuals (i.e. women with vaginal discharge).

ii. **POCTs landscape analysis and Target Product Profiles (TPP) for STIs** [16] (Annex 1)

In the last years, Maurine Murtagh and Colleagues, in collaboration with the WHO, wrote updated reports on the available and pipeline rapid diagnostics for curable STIs, namely syphilis, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and human papillomavirus (HPV).

The landscape analysis represents a comprehensive, easy to use report where all the publicly available information (i.e. published and unpublished

reports and prospectuses, interviews with developers and manufacturers) on the above-mentioned diagnostics are clearly listed and explained.

The aim is to provide researchers, clinicians and policy makers updated information on the STIs POCTs in terms of test's components, procedure and performance as declared in the package insert. In addition, in recent years, the POCT minimal and optimal requirements, as described in the TPP, have been used by diagnostic manufacturers as guidance for the POCT research and development process.

iii. **Systematic reviews on STIs POCTs**

Many systematic reviews have been carried out in order to summarise the body of knowledge in terms of operational characteristics and performance of POCTs for HIV/syphilis [34], urogenital gonorrhoeae [35], *C. trachomatis* infection [36] and HPV [37]. These data have been used, where possible, to evaluate the cost-effectiveness, acceptability and easiness to use of the POCT by end users. This set of knowledge was published in December 2017 in the *Sexually Transmitted Infections*' special issue on STIs Point-of-care tests. The special issue was specifically focused on the POCTs approach, providing assumptions and strategic objectives on the use of these new diagnostic tools, as well as some evaluation and/or piloting exercises of POCTs in real-life settings.

iv. **Field evaluation of two point-of-care tests for syphilis among men who have sex with men, Verona, Italy** [31]

This evaluation translated all previous research efforts and findings into the development of tools and procedures meant to collect data on both the performance and the acceptability of syphilis POCTs in a real-life setting.

The population amongst these two POCTs were evaluated was that of MSM as both they accounted for most of syphilis new cases in European countries and they were already approachable in the context in the Sialon II study.

The findings of this syphilis POCTs evaluation study were published in a paper which was one of those selected for the *Sexually Transmitted Infections*' special issue on the basis of the solid innovation brought by this evaluation [31]. Indeed, even though the theoretical background of POCTs

was well developed at that time and promising practices were in place, some core research questions were still open: the exact clinical performance of syphilis POCTs, [in this case, the SD Bioline Syphilis 3.0 (Standard Diagnostics, South Korea) and the DPP Syphilis Screen & Confirm Assay (Chembio Diagnostic Systems, USA)] and the potential impact of the human component when using a POCT. Indeed, one of the core assumptions of these POCTs is that they should be performed (pre-analytical, analytical and post-analytical phase) by end-users that, in this study, were the health care staff. To address this issue and provide new evidence, this study pointed out the need of weighted estimates for the detected infection (i.e. syphilis) for the precise assessment of POCTs performance (i.e. predictive values) and fine-tuned the identification system for the operator in charge of the POCTs execution and interpretation. The latter was key to properly assess the impact of the human component and potential subjectivity within the entire POCTs process.

The results of the field evaluation were promising, despite some clear limitations. POCT proved to be feasible and acceptable in terms of performance, considering the specific characteristics of the MSM population (i.e. syphilis prevalence). However, the general diagnostic performance of the syphilis POCTs was lower than that declared by the diagnostic companies. This result was essential as it contributed to an improved understanding of the actual performance of rapid diagnostic tests when used in real-life settings (i.e. STIs clinics), compared to the laboratory-based performance.

The contribution toward advancing knowledge in this field is significant as it was possible to confirm the potential of the POCT technology in specific contexts and targeting a specific population, and the importance of specific training for users and the adoption of supporting materials to reduce misinterpretation of the results due to human subjectivities was underlined. Tools and procedures developed and optimised in the context of this first syphilis POCTs real-life evaluation was adopted by the WHO and provided

a strong methodological background for the development of the ProSPeRo study.

The ProSPeRo study, launched by the WHO in 2017 and still ongoing, is made of 3 components, two of which are still ongoing: i) the laboratory-based arm with the aim of assessing the performance of NAATs POCTs (i.e. NG/CT Assay and *Trichomonas vaginalis* Assay) in a laboratory setting and challenging POCTs specificity with bacteria belonging to different genus of the same species. The findings of this study were published in late 2018 [38]; ii) the clinical-based component with the objective of evaluating STIs POCTs performance compared with that of laboratory tests amongst several STIs high-risk populations worldwide; iii) the clinical-utility component with the aim of assessing the feasibility and acceptability of the HIV/Syphilis POCTs among MSM in non-medicalised settings (such as NGOs) in four European Countries (Barcelona, Spain - Ljubljana, Slovenia - Riga, Latvia - Kiev, Ukraine).

As far as the clinical-based component in concerned, according to the research priorities established by the experts during the WHO technical consultations, it is compounded of 5 protocols which evaluate the real-life performance of 3 different STIs POCTs amongst 4 populations. To do so, the WHO developed 5 core protocols, which are:

1. The evaluation of dual HIV and syphilis POCTs among MSM and sex workers (both men and women) for screening purpose;
2. The evaluation of dual HIV and syphilis POCTs among pregnant women attending ANC for screening purpose;
3. The evaluation of NG/CT POCT among MSM in genital and extragenital sites for screening purpose;
4. The evaluation of NG/CT and TV POCT among women at risk of these infections for screening purpose;
5. The evaluation of NG/CT and TV POCT among women with vaginal discharge for case management.

All the clinical sites that agreed in implementing the study locally, had the opportunity to adapt slightly one or more of these five protocols to local context and need without modifying study outcomes, tools and procedures. So far, 22 sites in 10 countries worldwide implemented or are implementing the clinical-based component of the ProSPeRo initiative.

As in the European context, the most at risk population for HIV, syphilis as well as gonorrhoeae is that of MSM [4 9 10] we decided to implement the two clinical-based studies amongst MSM: the evaluation of the dual HIV/syphilis POCTs and that of the NG/CT POCT in genital and extragenital sites.
The findings of the two studies carried out at the HIV/STIs clinic of Verona, Italy between 2017 and 2018, are described in the next two parts of the thesis.

Project 1

Clinic based evaluation of SD BIOLINE HIV/Syphilis Duo and DPP HIV-Syphilis Assay for the screening of HIV and syphilis amongst MSM attending the STI screening facilities of Verona, Verona, Italy.

Background

In these last years there has been a growing body of evidence confirming the need for a STI testing paradigm shift. Indeed, addressing the efforts in detecting symptomatic STI cases only, would not reduce the burden of asymptomatic infections, which lie beyond the surface and which continue to represent a reservoir for the further transmission of the infection in the community [20] [39]. This is particularly evident when relatively small groups of people with high risk factors for HIV/STIs are taken into consideration. These people, known as key and vulnerable populations [32], carry a disproportionate burden of HIV/STIs mainly due to structural (i.e. lack of or suboptimal access to appropriate health care due to health inequalities) and psychological factors (i.e. stigma and discrimination due to sexual behaviour, ethnicity/race, gender) [40-43]. As for the MSM community, a paper published in *The Lancet* in 2012 [40], stated that HIV infection among MSM is heavily biologically determined as 98% of difference between HIV epidemics among MSM and the heterosexual population is basically related to the much higher per-act and per-partner transmission probability of HIV in unprotected anal intercourses than in unprotected vaginal ones and the existence of role versatility. Behavioural factors, such as the number of casual sex partners accounted only for 2% of the difference [40]. The higher HIV transmission probability per sexual act is both related to the biological features of anal intercourses and the prevalence of infection within the community. Indeed, as MSM sexual network is smaller than that of the heterosexual population, HIV/STIs can be transmitted within the community at great speed, increasing the prevalence and the related transmission/acquisition rate as in a loop [40].

As well as in other high income countries, in 2017 MSM accounted for 38% of all new cases of HIV in the EU/EEA [4]. Furthermore, in this high-risk group, the incidence of syphilis has increased every year since 2008 and in 2017, 67% of new cases were among MSM [10].

In Verona the EU-funded Sialon II project estimated an HIV and syphilis (treponemal test positivity) prevalence among MSM of 9,6% and 12,7% respectively [2], in line with the data available at national and European level [44].

As mentioned earlier, structural and psychological barriers could deeply reduce the seek for health care by vulnerable populations, having a major impact on their chance to receive appropriate diagnostic patters, linkage to care and treatment [40].
Undiagnosed and thus untreated asymptomatic STIs could anyhow cause long-term sexual and reproductive health complications and sequelae [45] and could increase the rate of HIV transmission and acquisition [46 47]. This chain of events could be tackled through early testing and subsequent treatment as recommended by the WHO [20].
To maximize the benefit and expanding health-services coverage with less direct costs to those who need the services, the WHO advocate the integration of new STIs screening services into existing HIV testing programs, allowing the progression towards the universal health coverage [20].
Despite the fact that the global health goals set in the Agenda for Sustainable Developments, to be reached by 2030, are ambitious [48], most of the needed tools are already available. Key element in this roadmap is the diagnostic improvement represented by the STIs POCTs [20] as they allow for diagnosis and prompt referral to care and treatment within the same clinical encounter [49].
Although STIs POCTs are promising and valuable tools to tackle HIV/STIs, there are still some concerns on their wide use. An important issue relates to the clinical performances of the POCT when adopted in specific settings as POCT results need to be considered in close relation with the epidemiological characteristics of the population in itself (i.e. prevalence of the infection), beyond the analytical characteristics of the test. The same POCT, indeed, might result in different performance when used to test different populations. This requires that specific validations need to be performed to better understand the performance of the POCT, in light of the specific epidemiological patterns within a specific target population.
To fill in this gap of knowledge as well as assess the acceptability of POCTs when included in routine clinical practice and the potential impact of the human component in performing and reading the POCTs, the RHR Department of WHO launched an international, multi-country evaluation of STIs POCTs among high risk populations. This is called Project on Sexually Transmitted Infections Point-of-care testing established by the Reproductive Health and Research Department of the WHO (ProSPeRo: https://www.who.int/reproductivehealth/topics/rtis/pocts/en/).

Objectives

The purpose of this study is to assess the performance of two HIV/syphilis POCTs (the SD Bioline HIV/Syphilis Duo – Abbott, and DPP HIV-Syphilis Assay - Chembio) when compared to that of laboratory-based gold standard tests when used to screen for HIV and syphilis the MSM population living in Verona.

Secondarily, the performance and the potential utility of these POCTs in identifying probable active syphilis cases (treponemal and non-treponemal test positivity) is evaluated.

This paper will describe the Italian component of ProSPeRo evaluation.

Methods

Study sites and population

From May 2018 to February 2019, consecutive MSM presenting at the two main HIV/STIs screening facilities in Verona (Infectious Diseases and Tropical Medicine Unit of the Verona University Hospital, Infectious Diseases Unit of the Verona Health District 9-Scaligera) were enrolled prospectively in the study after the signing of the informed consent. In addition, participants were asked to join the study only if they were male of 18 years or older and they had had sex with at least one man over the last 12 months.

Enrolled MSM received an automatically generated unique bar code to participate anonymously and to link participants to their rapid and laboratory test results. According to WHO core protocol of the study, a structured questionnaire was used to collect information about demographic and behavioural characteristics, participant's past HIV and syphilis history and acceptability of the POCTs approach.

Considering the sensitivity and specificity declared by the POCT manufacturers on the package insert documentation we assumed a reduced sensitivity and specificity: respectively 90% and 95% for HIV and 80% and 90% for TPPA. It has to be noticed that, in particular for the treponemal component, we adopted a very conservative approach as we assumed a POCTs performance even lower than the minimal acceptable performance suggested by WHO in its TPP (sensitivity > 85% and specificity > 95%) [50]. Under this assumption a confidence interval width of 10% was assumed. The formula used for the sample size calculation is based on the 2006 WHO/TDR expert panel document on the evaluation of new diagnostic methods and techniques [51]. The positive cases to be therefore recruited by our study were 35 for HIV and 62 for syphilis.

As standard approach, the sample size is adjusted for the infection prevalence using the formula (100/prevalence x sample size using sensitivity/specificity only) [51]. Based on these data, assuming a HIV prevalence of 9,6%, a treponemal positive prevalence of 12,7%, with a TPPA positive and RPR positive prevalence of 5,1%, as reported by the SIALON II bio-behavioural survey [2], the estimated number of patients to be recruited was 350 for HIV and 475 for syphilis.

POCTs under evaluation

The tests evaluated in this study were SD Bioline HIV/Syphilis Duo (Abbott Diagnostics - United States; hereafter termed Bioline POCT) and the Chembio Dual Path Platform (DPP) HIV–Syphilis Assay (Chembio, United States; hereafter termed Chembio POCT). Both are single-use qualitative immunochromatographic assays for the simultaneous detection of HIV and syphilis (treponemal component) in human serum, plasma, whole venous or fingerpicked blood.

The SD BIOLINE HIV/Syphilis Duo test detects antibodies of all isotypes (IgG, IgM, IgA) against HIV specific antigens (HIV-1 gp41, sub O, HIV-2 gp36) and specific IgG and IgM antibodies against a 17 kilodalton recombinant *Treponema pallidum* antigen (*rTp*17 kDa). In 2015 this test was accepted for the WHO list of prequalified in vitro diagnostics [52].

The Chembio DPP HIV–Syphilis Assay detects specific antibodies against HIV types 1 and 2 (HIV 1/2) and *Treponema pallidum* [53]. Recently, Chembio company developed the DPP Micro Reader (MR) to complete the Chembio DPP technology and minimise human errors due to subjective visual interpretation. The MR is a portable, blue, battery-powered cubic reflectance reader with a small liquid crystal display and a single button on the top. The display shows the status of the instrument and shows the test result to the operator whereas the multi-function button turns on the MR and guide the operator in the next steps. This device has to be applied on the Chembio POCT through the use of a specific holder. The MR scans the Chembio POCT cartridge, verifies the presence of line(s) at the control and each of the test line positions and measures numerically the optical density of each test line(s). The device interprets the results comparing the optical density with that of a scoring algorithm, translating it into a numerical value. This number is compared with that of the set cut-off ($\geq$ 20 for the HIV component, $\geq$ 10 for the treponemal component) and if higher, the POCT result is displayed as reactive (R), otherwise, as non-reactive (NR). If the MR reading phase is not successful, the displayed result is invalid (I).

Both Bioline and Chembio POCTs were performed by trained health-care staff following manufacturer's instructions. According to the latter, during the visual read out, the presence of any visible band in the positive region was considered as

a reactive result for HIV and/or syphilis, irrespective of the strength of the band. Test results were recorded on separate sheets for both the two visual readouts. As far as the MR is concerned, the qualitative result (R, NR, I) and the number displayed on the electronic screen is written by the operator in a specific form.

The POCTs under evaluation were donated by the manufacturers to WHO in the context of the ProSPeRo initiative. Manufacturers were not involved in any part of the study (study design, data collection, data analysis, data interpretation and writing of the paper).

Reference laboratory tests

Bioline and Chembio POCTs results were compared with those of the HIV and syphilis serological laboratory assays.

As for the HIV component, two screening tests and one confirmatory test were used: the ADVIA Centaur HIV Ag/Ab combo assay (Siemens Healthcare, Erlangen, Germany) and the VIDAS HIV DUO QUICK (BioMérieux, Marcy-l'Étoile, France) whereas the confirmatory test was the INNO-LIA HIV I/II Score (INNO, Fujirebio, Ghent, Belgium). As per WHO core protocol, laboratory reference tests were the HIV Ag/Ab assay in case of negative result and the HIV confirmatory test result in case of positive result of one of the HIV screening 4th generation assays.

For the treponemal component, two treponemal tests were used: the CLIA (ADVIA Centaur Syphilis assay – Siemens Healthcare, Erlangen, Germany) and TPPA (SERODIA-TP·PA - Fujirebio Diagnostics, Inc, Malvern, PA, USA). As far as the non-treponemal component is concerned, RPR (Syphilis RPR test - HUMAN Diagnostics Worldwide, Wiesbaden, Germany) was used as reference test. As per WHO core protocol, only the TPPA result was considered for comparison with the syphilis component of the POCTs.

According to the standard laboratory procedure the titration for both TPPA and RPR was also recorded.

Quality Assurance

Throughout all the duration of the study, both the clinic and the laboratory entered into an internal quality control (IQC) and external quality assurance (EQA) testing programme.

As far as internal controls is concerned, every 20 participants enrolled in the study, researchers at clinical sites were asked to perform the POCTs under evaluation using well characterised sera (one dually HIV/treponemal negative and one dually HIV/treponemal positive) supplied by the central laboratory. As internal controls were not meant to challenge the POCTs under evaluation, but to assure HIV/syphilis POCTs are performed according to specifications, reference laboratory have to select sera samples with high titres of VDRL ($\geq$1:16) to generate clear treponemal positive, as detailed in the standard operational procedures of the study. In the event of inconsistencies between expected and achieved POCT results, providers at clinical site were asked to repeat immediately the POCTs using the same serum and, whether the result is still not concordant, to report the event to the local laboratory for a double check and to the ProSPeRo research team.

HIV and syphilis EQA were performed every 6 weeks during the study period, as detailed in the standard operational procedures of the project. To do so, the WHO, in collaboration with Centers for Diseases Control and Prevention (CDC - Atlanta, USA), provided the local laboratory with 8 rounds of dried tube specimens (DTS) from HIV and treponemal antibody positive and negative sera. The reference laboratory has to rehydrate three panels of DTS every 6 weeks and send them to clinical sites. Both the staff at the peripheral sites and the local reference laboratory were blind to the patterns of seroreactivity of the various DTS panels. EQA results were recorded on a specific form and sent to CDC for their feedbacks.

Specimen collection, testing procedures and POCTs results reading

Tools and testing procedures were developed during the research and pre-implementation phase of the ProSPeRo initiative, based on the experience learnt in our previous syphilis POCTs validation exercise [31], on the training provided us by POCT manufacturers and on their publicly available instructions [54 55]. Considering the latter, both the required amount of capillary (using manufacturer's pipettes and

loops) and venous blood (5 mL), collected by trained healthcare staff of the Verona University Hospital, and the waiting time before the beginning of the reading phase were meticulously followed.

During the reading phase, the presence of the line(s) was evaluated independently by the naked eye by two trained readers who were blind to each other's results and to the clinical history of the study participants. It has to be noticed that only the results of the first reader (R1) were then considered for the evaluation of the POCT performance as, in real life, POCTs are meant to be performed and read by only one operator. The visual readout of the second reader (R2) is considered in the analysis of concordance between the two readers.

In addition, according to study procedure and WHO core protocol, the MR was used and its result read by R2 only after that the results of the POCTs visual readouts, by both readers, were registered on the paper.

Pre- and post-test counselling was provided to all participants, according to the WHO recommendations and local clinical practice.

Whereas the two POCTs performed on fingerstick blood were read immediately (R1-R2-MR) at the testing sites, blood tubes were sent to the Microbiology Unit of the Verona University Hospital. At the laboratory, blood samples were centrifuged to obtain serum and to perform the laboratory-based HIV and syphilis serological tests. The specimens that could not be processed immediately were stored at 4°C and processed within 3-4 days to allow study participants to withdraw their HIV and syphilis definitive results, using the bar code provided at enrolment.

In line with local protocol, linkage to care and treatment was made available to participants during the post-test counselling, in the case of a positive result of laboratory test for HIV and/or syphilis.

Ethics

Before the enrolment started, research protocols were approved by both the local Ethics Committee (*Comitato Etico per la Sperimentazione Clinica - CESC delle province di Verona e Rovigo,* 1309CESC), the WHO Research Project Review Panel (RP2) and the WHO Research Ethics Review Committee (ERC).

In addition to the local standard procedure to guarantee anonymity and confidentiality of respondents' data, the bar-code system developed in the validation exercise previously conducted in Verona [31] was adopted to allow an appropriate link between the different types of data collected for each individual (demographic and behavioural information, biological samples) and the withdrawal of reference test results few days after the enrolment.

Although the provision of treatment was directed only by a positive result of at least one of the laboratory tests, participants were informed on the POCT results in order to give them the chance not to transmit further the infection in the community in the time between the testing day and that in which the laboratory results were made available. In case of laboratory positive result(s), MSM received further information about the infection(s) and the relative treatment during post-test counselling. The treatment provided was in line with national guidelines and standards.

Finally, in line with the protocol, samples were stored at -80°C at the Microbiology Unit of the Verona University Hospital for the overall completion of the study (including publications).

Statistical Analysis

The overall sample size is calculated considering the HIV and syphilis prevalence amongst MSM living in Verona [2].

Sensitivity, specificity, PPV and NPV for each rapid test were estimated comparing the POCT results with the gold standard laboratory test results [56]. It has to be specified that we decided to assess POCTs performance comparing laboratory results with the POCTs visual readouts as reported only by one of the two readers (R1) as, beyond the validation exercise, in routine clinical practice, POCTs are meant to be performed and read only by one operator. The comparison was then made between serological tests and POCT MR results.

The concordance between R1-R2, R1-MR readings and R2-MR readings was estimated with Cohen's Kappa for binary variables considering also the percentage agreement (concordance) [57].

Cohen's Kappa represents a measure of inter-rater agreement, ranging from −1 to +1, where 0 is the level of agreement that can be expected in case of random chance. According to the literature, thresholds for Kappa are usually categorized as follows: <0.0 (poor agreement), 0.0–0.2 (slight), >0.2–0.4 (fair), >0.4–0.6 (moderate), >0.6–0.8 (substantial), and >0.8–1.0 (almost perfect agreement) [58].

STATA Version 16.1 was used for all analyses (College Station, TX: StataCorp LP).

Results

Study population

From May 2018 and February 2019 (9-months period) 492 MSM were enrolled in the study.

All participants provided behavioural and clinical information. The collection of venous blood for HIV and syphilis serological testing was performed on each study participant. All but 1 participant provided fingerstick whole blood for the HIV/syphilis POCTs under evaluation.

The mean age of the participants was 41,0 (Median 39; SD 13,5; min 19, max 7, iqr 25).

As for the presence of signs and symptoms, 473/492 MSM (96,1%) were fully asymptomatic and 19 (3,9%) complained the presence of sore(s) or ulcer(s) at genital site. Among all symptomatic individuals and six asymptomatic ones, a genital examination was performed (25/492, 5,1%). The physical examination confirmed the presence of genital lesions in 40% (6/15) of the cases, while the presence of maculopapular rash was reported in 28,6% (4/14) of the study participants.

Among study participants, 459 (93,3%, CI95% 90,9-95,4) were already been tested for HIV before the enrollment in the study. The mean time from last HIV test was 27,7 months (median 7; SD 62,9; min 0,25, max 442, iqr 15). Excluding the 47 participants already aware of being HIV positive at the enrollment time, 79,1% (CI95% 74,9-82,8) of those already tested for HIV had a previous HIV test performed within last 12 months, whereas 12,4% (CI95% 9,5-15,4), 6,5% (CI95% 3,5-12,4), 1,5% (CI95% 0,6-3,2) and 0,5% (CI95% 0,1-1,9) between 1-2, 2-5, 6-10 and more than 10 years earlier, respectively.

All the 47 individuals aware of being HIV positive were on highly active antiretroviral treatment (HAART). Three of them (6,3%) were not aware of the result of their last HIV viral load assessment, whereas, all the others reported a HIV viral load below 200 cp/mL with full undetectability (HIV-RNA<50cp/mL) in 93,2% of them (CI95% 80,1-97,9).

As far as syphilis is concerned, among the overall sample, 369 respondents reported a previous syphilis test (75,8%, CI95% 71.7-79.4) with 158 of them (42,8, CI95%

37,8-47,9) resulted positive. The mean time from last syphilis diagnosis was 1,8 years (median 1; SD 2,2). Indeed, 40,5% (CI95% 33,1-48,4) of enrolled individuals reported a syphilis diagnosis during last 12 months, 21,5% (CI95% 15,7-28,7), 17,1% (CI95% 9,1-31,8) and 8,9 (CI95% 5,3-14,5) between 1-2, 2-5 and 6-10 years earlier and 12,0 (CI95% 7,8-18,2) more than 10 years earlier.

All but 4 (97,5%, CI95% 93,4-99,1) of those diagnosed with syphilis have been treated. The most frequent treatment reported by those who remembered the type of treatment they received (149, 96,7%) was intra muscle injection of penicillin (92,6%, CI95% 87,8-95,9) followed by oral treatment, such as doxycycline, (6,7%, CI95% 3,6-12,1), and intra venous treatment with ceftriaxone (0,7%, CI95% 0,9-4,7). In terms of syphilis follow-up, 127 MSM (83,6%) reported serological testing for *Treponema pallidum* within the first 12 months after syphilis treatment.

Among study participants, 62 (12,6%) reported any antibiotic treatment on the 3 weeks before the day of enrollment.

POCTs acceptability

Study participants have been asked about their willingness to wait for POCT result in case this kind of test would be routinely available at the clinic.

The majority of them (466/492; 94,7%) reported to be willing to wait, with 146 (31,3%) willing to wait up to one hour, 126 (27,0%) up to 30 minutes and 51 (10,9%) up to 20 minutes respectively. 101 (21,7%) MSM declared to be able to wait up to 2 hours and 25 (5,1%) even for more time.

When participants have been asked about their preference about being tested for HIV and syphilis using two single POCTs, each one specific for one infection, or one dual POCT, 65,0% (320/492) reported to prefer a dual test, 14,0% (69/492) two single tests and for 17,9% (88/492) of them it was exactly the same. To those who preferred a single POCT, we asked whether it was due to their unwillingness to be tested for HIV or syphilis. Only 10 participants (14,5%) reported that that was the reason, whereas most of them declared concerns on dual POCTs performance.

Results of gold standard laboratory tests

As for the HIV component is concerned, 55 enrolled respondents had a positive result both at the screening and confirmatory HIV tests.

Among those participants whose HIV laboratory tests resulted positives, 53 have been previously tested for HIV (96,4%). Among the latter, 47 (88,7%) were already aware of the diagnosis whereas 6 (11,3%) were newly diagnosed. Amongst the latter, no one had an acute infection as no HIV-antigen positive and HIV-antibody negative laboratory test result was recorded. All HIV positive individuals newly diagnosed were linked to care, however, one of them was then lost to follow-up.

For the treponemal component, two treponemal tests were used: the chemiluminescence immunoassay (CLIA) and *Treponema pallidum* particle agglutination (TPPA). For the non-treponemal component, rapid plasma reagin (RPR) was used as reference test.

Based on TPPA, 169 samples were found to be positive (34,3%, CI95% 30,3-38,7). It has to be noticed that CLIA test resulted positive in 171 cases (34,8%, CI95% 30,7-39,1), two cases had therefore treponemal tests discordant results.

RPR test was reactive in 53 participants (10,8%, CI95% 8,3-13,8). Among them, RPR titre was $\geq$ 1:8 in 13 individuals (24,5%).

All but one (52/53, 98,1%) RPR positive samples were also TPPA positive.

Performance of the POCTs

During the 9-month enrolment period, 491 (99,8%) Bioline and Chembio HIV/syphilis POCTs were performed at the HIV/STIs testing sites in Verona.

POCTs sensitivity and specificity, positive and negative likelihood ratio (LR) as well as predictive values, compared to the golden standard laboratory tests, varied considerably according to the type of infection (see Table 1).

As for HIV infection is considered, the Bioline POCT was found to have a sensitivity of 100% (CI95% 88,8-100) and a specificity of 99,6% (CI95% 97,8-100) at the human reading. The positive LR was 167 (CI95% 33,8-822) whereas the negative one was found to be 0,02 (CI95% 0,0-0,2). The PPV of the Bioline test was 96,6% (CI95% 79,0-98,9), while the NPVs was 100% (CI95% 97,3-100). With regards to the treponemal component, the sensitivity of the Bioline test was 78,5%

(CI95% 68,8-86,3), whereas the specificity was 99,5% (CI95% 97,1-100). LR was found to be 100 (CI95% 20,3-495) when positive and 0,2 (CI95%0,15-0,3) when negative. The PPV of the Bioline POCT was 95,7% (CI95% 75,2-98,7), while the NPV was 96,9% (CI95% 95,4-97,8).

With regards to the Chembio test, as for the HIV component, it yielded a sensitivity of 100% (CI95% 88,8-100) for both the reader and the MR. As far as the specificity is concerned, it was 98,0% (CI95% 95,4-99,4), 99,2% (CI95% 97,2-99,9) for reader and MR, respectively. Positive LR was 45,4 (CI95% 19,9-104) and 100 (CI95%29,1-344) for reader and MR respectively, whereas the negative one was 0,02 (CI95% 0,0-0,2) for both reader and MR. The PPVs were 84,9% (CI95% 68,8-92,0) and 93,4% (CI95% 76,4-97,4) for the reader and the MR respectively, whereas the NPVs were perfect for both of them (100%, CI95% 97,3-100). With regards to the treponemal component, the Chembio POCT registered a sensitivity of 81,7% (CI95% 72,4-89,0) and 78,5% (CI95% 68,8-86,3) for the reader and MR, respectively. This POCT yielded a 99,5% (CI95% 97,1-100) for the reader and the MR when the specificity is considered. The positive LR was 104 (CI95%21,1-514) and 100 (CI95%20-3-495) for the reader and MR respectively, while the negative LR was found to be 0,2 (CI95% 0,1-0,3) for both reader and MR. PPVs of 95,9% (CI95% 75,9-98,7) and 95,7% (CI95% 75,2-98,7) were calculated for the reader and MR respectively. NPVs were 97,3% (CI95% 95,9-98,2) for the reader and 96,9% (CI95% 95,4-97,8) for MR.

Table 1 – POCTs Sensitivity, Specificity, Positive and Negative Likelihood Ratio (LR+/LR-) (Part A), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) (Part B) per type of infection (Estimated HIV Prevalence: 10%; Estimated Syphilis Prevalence: 13%), according to the assessment of the reader (R) and the microreader (MR), compared to the lab-based golden standard (HIV confirmatory test (WB) for HIV component, TPPA for syphilis component)

Part A

			Sensitivity	**CI 95%**		**Specificity**	**CI 95%**		**LR+**	**CI 95%**		**LR-**	**CI 95%**	
BIOLINE	**HIV**	**R**	100,0%	88,8%	100,0%	99,6%	97,8%	100,0%	167	33,8	822	0,02	0,0	0,2
	SYPHILIS	**R**	75,8%	68,8%	86,3%	99,5%	97,1%	100,0%	100	20,3	495	0,2	0,1	0,3
CHEMBIO	**HIV**	**R**	100,0%	88,8%	100,0%	98,0%	95,4%	99,4%	45,5	19,9	104	0,02	0,0	0,2
	SYPHILIS	**R**	81,7%	72,4%	89,0%	99,5%	97,1%	100,0%	104	21,1	514	0,2	0,1	0,3
CHEMBIO	**HIV**	**MR**	100,0%	88,8%	100,0%	99,2%	97,2%	99,9%	100	29,1	344	0,02	0,0	0,2
	SYPHILIS	**MR**	78,5%	68,8%	86,3%	99,5%	97,1%	100,0%	100	20,3	495	0,2	0,1	0,3

R = Reader; MR = MicroReader

Part B

			Prevalence (%)	**PPV**	**CI 95%**		**NPV**	**CI 95%**	
BIOLINE	**HIV**	**R**	9,6	96,6%	79,0%	98,9%	100,0%	97,3%	100,0%
	SYPHILIS	**R**	12,7	95,7%	75,2%	98,7%	96,9%	95,4%	97,8%
CHEMBIO	**HIV**	**R**	9,6	84,9%	68,8%	92,0%	100,0%	97,3%	100,0%
	SYPHILIS	**R**	12,7	95,9%	75,9%	98,7%	97,3%	95,9%	98,2%
CHEMBIO	**HIV**	**MR**	9,6	93,4%	76,4%	97,4%	100,0%	97,3%	99,9%
	SYPHILIS	**MR**	12,7	95,7%	75,2%	98,7%	96,9%	95,4%	97,8%

R = Reader; MR = MicroReader

RPR and TPPA titration values

Among study participants, 171 resulted CLIA positive, 169 TPPA positive and 53 RPR positive. All RPR positive individuals but one resulted TPPA positive.

Among the 55 HIV positive individuals enrolled in the study, 31 had a TPPA positive result (31/55, 56,4%). Amongst the latter, the RPR resulted negative in 17 cases and positive in 14 of them (14/31, 45,2%). Two MSM with both TPPA and RPR positive results, although they were both aware of this previous, recent syphilis infection, were newly diagnosed with HIV.

As far as the *Treponema pallidum* infection is concerned, 154 (94,5%) of those resulted TPPA positive were already aware of their previous syphilis infection. In addition, 151 (98,1%) of them were previously treated for syphilis, whereas 9 individuals were newly diagnosed. Among the latter, 3 were also RPR positive.

POCTs results and laboratory titrations

Table 2 shows the TPPA titration in relation to the number of reactive POCTs.

For each titration level, the percentage of Bioline positive POCTs reached 83,3% or above when the TPPA titre was more than or equal to 1280. In contrast, when the TPPA titres were lower than or equal to 640, the percentage of positives was 50,0% or lower. As far as the treponemal component of the Chembio test is concerned, with TPPA titres higher than or equal to 1280 the positivity rate was 88,9% or above for both reader and microreader. With lower TPPA titres the percentage of reactive results was 68,5% or lower and 62,5% or lower for reader and microreader respectively (Table 2).

When the RPR titration is considered, for low titres (< 1:8) the misclassification rate for Bioline test was 2,5% whereas for the Chembio test, the human reader and the microreader yielded a misclassification rate of 2,5% and 10,0%, respectively.

When RPR titration values are equal to or more than 8, there was no discrepancy between POCTs treponemal result and that of the laboratory test (Annex 2).

Among the four TPPA+/RPR+ cases which would have been missed if only the POCTs had been used, 3 reported a previous syphilis diagnosis and treatment (possible serofast state), whereas one had not been treated previously.

Table 2 – SD Bioline and Chembio POCTs treponemal component reactive results according to TPPA titre

TPPA Titre	**Bioline Reader**			**Chembio Reader**			**Chembio MR**		
	Tot	*Reactive*	*%*	*Tot*	*Reactive*	*%*	*Tot*	*Reactive*	*%*
20480	45	44	97,8	45	44	97,8	45	42	93,3
10240	22	20	90,9	22	20	90,9	22	19	86,4
5120	26	25	96,1	26	25	96,1	26	25	96,1
2560	19	16	84,2	19	17	89,5	19	18	94,7
1280	18	15	83,3	18	16	88,9	18	16	88,9
640	16	8	50,0	16	11	68,7	16	10	62,5
320	12	0	0	12	1	8,3	12	2	16,7
160	4	0	0	4	1	25,0	4	0	0
80	8	1	12,5	8	2	25,0	8	1	12,5
Tot	170	129	75,6	170	137	80,6	170	133	78,2

R1-R2-MR concordance (Cohen's Kappa) and POCTs agreement

The concordance and the agreement between human readers and, where available, the two readers and the Micro Reader for both the Bioline and Chembio POCTs is shown in Table 3.

For both HIV and syphilis component of both POCTs, the agreement resulted almost perfect (min 98,0% and 99,8% regardless the type of infection) with Cohen's Kappa [58] that ranged from 0,95 to 0,99.

As for the Bioline POCT, the agreement between readers (R1-R2) was 99,8% for HIV and 99,0% for syphilis. With regards to the HIV component of the Chembio test, the agreement between readers (R1-R2) and readers and Micro Reader (R1-MR; R2-MR) was 99,4%, 99,0% and 99,2% respectively. As far as the treponemal component of the same POCT is concerned, the percentage of agreement was 99,4% between readers (R1-R2) and 98,0% and 98,6% between R1-MR and R2-MR respectively (Table 3).

Table 3 – Agreement between readers (R1-R2) and, where available, readers and microreader (R1-MR; R2-MR) for both HIV and syphilis component of the Bioline and Chembio POCTs: Agreement and Cohen's Kappa values (per infection)

			Agreement	Expected	Kappa	Z	Prob>Z
BIOLINE	**HIV**	**R1-R2**	99,80%	79,95%	0,99	21,93	0,00
	SYPHILIS	**R1-R2**	98,98%	60,97%	0,97	21,58	0,00
CHEMBIO	**HIV**	**R1-R2**	99,39%	78,70%	0,97	21,52	0,00
		R1-MR	98,98%	79,01%	0,95	21,09	0,00
		R2-MR	99,19%	79,16%	0,96	21,30	0,00
	SYPHILIS	**R1-R2**	99,39%	59,68%	0,98	21,82	0,00
		R1-MR	97,96%	59,94%	0,95	21,04	0,00
		R2-MR	98,57%	60,04%	0,96	21,37	0,00

R1 = Reader 1; R2 = Reader 2; MR = Micro Reader

Discussion

POCT performance evaluation

In recent years, the body of literature on HIV and syphilis POCT evaluations and comparisons with laboratory tests has increased, providing valuable information on performance in different settings, with different populations and for different purposes.

Although the POCTs analytical characteristics and their procedures do not appear to be affected by the context, the literature suggests that the epidemiological characteristics of the population may have a significant impact on the information provided by POCTs' results [29].

A strength of our study is that the ProSPeRo core protocol was developed following the QUADAS criteria [59 60]. In particular, the population among whom our study was conducted was well characterised and homogeneous so that our findings might be generalisable to all MSM living in Italy and, in a broader perspective, in other high-income Countries. In addition, the prevalence of the considered infections was not inferred from study findings but assessed directly in a bio-behavioural survey carried out in Verona few years ago [2]. Furthermore, the laboratory tests, to which the POCTs under evaluation were compared, were fully independent from the index tests and represents the gold standard reference tests as described in detail in the literature and in STIs international diagnostic guidelines [61 62].

In 2017 a systematic review by Gliddon et al. was undertaken [34]. The purpose of this review was to assess the diagnostic accuracy, acceptability and cost-effectiveness of the SD Bioline HIV/Syphilis Duo test, Chembio DPP HIV/Syphilis Assay and MedMira Multiplo Rapid TP/HIV Antibody Test. It should be noted that among the studies considered in the review, only six [63-68] had the aim of assessing the performance of the considered POCTs among MSM, the population that accounted for the majority of HIV and syphilis new infections yearly in high-income countries.

In addition, as pointed out in the review, the setting in which the POCTs evaluation is carried out plays a major role as in the field the control over experimental variables might be much lower than in the laboratory. As STIs POCTs are meant to be used outside the ideal environment of the laboratory, clinical validation exercises

may represent the best way to assess the performance of the diagnostic tool in a real-life setting.

Among the studies considered in the review, only four were carried out in the field on fingerpicked whole blood [63 66-68]. Additionally, only the paper written by Bristow et al in 2016 was specifically and exclusively addressed to MSM (including transgender women) [67] whereas the other papers included other populations with higher risk for STIs.

In 2018, Withers et al published a field evaluation of the SD Bioline HIV/Syphilis POCT among MSM and pregnant women living in Hanoi (Vietnam) [68]. As the MSM enrolled in the study accounted for all HIV and treponemal positive results of both POCT and reference test, we can assume the POCT performance assessed in the study as that in of the MSM population enrolled in the study.

Comparing our findings with those reported in the literature, the performance of the HIV component of both the SD Bioline HIV/Syphilis Duo test and Chembio DPP HIV/Syphilis Assay was found to be optimal and in line with that previously described.

As far as the treponemal component of the evaluated POCTs is concerned, there was a considerable variation when it comes to whether specificity was found to be 98% or higher sensitivity.

The SD Bioline treponemal sensitivity was lower in our study than in the paper written by Bristow et al in 2016 [75,8 (95%CI 68,8-86,3) *vs* 89,2% (95%CI 83,5-93,5)] [67]. In addition, the recently published systematic review [34] on HIV and syphilis POCTs performance amongst different populations, found that the treponemal sensitivity of the Bioline POCT ranged from 89% to 100%, being more aligned to that reported by Bristow et al that report by our study. However, our datum of a lower sensitivity of the treponemal component of the Bioline POCT is similar to that described by Withers et al, particularly when confidence intervals are taken into consideration [sens 83.1% (95% CI: 71.0–91.6%)] [68].

There are a number of explanations of the possible causes of this discrepancy in Bioline POCT treponemal sensitivity. First of all, our datum might be due to errors in performing the POCT itself and/or interpreting its results. However, given our previous experience in evaluating syphilis POCTs [31], the training provided us by

POCTs' manufacturers at the beginning of the enrolment phase as well as the optimal performance in the internal and external quality control tests throughout the whole study period and the high sensitivity of the HIV component of the same POCT, it is unlikely that this is the case. A second possible explanation could be the lower proportion of probable active syphilis cases in the study sample. Indeed, although in the study published by Bristow et al the TPPA positive rate was quite similar to that found by us [167/415 (40,2%) vs 169/492 (34,3%)], the frequency of RPR positive results and, in particular, those with high titre (> 1:4) was much lower in our study [RPR positive results: 143/167 (85,6%) *vs* 53/169 (10,8%), high titre RPR: 53/143 (37,1%) *vs* 13/53 (24,5%)]. Even in the study by Withers et al, all the treponemal positive results were in individuals already aware of being infected by *Treponema pallidum* and already treated [68]. In this study, although non-treponemal laboratory tests were not performed, it is highly likely that the number of individuals with high titre RPR positive results would have been very low if not null.

Comparing our findings with those already published in similar studies and evaluating the discordant results is a productive exercise as it helps in outlining a trajectory by which treponemal POCTs seem to be adequate tools for active syphilis cases, whereas they might be suboptimal for latent syphilis cases. It is well known that there is a link between treponemal and non-treponemal test positivity and titration. Active syphilis cases are serologically defined by RPR titres higher than or equal to 1:8 and, in these cases, TPPA titre is usually far higher than 1:1280.

In these conditions, syphilis single rapid tests and the treponemal component of dual HIV/Syphilis POCTs show better sensitivities and misclassification rate is rather negligible [31 67].

As for the treponemal sensitivity of the Chembio POCT, it is almost doubled in our study with respect to that reported by Hess et al in 2014 [81,7% (CI95% 72,4-89,0) *vs* 47,4 (CI95% 36,0-59,1)] [63]. Even considering the HIV/Syphilis POCTs systematic review published in 2017, the sensitivity of the treponemal component of the Chembio POCT varied largely (even excluding the performance reported in the Hess study, that was included in the review, CI95% ranged from 57% to 99%) [34].

As described for the Bioline POCT, a probable explanation for this wide range of treponemal sensitivity is the fact that, considering different STIs high risk population along with MSM, the prevalence of probable active syphilis cases might decrease and, reducing the number of high titre RPR cases, the misclassification rate of the POCTs can be higher (false negative results).

To the best of my knowledge, only one study evaluated the performance of the Chembio DPP HIV/Syphilis Assay using the MR [65]. Although this study has several similarities to ours in terms of procedures, reference tests and population, it was carried out in a lab setting using stored serum samples. In terms of MR evaluation, using the same MR cut-offs used in our study, Leon et al reported better performance than ours for the treponemal component of the Chembio POCT. Indeed, as sensitivity and specificity for HIV were optimal in both studies [Leon et al: sens 100% (CI95% 97,6-100), spec 100 (CI95% 98,8-100); our study: sens 100,0% (CI95% 88,8- 100), spec 99,2% (CI95% 97,2-99,9)], for the treponemal component our sensitivity was lower than that previously reported [Leon et al: sens 94,7% (CI95% 89,8-97,7), spec 99,7 (CI95% 98,2-100); our study: sens 78,5% (CI95% 68,8- 86,3), spec 99,5% (CI95% 97,1-100)]. As the population in the two studies is homogeneous, although the rate of sera with RPR titres higher than 1:4 is not specified in the Leon's study, the reason for this discrepancy could be due to the different specimen taken into consideration. Indeed, in most cases, syphilis POCTs performance, especially in terms of sensitivity, is higher on serum than on blood [34]. It should be noted , however, that, in the context of a previous syphilis POCT validation study conducted among MSM living in Verona on both fingerpicked blood and sera, the performance of the Chembio DPP Syphilis Screen & Confirm Assay did not vary significantly according to specimen type [31].

To my knowledge, there have been no studies to date that have evaluated the performance of the Chembio DPP HIV/Syphilis Assay on fingerpicked blood and serum concurrently.

Public health considerations

As the major public health benefit in the use of HIV/syphilis POCTs among high risk groups is the lowering of the HIV/STIs testing threshold to implement

diagnosis and treatment, the performance of POCTs should be evaluated not only considering the analytical characteristics of the test themselves, but, in a broader perspective, considering their specific characteristics, such as being performed in non-medicalised, non-traditional diagnostic settings by non-medical staff and being able to attract anxious, never tested individuals.

In addition, although in our study the sensitivity of the treponemal component of both the POCTs under evaluation was lower than that declared both by manufacturers [52 53] and described in WHO TPP [69], as predictive values are influenced by the prevalence of the infection in the community, the HIV/syphilis POCTs NPVs are satisfactory. It means, in other words, that Bioline and Chembio POCTs might not be the best choice to screen populations with low HIV and syphilis prevalence, as their ability in ruling out the infection (negative predictive value) is inadequate, but they could be considered as alternative solution to laboratory tests to routinely screen all people belonging to high risk/high prevalence groups.

As reported in our study, the performance of the POCTs under evaluation was sufficiently adequate to guarantee the detection of new cases of both HIV and active syphilis.

Although TPPA usually remains permanently positive throughout individual's life despite the provision of an adequate syphilis treatment, in some cases treponemal titre might decrease over time. This is more likely to happen, for instance, in syphilis cases in which the time between syphilis acquisition and diagnosis is short and treatment is provided promptly. TPPA titre at diagnosis, indeed, is more likely not to be so high and it might reduce over time, achieving a full clearance during the following years. From this perspective, it should be pointed out that, in our MSM population, in which there is both a high syphilis prevalence and a quite good syphilis testing frequency, the rate of RPR-negative/low-titre TPPA positive results is significant (33,1%).

A reduction in TPPA titration under 1:1280 is usually sufficient not to be detected by treponemal POCTs, but not to affect laboratory TPPA detection. Nevertheless, in our study, 4 participants (2,5%) among those who reported a previous syphilis

diagnosis and treatment, resulted negative both to the treponemal component of the two POCTs under evaluation and the TPPA performed at the lab.

The clearance of laboratory TPPA or its persistence with low titres is more likely to occur in populations where there are high rates of screening, and this might be the case of the 33,1% of respondents enrolled in our study.

From a public health perspective, recommendations on the use of syphilis POCTs should be considered in a broad perspective in which several elements, such as syphilis prevalence and the related infection and re-infection rate, syphilis testing frequency and options on treatment provision are included. Indeed, all POCTs stakeholders, such as public health institutions and authorities, clinicians and researchers, should outline different scenarios and trajectories in which the proper balance between individual and collective health benefits is carefully evaluated and the proper screening tool is suggested. In the population enrolled in this study, for instance, the availability of laboratory treponemal and non-treponemal tests as well as the high proportion of individuals previously diagnosed and treated for syphilis, might have reduced the impact of POCTs. However, for those MSM living in Verona not aware of the services provided in our HIV/STIs clinic or not willing to overcome the psychological barrier represented by the medical setting or not perceiving themselves as at risk for syphilis or not willing to deal with their fear of needles, POCTs performed on fingerpicked blood in a non-medical setting might be the only way to have them tested. In addition, the impact of syphilis POCTs might be maximised when these tools are used to screen people never diagnosed with syphilis. This approach, indeed, would both reduce the chances of positive results due to the persistence of low treponemal titres even after an adequate syphilis treatment, and be more cost-effective, reserving syphilis rapid tests for those individuals for whom a treponemal positive result can be sufficient for receiving the treatment.

Finally, we believe that implementing STIs screening through the use of dual POCTs, according to the high acceptance rate of our study and the rapid turnaround of test results, might help MSM who have never been tested for HIV/STIs or that are willing to be tested only for a specific STIs, to enlarge their screening to other STIs (i.e.: HIV, hepatitis, syphilis).

Conclusions

- Every year, MSM accounted for the majority of new diagnoses of HIV and syphilis in Europe as well as in other high-income countries. To allow for the progression towards the universal health coverage, the integration of syphilis and other STIs screening services into existing HIV testing programs is a key element.
- HIV and syphilis dual POCTs are valuable tools to tackle these infections both in high income and in low-middle income countries. The routine use of HIV/Syphilis POCTs among target populations has to be considered in close relation with the epidemiological characteristics of the population itself, beyond the analytical characteristics of the test.
- Whereas HIV POCTs, both single and dual, have optimal performance and might be safely used among low-risk and low-prevalence populations, treponemal (syphilis) POCTs, both single and dual, should be used to test high risk individuals as the high prevalence of the infection would guarantee high predictive values.
- Although the Chembio Micro Reader would have reduced the risk of human misinterpretation of the POCT results, in this study the visual readout proved to be the same or slightly better, although not statistically significant, than the electronic reading.

Project 2

Clinic based evaluation of the dual GeneXpert CT/NG for the screening of extragenital chlamydial and gonococcal infections amongst MSM attending the Infectious Diseases and Tropical Medicine Unit, Verona University Hospital, Verona, Italy.

Background

Four fully curable STIs accounted for 1 million of new infections daily that turns into 376 million cases per year, according to the most recent WHO estimates [70]. In order of frequency, *Trichomonas vaginalis* accounted for 156 million cases, *Chlamydia trachomatis* for 127 million, *Neisseria gonorrhoeae* (gonorrhoeae) for 87 million and *Treponema pallidum* (syphilis) for 6,3 million of new cases in 2016 [45 70]. Peculiar characteristic of these STIs is that they are curable with a short course of anti-infective treatment which is cheap, available worldwide and, in most cases, administered orally.

As treatment is the natural consequence of a clinical suspicion or a microbiological diagnosis, the high rate of asymptomatic infections and the lack of a capillary distribution of diagnostic technologies represent the basis by which these four STIs might continue to spread, year after year, in the community. Unfortunately, the complete lack of signs and symptoms is quite a common feature of these four STIs as it has been reported that up to 50-80% and 10-40% of genital infections are fully asymptomatic in women and men, respectively [71]. It should be pointed out that women are disproportionally affected by sexual and reproductive health long-term consequences and sequelae. Indeed, these undiagnosed, and consequently untreated, infections could be responsible for chronic inflammation and subsequent scars which can lead to pelvic inflammatory disease [72] and hampered pregnancy (i.e. poliabortivity, ectopic pregnancy, infertility, pre-term delivery, premature rupture of membranes) [71].Moreover, new-borns might suffer from acute conjunctivitis that progressively can lead them to blindness [73]. In addition, it has to be considered that, in extragenital sites, such as rectum and pharynx, both in women and men, CT and NG infections are almost always asymptomatic [13 74] and, as far as syphilis is concerned, the chancre has more chances not to be seen.

Considering only symptomatic cases of syphilis, *Trichomonas vaginalis*, CT and NG infections underestimates their true prevalence and their real burden worldwide. It is however to be said that collecting reliable data on the prevalence of asymptomatic infections is challenging as the lack of signs and symptoms, that force the individual to look for medical assistance and guide the healthcare staff in prescribing the proper diagnostic-therapeutic pathway, make these infections be

diagnosed and treated only when STIs screening approach is widely implemented. On top of this it has to be noticed that screening policies, even where implemented, might not show the real prevalence of STIs. Indeed, as for CT infection, screening practice is far more common amongst women than men and, in both genders, it is considered for genital sites but not for extragenital ones [75], such as rectum and pharynx, where this infection is almost always asymptomatic. Unfortunately, *C. trachomatis* infection has shown to noticeably affect both genital and rectal site in women and men reporting extragenital exposures [12 76].

Although the greatest burden of the four above-mentioned curable STIs is carried by low and middle income countries [45], in EU/EEA, as well in the United States, Canada and Australia, few groups of people accounted for the vast majority of STIs such as HIV, syphilis and gonorrhoeae. Among the so-called key populations, as defined by the WHO in 2016 [32], MSM carry the highest burden of these infections in high income countries [4 9 10 77].

Ultimately, asymptomatic infections represent a never-ending reservoir for the spread of these pathogens in the community and in augmenting the risk of HIV transmission [47 78 79]. Screening and appropriate treatment for these infections are key elements in preventing this cascade.

MSM, as well as other populations at higher risk for STIs, often experienced discrimination, stigma and inequalities in several aspects of their life, including health care environment.

The relationship between HIV/STIs epidemic and structural, interpersonal and internalized stigma towards minorities (ethnic, racial, sexual orientation, religion, etc.) has already been described [80]. Indeed, individuals living in highly stigmatising contexts are more likely not only to engage in higher risk sexual behaviours, but also to receive insufficient health care access. The latter is caused by both the individual's personal fear and the objective chance of being judged, discriminated and rejected from health care staff, who quite often, lacks of sufficient education [81 82]. In this context, it is easy to understand that if people at higher risk for STIs are prevented to look for health services even when forced by symptoms, this becomes even more obvious with asymptomatic STIs, which is the far majority of cases in extragenital sites [83].

Despite the fact that psychosocial barriers to HIV/STIs testing and care are still tangible, representing a block in the HIV treatment cascade as well as in the unalienable human right to health [82], from a technical viewpoint, the screening for some of the most frequent STIs, such as gonorrhoeae and chlamydial infection, has become far easier with the arrival of nucleic acid amplification tests (NAATs). This technique is nowadays the golden standard for diagnosing these infections [61 84] as it has almost perfect analytical characteristics and collecting and transporting biological samples is far easier than for classical microbiological methods. On the downside, this technique is complex and it is not widely available in most healthcare settings in developing countries as it needs expensive equipment, specialized facilities, highly trained technicians and regular maintenance. Consequently, although the performance of laboratory tests is almost perfect, they are inaccessible to most people in the world [23] and relatively few of them can benefit from appropriate diagnosis and treatment.

In order to overcome the lack of accessibility to STIs facilities and testing sites, new diagnostic tests have been made available in the last decades. All these new rapid tests are characterised by the fact of being performed outside the laboratory setting and by a rapid turnaround time for test result (maximum 90 minutes). These features allows for two main achievements: the shift of testing places from the lab to POC or near-to-the-patient testing sites (i.e. patient's bedside, medical office, non-medicalised testing venues) and the chance of testing the client and providing him/her a proper treatment within the same clinical encounter.

To guide manufacturers in the development of the "optimal" STIs POCTs, in 2006 the WHO published the ASSURED criteria [23] and, since 2017, updated records on the features of available and in the pipeline STIs POCTs are published by the WHO on its STI POCT webpage [16].

Despite this huge effort in categorising and evaluating POCTs, there are still concerns on their use in clinical practice and in the potential replacement of standard laboratory methods with the POCTs approach. Clinicians and policy makers such as other stakeholders feel indeed the need of a more comprehensive assessment of STIs POCTs in improving patient outcomes. As so far no one STIs POCTs is ideal, scoring the best in all the ASSURED criteria, finding the best

balance amongst these characteristics considering, as a key element, the complex scenario in which the POCTs have to be used is paramount. In other words, there is the urgent need of evaluating STIs POCTs performance considering at the same time the different prevalence of the considered infection in that specific population and the impact of POCTs use in terms of access to care, reduction in loss to follow-up, improvement of linkage to care and rapidness of treatment [49]. Concretely, accuracy and rapid turnaround time for results might be the most important features a STIs POCTs should have in high income countries. Otherwise, in low and middle income countries accessibility, both in terms of cost and deliverability, could prevail over the performance [21]. Finally, finding the best trade-off between acceptable risks and incremental clinical benefit cannot be disentangled from the features of the population we are considering and the socio-geographical context in which it lives [85 86].

As for gonorrhoeae and chlamydial infection is concerned, traditional POCTs, which are lateral flow immunochromatographic tests (ICTs) or optical immunoassays (OIAs), have shown low performance, especially in terms of sensitivity [35 87]. In addition, they need a significant number of steps for preparing the sample and performing the test and they require human visual interpretation. More recently, a new CT/NG POCT, which relies upon NAA technology, have been introduced on the market. Beyond the fact that this POCT needs electricity, it showed optimal performance for both pathogens using genital biological samples both in laboratory and small real-life evaluation studies [35], opening new boundaries for its extensive use in the field and for the detection of extra-genital NG and CT infections.

The aim of the present study is to assess the acceptability, the operational characteristics and the performance in a real-life clinical setting of the dual GeneXpert CT/NG for the screening of genital, anorectal and pharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections amongst MSM compared to that of laboratory NAATs. Our study is the Italian component of the broader ProSPeRo initiative as previously described.

Objective

The main purpose of this study is to determine the performance of the GeneXpert® CT/NG assay (Cepheid) for the screening of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections at genital and extra-genital sites (anorectal and pharyngeal) amongst MSM attending our HIV/STIs screening facility compared to that of the laboratory based nucleic acid amplification tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Secondarily, operational characteristics and acceptability to end-users (clients) of the above mentioned POCT are assessed.

Methods

Study sites and population

From May 2018 to February 2019, consecutive MSM presenting at the HIV/STIs screening facility of the Infectious Diseases and Tropical Medicine Unit of the Verona University Hospital were enrolled prospectively in the study after the signing of the informed consent. Participants were asked to take part in the study only whether they were adult male (≥18-year-old) and they had had sex with at least another man over the last 12 months.

Enrolled MSM received an automatically generated unique bar code to participate anonymously and to link participants to their own test results. According to the WHO core protocol of the study, a structured questionnaire was used to collect information about demographic, behavioural characteristics, participant's STIs history and acceptability of the POCT approach.

Considering that there are no data on NG and CT prevalence at extragenital sites among MSM, the prevalence used for the POCT evaluation was based the results of a systematic review published by Chan et al in 2016 on CT and NG extragenital infections [88]. In this review, the median prevalence of NG infection was 4,6% and 5,9% at pharyngeal and rectal site respectively, while for CT the median prevalence was 1,7% in the pharynx and 8,9% in the rectum [88].

We assumed, for both NG and CT, a specificity of 98% and a reduced sensitivity (80%). It has to be noticed that, in terms of sensitivity, we adopted a very conservative approach as we assumed a POCT sensitivity far lower than that both declared by the manufacturer [89] and recommended by WHO standards for NG and CT rapid diagnostic tests [90]. Under this assumption a confidence interval width of 10% was assumed. The formula used for the sample size calculation is based on the 2006 WHO/TDR expert panel document on the evaluation of new diagnostic methods and techniques [51].

As standard approach, the sample size is adjusted for the disease prevalence using the formula (100/prevalence x sample size using sensitivity/specificity only) [51]. Based on these data, assuming a NG and CT infection (all anatomical sites) prevalence of 10%, the estimated number of patients to be recruited was 615.

Considering this data, the positive cases to be therefore recruited by the study were 61 for NG and 61 for CT.

According to the contribution of participants as foreseen by the ProSPeRo initiative, we enrolled 300 MSM. This sample size, according to the above-mentioned prevalence, provided 30 cases of *C. trachomatis* and *N. gonorrhoeae* infections, respectively.

POCT under evaluation

The test evaluated in this study was the GeneXpert CT/NG assay (Cepheid, Sunnyvale, California, United States; hereafter termed CT/NG POCT).

The GeneXpert Instrument System platform is a simple, cartridge-based system that automatically integrates all the real-time PCR steps, from sample processing to DNA extraction, amplification and detection. Once collected, the specimen is transferred, using the pipette provider by the manufacturer, to the cartridge that then is directly loaded onto the GeneXpert System.

The CT/NG assay is designed to detect presence of one chromosomal target for *C. trachomatis* (CT1) and two separate, highly conserved, genomic DNA targets that are unique to *N. gonorrhoeae* (NG2 and NG4) and not found in other Neisseria species. CT/NG POCT results (reactive, non-reactive, invalid, error) are obtained in 90 minutes and displayed separately for CT and NG.

Although CT/NG POCT was conceived for the use with genital and urine specimens (first catch urine in men, urine, endocervical specimens and vaginal swab specimens in women), Food and Drug Administration cleared its use with extragenital specimen (pharyngeal and anorectal swabs) in May 2019 [91].

The CT/NG POCT was donated by Cepheid to the WHO in the context of the ProSPeRo initiative. The manufacturer was not involved in any part of the study (study design, data collection, data analysis, data interpretation and writing of the paper).

Reference laboratory test

CT/NG POCT results were compared with those of the laboratory Cobas 4800 CT/NG test. This is an *in vitro* NAAT for the qualitative detection of *C. trachomatis*

and *N. gonorrhoeae* in patient specimens. The DNA targets include all 15 major CT serovars, including the Swedish CT mutant (nvCT), and both wild-type and variant DR-9 sequences of NG [92].

Specimens were collected in Cobas PCR media and tested according to the manufacturer's instructions [92].

Quality Assurance

Both the clinic and the Laboratory entered into both internal quality control (IQC) and external quality assessment (EQA) testing programme throughout all the duration of the POCT evaluation.

As far as internal controls is concerned, CT/NG POCT has already an internal quality control system based on the presence, in the cartridge itself, of three controls: the Sample Processing Control, which has been added to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction, the Sample Adequacy Control, which detects the presence of a single copy human gene and monitor whether the specimen contains human DNA, and the Probe Check Control, that verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability [89].

As for the laboratory reference tests, to monitor the entire process and guarantee an adequate quality assurance, internal controls containing CT and NG DNA were added to all sample during preparation, amplification and detection phase.

IQC tests were conducted on a monthly basis using dry swabs with known bacterial loads (i.e. NG positive/CT negative, NG negative/CT positive, double negative, double positive) both at the clinic and at the lab using the CT/NG POCT and the reference test, respectively.

POCT and laboratory EQA tests were performed twice during the study period using a panel of 5 dry swab samples, provided us by WHO, with unknown result.

Specimen collection and testing procedures

Tools and testing procedures were developed during the research and pre-implementation phase of ProSPeRo, based on Cepheid's training and instructions

[93]. Considering the latter, the proper amount of specimen was collected using manufacturer's swabs, transfer pipettes and transport tubes.

For each study participant three types of specimens were collected simultaneously: urine, two oropharyngeal swabs collected by trained healthcare staff and two anorectal swabs self-collected by enrolled respondents according to clinic's standard of care. One swab per anatomical site and a proper amount of urine was used to perform the CT/NG POCT at the HIV/STIs clinic whereas the remaining swabs and urine were sent to the Microbiology Unit of the Verona University Hospital. At the laboratory, those specimens that could not be processed immediately were stored at 4°C and processed within 4 days maximum.

As soon as the CT/NG POCT displayed its result in the View Results window of the GeneXpert System, the health care staff recorded the result for both NG and CT in each considered anatomical site on a specific form. Specimens generating an error or invalid POCT result were retested whether specimen leftover was sufficient.

Study participants could withdraw their *N. gonorrhoeae* and *C. trachomatis* laboratory results few days later, using the bar code provided at the enrolment.

Pre- and post-test counselling was provided to all participants, according to the WHO recommendations and local clinical practice.

During the post-test counselling, in the case of laboratory test positive result, linkage to care and treatment was made available to the participant according to local protocols and standard of care.

Ethics

Before the enrolment started, research protocols were approved by both the local Ethics Committee (*Comitato Etico per la Sperimentazione Clinica - CESC delle province di Verona e Rovigo*, 1310CESC), the WHO Research Project Review Panel (RP2) and the WHO Research Ethics Review Committee (ERC).

In addition to the local standard procedure to guarantee anonymity and confidentiality of respondents' data, the bar-code system developed in the validation exercise previously conducted in Verona [31] was adopted to allow an appropriate link between the different types of data collected for each individual

(demographic and behavioural information, biological samples) and the withdrawal of reference test results few days after the enrolment.

Although the provision of treatment was directed only by a positive result of one or more of the laboratory tests, participants were informed of POCT results in order to give them the chance not to transmit further the infection in the community in the time between the testing day and that in which the laboratory results were available. In case of positive results of the laboratory tests, MSM received further information about the infection(s) and the related treatment during post-test counselling. The treatment provided was in line with national guidelines and local standards.

Finally, in line with the protocol, samples were stored at -80°C at the Microbiology Unit of the Verona University Hospital for the overall completion of the study (including publications).

Statistical Analysis

As there were no official data on NG and CT prevalence at extragenital sites amongst Italian MSM, the prevalence used for the POCT evaluation was based the results of a systematic review published by Chan et al in 2016 on CT and NG extragenital infections [88]. In this review, the median prevalence of NG infection was 4,6% and 5,9% at pharyngeal and rectal site respectively, while for CT the median prevalence was 1,7% in the pharynx and 8,9% in the rectum [88].

Sensitivity, specificity, Positive and negative likelihood ratio (MR), positive predictive value (PPV) and negative predictive value (NPV) for the index test in each anatomical site was estimated comparing the CT/NG POCT results with those of the gold standard laboratory test [56].

STATA Version 16.1 was used for all analyses (College Station, TX: StataCorp LP).

Results

Study population

From May 2018 and February 2019 (9-months period) 300 MSM were enrolled in the study.

All participants provided behavioural and clinical information and agreed to donate the biological specimens required by the study (urine, pharyngeal and rectal swabs).

The mean age of the participants was 37,3 (Median 33,5; SD 12,4; min 19, max 74) and, as shown in Figure 1, with a positive skewed distribution towards older age.

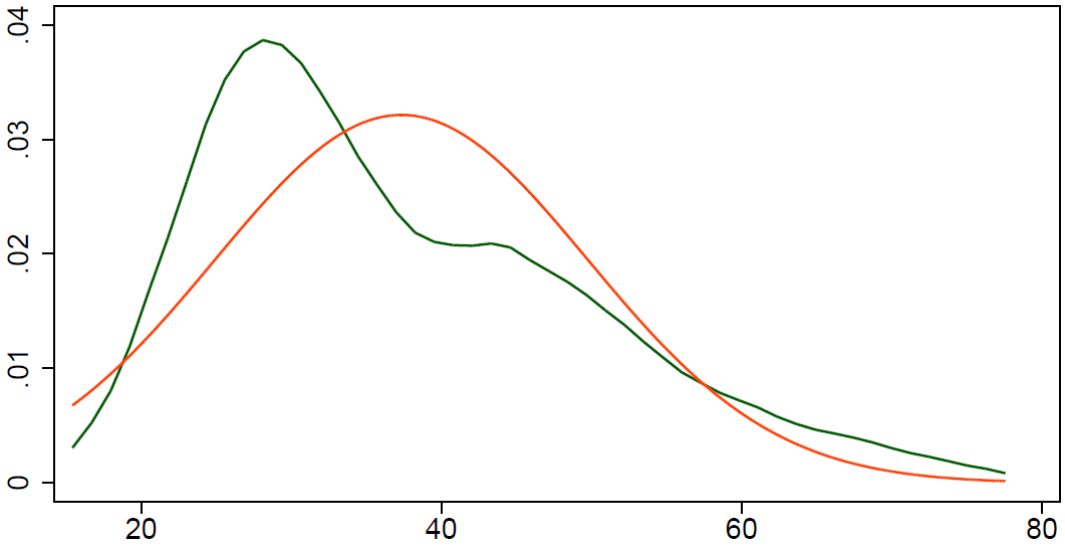

Figure 1: This graph shows age distribution amongst study participants: the green line represents the Kernel density estimate whereas the orange one shows the normal density.

As far as the presence of signs and symptoms is concerned, 58/300 MSM (19,4% CI95% 15,3-24,3) complained the presence of STIs symptoms at genital and/or extra-genital sites. In particular, 10,3% (31/300 CI95% 7,4-14,4) reported the presence of urethral symptoms such as dysuria and/or urethral discharge, 7,4% (22/300 CI95% 4,9-10,9) complained about the presence of anal/perianal discomfort (pain, discharge, tenesmus and bleeding) whereas 2,1% (6/300 CI95% 0,9-4,6) reported the presence of pharyngitis.

The majority of the study participants was fully asymptomatic (241/300, 80,6% CI95% 75,7-84,7) and a physical examination was performed only in 26/300

individuals (8,7% CI95% 6,0-12,4). Among them, 10 had a positive assessment (38,5%): 4 with urethral discharge, 4 with anorectal inflammatory signs such as anal discharge, ulcers and/or bleeding and 2 with pharyngeal erythema and white spots on the tonsils.

As far as participants' previous STIs history is concerned, 58,3% (175/300 CI95% 52,6-63,8) of enrolled MSM had been previously diagnosed with at least one STIs, 18,3% (CI95% 14,3-23,1) with two STIs, while 4,7% (CI95% 2,8-7,7) and 0,7% (CI95% 0,2-2,6) were previously diagnosed with three or more STIs, respectively.

The most frequent previously diagnosed STIs was syphilis (29,7% CI95% 24,7-35,1), followed by HIV (21,7% CI95% 17,3-26,7), gonorrhoeae (20,0% CI95% 15,9-25,0), condylomatosis (13,3% CI95% 9,9-17,7), *Chlamydia trachomatis* infection (11,4% CI95% 8,2-15,5), hepatitis A and B (4,0% CI95% 2,3-6,9 each), genital herpes (3,3% CI95% 1,8-6,1), hepatitis C (3,0% CI95% 1,6-5,7) and *Mycoplasma genitalium* infection (2,0% CI95% 0,9-4,4).

In terms of antibiotic exposure for any reason during the previous three weeks, 44 study participants (14,7% CI95% 11,1-19,2) answered positively.

POCT acceptability

Study participants have been asked about their willingness to wait for POCT result if that type of test would be routinely available at the screening facility. The majority of MSM reported to be willing to wait for the POCT result (280/300; 93,3% CI95% 89,9-95,7), with 25 (8,9% CI95% 6,1-12,9) willing to wait up to 20 minutes, 50 (17,9% CI95% 13,8-22,8) up to 30 minutes, 102 (36,4% CI95% 31,0-42,3) up to one hour, 78 (27,9% CI95% 22,9-33,4) two hours and 18 (6,4% CI95% 4,1-10,0) willing to wait for more than 2 hours.

Results of gold standard laboratory tests

Cobas CT/NG test yielded 37 positive results for *N. gonorrhoeae* overall (all anatomical sites) with a prevalence of 8,0% (CI95% 5,4-11,7). In terms of number of NG infections per site, 8 cases were diagnosed at genital site (urethra), 12 at pharyngeal site and 17 at rectal site.

As far as *Chlamydia trachomatis* infection is concerned, reference test reported a positive result in 32 study participants. Most of CT cases were diagnosed at rectal site (N=21) followed by urethral (N= 6) and pharyngeal (N=5) infections.

As NG and CT infection might be detected in more than one site for the same participant, the anatomical distribution of these infections is shown in Figure 2. In addition, some participants were diagnosed with concurrent NG and CT infection in the same site: 6 individuals were found to have both NG and CT infections at rectal site, 1 MSM were diagnosed with a double infection at pharyngeal site and 1 more at genital site.

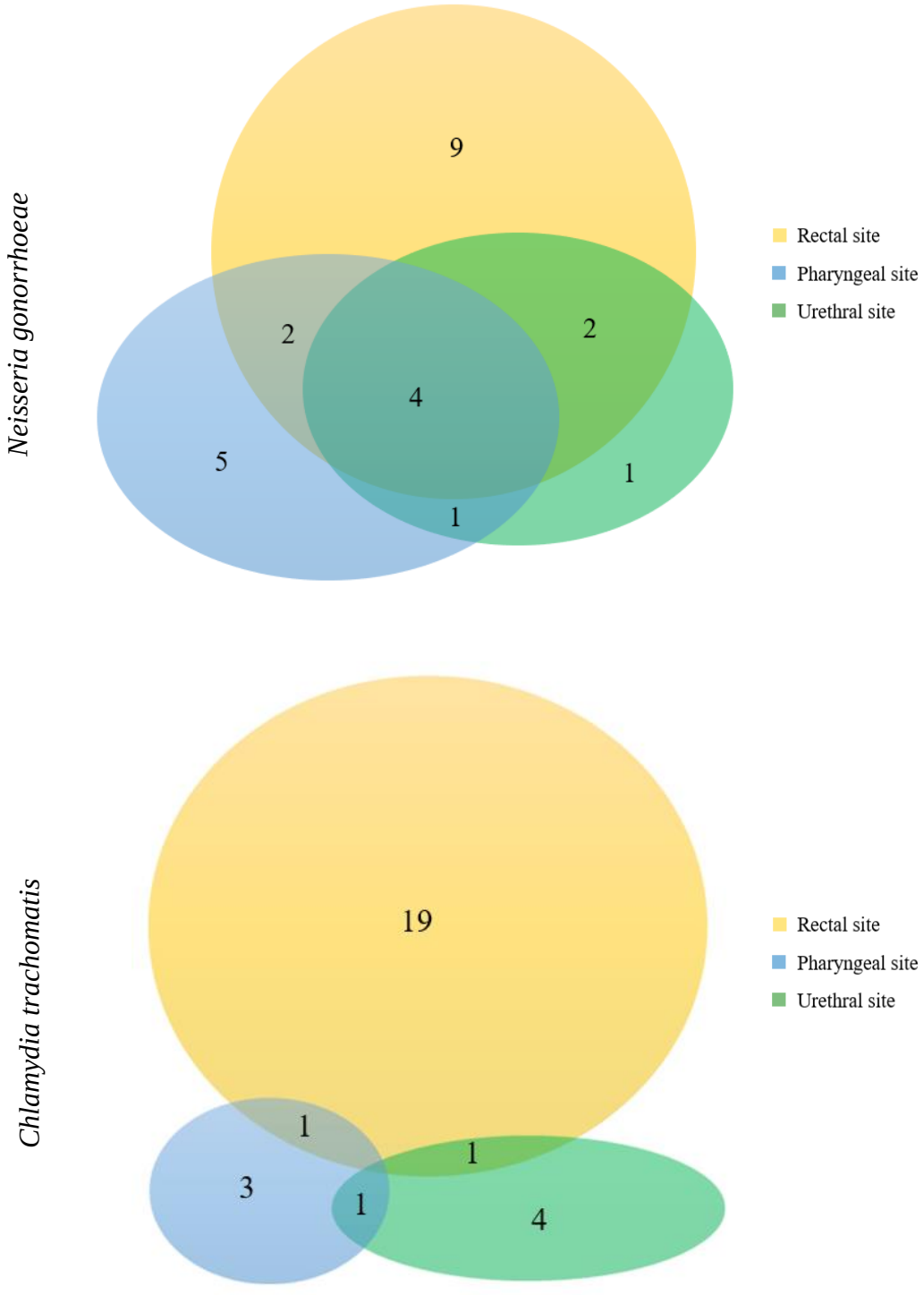

Figure 2: Number of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections per anatomical site.

POCT performance

During the 9-month enrolment period, 300 CT/NG POCT were performed at the HIV/STIs facility in Verona.

POCT sensitivity specificity, positive and negative LR as well as PPVs and NPVs, compared to the golden standard laboratory tests varied considerably according to the type of infection and the anatomical site considered (see Table 1).

As far as gonococcal infection is concerned, at genital site, the index test proved to have a sensitivity of 87,5% (CI95% 47,3-99,7) and a specificity of 99,7% (CI95% 98,1-100). The positive LR was 225 (CI95% 35,4-1833) while the negative one was 0,1 (CI95% 0,0-0,8). PPV was 87,5% (CI95% 47,3-99,7), while the NPVs was 99,7% (CI95% 98,1-100). With regards to the extra-genital sites, on rectal specimens sensitivity, specificity were the same as above [sens 87,5% (CI95% 61,7-98,4), spec 99,6% (CI95% 98,0-100)] whereas likelihood ratio and predictive values were higher according to the higher prevalence of NG infection at this site [LR+ 239 (CI95% 33,5-1705); LR- 0,1 (CI95% 0,0-0,5); PPV 93,3% (CI95% 68,1-99,5), NPV 99,3% (CI95% 97,4-99,9)]. At the pharynx POCT although specificity and the related NPV proved to be in line with those listed above (spec: 98,3% CI95% 96-99,4; NPV: 98,9% CI95% 96,9-99,8), sensitivity and PPV were lower (sens: 75,0% CI95% 42,8-94,5; PPV: 64,3% CI95% 35,1-87,2). As for the positive LR it was 42,9 (CI95% 17-109) while the negative one was 0,3 (CI95% 0,1-0,7).

As for *Chlamydia trachomatis* infection, at genital site, CT/NG POCT was found to have a sensitivity of 83,3% (CI95% 35,9-99,6) and a specificity of 98,6% (CI95% 96,5-99,6). The positive and negative LR was 61 (CI95% 21,5-172) and 0,2 (CI95% 0,0-1,0) respectively. PPV was 55,6% (CI95% 21,2-86,3), while the NPVs was 99,7% (CI95% 98,1-100). With regards to the extra-genital sites, on rectal swabs sensitivity was 90,0% (CI95% 68,3-98,8) and specificity was 98,5% (CI95% 96,2-99,6). Positive LR was found to be 60,3 (CI95% 22,6-161) whereas the negative LR was 0,1 (CI95% 0,0-0,4). According to the CT prevalence at this anatomical site (6,9%), PPV was 81,8% (CI95% 59,7-94,8) and the NPV was 99,2% (CI95% 97,3-99,9). At pharyngeal site, the POCT was found to have perfect performance although sensitivity positive LR and PPV's confidence intervals were pretty wide [sens: 100% (CI95% 47,8-100), spec: 100% (CI95% 98,7-100), LR + 539 (CI95%

33,4-8688) LR - 0,1 (CI95% 0,0-1,19), PPV 100% (CI95% 47,8-100), NPV: 100% (CI95% 98,7-100)] (Table 1).

Table 1 – POCT Sensitivity and Specificity (Part A), Prevalence, Predictive Values (Positive and Negative), Likelihood Ratio (Positive and Negative) (Part B) per type of infection and anatomical site, compared to the lab-based golden standard NAAT.

Part A

SITE		Sensitivity (%)	CI 95%		Specificity (%)	CI 95%		LR +	CI 95%		LR -	CI 95%	
URETHRA	**NG**	87,5	47,3	99,7	99,7	98,1	100	255	35,4	1833	0,1	0,0	0,8
	CT	83,3	35,9	99,6	98,6	96,5	99,6	61	21,6	172	0,2	0,0	1,0
RECTUM	**NG**	87,5	61,7	98,4	99,6	98,0	100	239	33,5	1705	0,1	0,0	0,5
	CT	90,0	68,3	98,8	98,5	96,2	99,6	60,3	22,6	161	0,1	0,0	0,4
PHARYNX	**NG**	75,0	42,8	94,5	98,3	96,0	99,4	42,9	17	109	0,3	0,1	0,7
	CT	100	47,8	100	100	98,7	100	539	33.4	8688	0,1	0,0	1.19

NG= *Neisseria gonorrhoeae*; CT= *Chlamydia trachomatis*

Part B

SITE		Prevalence (%)	PPV (%)	CI 95%		NPV (%)	CI 95%	
URETHRA	**NG**	2,7	87,5	47,3	99,7	99,7	98,1	100
	CT	2,0	55,6	21,2	86,3	99,7	98,1	100
RECTUM	**NG**	5,5	93,3	68,1	99,8	99,3	97,4	99,9
	CT	6,9	81,8	59,7	94,8	99,2	97,3	99,9
PHARYNX	**NG**	4,0	64,3	35,1	87,2	98,9	96,9	99,8
	CT	1,7	100	47,8	100	100	98,7	100

NG= *Neisseria gonorrhoeae*; CT= *Chlamydia trachomatis*

Discussion

NG and CT infections prevalence

As at National level there is no agreement on NG and CT screening in extragenital sites, there are no official data about the prevalence of these infections amongst MSM. To the best of my knowledge, only the study published by Foschi et al in 2017 reported the prevalence of rectal NG ad CT infection among MSM attending their STI clinic in Bologna [94].

At international level, many studies were published on this issue in the last decade, but NG and CT prevalence varied significantly in terms of both type of infection and anatomical distribution. In 2016, Chan et al published a systematic review to better assess the prevalence of pharyngeal and rectal CT and NG infections among women and men[88]. As for MSM, the median prevalence of NG infection was 4,6% (min 0,5% max 16,5%) in the pharynx and 5,9% (min 0.2% max 24%) in the rectum, while for CT the median prevalence was 1,7% (min 0 max 3,6%) and 8,9% (min 2,1% max 23%) for the two above-mentioned sites respectively [88].

Globally speaking, the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections found in our study seems to be consistent with that reported by Chan et al. In addition, if we exclude the non-European studies from the comparison, our results differ significantly only from the findings described by Diaz et al in 2013 (Rectal NG 21,1%, Genital NG 58,3%, no data for CT) [95]. We also compared our data with two very relevant studies that have been published since 2016, namely Sultan et al in England [96] and Foschi et al in Italy [94]. Both these studies reported NG and CT prevalence far higher than the median ones described in the systematic review and those found in our study.

It should be underlined that , in most cases, the MSM enrolled in the three above-mentioned European studies with the highest NG and CT prevalence, were mostly symptomatic, (presence of symptoms: 32,1% [94]- 72% [96]- 89,2% [95] vs 19,4% in our study). As the number of CT/NG positive cases is higher among symptomatic patients than among the asymptomatic ones, the difference in prevalence reported above may thus be explained. From this perspective, it would appear that focusing *N. gonorrhoeae* and *C. trachomatis* tests only on MSM reporting symptoms maximises the cost-benefit ratio. However, this would mean that extragenital

gonococcal and chlamydial infections are missed as they are fully asymptomatic in the majority of cases (25-100%) [88]. In addition, if MSM are tested for bacterial STIs only at urethral site (collecting urine and/or urethral swab), a significant number of rectal and pharyngeal CT/NG infections (14-85% [88]) would be missed representing a threat for both individual and public health. Lastly, undiagnosed and therefore untreated CT/NG infections might increase the risk of HIV transmission for the unaware infected individual and constitute a reservoir for the further transmission of the pathogen(s) in the community.

Pattern of CT/NG infection by site

The probabilities of detecting *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are strictly related to both the presence of signs and symptoms and, when they lack, the availability of multisite screening. Indeed, although nowadays NAA tests represent the reference method to ascertain the presence of *N. gonorrhoeae* and *C. trachomatis* with high accuracy, their result has anyhow to be attributed only to that specific anatomical site. Indeed, for CT/NG NAATs there is no available a single biological specimen that can be representative of all the potentially infected anatomical sites, as it is blood for serological tests. That is the reason for which STIs diagnostic recommendations suggest multisite testing for NG and CT amongst high-risk individuals.

As mentioned earlier, one of the elements that characterise CT and NG infections in extragenital sites, regardless gender and sexual behaviour, is the lack of specific symptoms and signs. Additionally, microbiological characteristics of the two pathogens themselves have to be considered. Looking at anatomical distribution of NG and CT infections, indeed, it is quite clear how their transmissibility, pathogenicity and fitness might change according to the mucosal surface involved (i.e. urethral, anorectal, oropharyngeal). The pattern of CT/NG infections by site as reported in this study confirms that chlamydial infections usually involve one anatomical site per individual, whereas *N. gonorrhoeae* is more often detected concurrently in different sites (multisite infection). This distributional pattern is consistent with that previously described in the literature where the percentage of

multisite concurrent infection ranges from 8,8% to 58% and from 10,2% and 28% for NG and CT respectively [97-101].

Quality assurance and control programme

As specified in the methodological component of this paper, a key element of our study was the assurance of optimal standard for the whole CT/NG POCT procedure. Cepheid provided our healthcare staff with a specific training at the beginning of the study ensuring strict adherence to the manufacturer's instructions In addition, the quality assurance (QA) testing programme and both internal and external quality control (QC) tests were regularly performed, according to the ProSPeRo standard operational procedures.

The need for QA and QC programmes for STIs POCTs, likewise for all other POCTs, is rooted in the subtle difference between providing reliable results or just results. For the latter, the ability of the POCT showing a result is enough, whereas the former requires standard operating procedures with the aim of preventing errors at each stage of the testing procedure and monitoring tester's ability in performing properly the POCT [102]. However, it should be pointed out that in contrast from laboratory QA and QC, STIs POCTs ones have to address end users who are often health-care personnel with very little or no experience in laboratory medicine and/or non-health care staff. For this reason, quality assurance and control programmes for STIs POCTs should be understandable for non-laboratory people and their workload should not be too heavy, so as not to discourage STIs POCTs users in participating regularly to the QA/QC programmes [103]. As far as our experience with STIs POCTs is concerned, the frequency and type of QA/QC tests were not so invasive with respect to the daily workflow. In particular, in the case of the CT/NG POCT, as three internal controls are already included in each cartridge, the internal QC of the ProSPeRo study were reduced to a monthly basis. External QC were performed twice during the study.

In addition to what is above stated, being part of a QA/QC programme has a psychological impact on end-users irrespective of background (health-care staff, clients, NGO volunteers, etc.). Having positive feedback from the laboratory, which is in charge for the QC programme, helps testers in developing the needed trust in

their ability of performing properly the POCT as well as stimulating their attitude in optimising further their performance, as in a virtuous cycle.

POCT performance evaluation and POCT clinical utility

Very little data are available on the performance of CT/NG POCT when used with extra-genital swabs. To fill this gap of knowledge, a laboratory validation to assess CT/NG POCT sensitivity and challenge its specificity with different species of the same genus (i.e. *non-NG Neisseria, non-CT Chlamydia, and non-TV Trichomonas species)* has recently been published. Authors concluded by supporting the use of CT/NG POCT not only for urogenital but also for anorectal and oropharyngeal specimens [38].

To the best of my knowledge this is the first study evaluating the performance in the field of the CT/NG POCT used with samples collected from genital and extragenital sites.

At genital site, this study provided lower sensitivity for both NG and CT than those available in the literature. The systematic reviews published on the Sexually Transmitted Infections Journal Special Issue in December 2017 found an overall sensitivity of the CT/NG POCT performed on urogenital samples of 97,5% (95%CI 91,4-99,7) for *C. trachomatis* and 98,0% (95%CI 88,4-99,9) for *N. gonorrhoeae* [35] [36]. Causer et al in 2015 published a field evaluation of the CT/NG POCT in which sensitivity was found to be 100% for both infections (95%CI 75,9-100 for CT and 56,1-100 for NG).

The reason for our discrepancy in sensitivity, might be due to errors in performing the POCT and/or reporting its results on the paper. However, as internal and external QCs were routinely performed during the whole study period and proved to be adequate, it is likely that there were no errors in the POCT procedure. A second reason that might explain the lower specificity could be related to the insufficient number of participants enrolled in this study. For this reason, additional studies may be required to achieve an adequate sample size and in fact, the broader PRoSPeRo initiative, of which our study is part, will enroll an adequate number of individuals worldwide as to achieve sufficient statistical power and narrow confidence intervals to guarantee the generalisability of the study results.

As for CT/NG POCT performance in extragenital sites, it is not possible to compare our results on pharyngeal samples as there is no available data on this area in the literature.

As far as the anorectal site is concerned, our findings are coherent with those already published. Two studies assessed the performance of the CT/NG POCT when used on rectal samples. The first one was published by Goldenberg et al in 2012 and, although rectal swab specimen had been collected from STI clinics attendees in London, the study was performed at the lab using the specimens' leftover from the laboratory reference test. The CT/NG POCT performance as reported in that study is quite similar to that found in our study for both chlamydial and gonococcal infections [CT: sens 86% (CI95% 72,1-94,7), spec 99,2% (CI95% 97,6-99,8); NG: sens 91,1% (CI95% 80,4-97), spec 100% (CI95% 99-100)] [104]. More recently, Badman et al assessed the performance of the CT/NG POCT among people with high risk for STIs in Papua New Guinea [105]. Surprisingly they reported very high sensitivity for both CT and NG [CT: 96,7% (CI95% 92,3-98,9), NG: 93,0% (CI95% 86,1-97,1) respectively], a perfect specificity for *N. gonorrhoeae* (100%, CI95%) and a slightly lower one for *C. trachomatis* (95,5%, CI95% 91,3-98).

A possible reason for the suboptimal sensitivity of the CT/NG POCT reported in the study carried out by Goldenberg et al, as acknowledged by Authors, was the need of diluting residual specimen transport media as they was supposed to contain interfering or inhibiting substance for the CT/NG POCT procedure. However, although in our study specimens were collected in the Cepheid transport reagent tube as per manufacturer instructions, the performance, above all in terms of sensitivity, was lower than expected and in line with that reported by Goldenberg et al. Nevertheless, it is important to state that, as for this study, sensitivity for both infections at rectal site was no lower than that found at genital site.

Whichever the CT/NG POCT performance, there are two additional perspectives that should be mentioned in the evaluation of a rapid diagnostic tool.

Firstly, the role of the CT/NG POCT has to be evaluated in a broader perspective, such as that described by the term "clinical utility". This new complex concept reduces the role of test's analytical characteristics increasing that of its impact in

improving health outcomes [106]. From this wider perspective, indeed, POCT evaluations should assess whether POC testing actually improves healthcare quality (i.e. treatment provision during the same clinical encounter, no/reduced loss to follow-up, no failure in withdrawing test results, better linkage to care), efficiency and cost-effectiveness [49 106]. To address this issue, POCT performance should be described in terms of likelihood ratio and test efficacy rather than sensitivity and specificity [49].

The results of the study confirm the role of the CT/NG POCT in guiding clinicians in not going ahead with additional tests or antibiotic prescriptions in case of negative result. Indeed, thanks to the high negative likelihood ratio of the CT/NG POCT, a negative result can rule out the presence of both infections regardless of the anatomical site considered (Figure 3). As for the positive likelihood ratio and the post-test probability, they varied largely across sites. They are indeed reliable diagnostic tools for gonococcal infection at genital, for both NG and CT at rectal sites and for CT only at pharyngeal site, whereas in genital samples for *C. trachomatis* and for gonococcal pharyngitis a positive result of the CT/NG POCT would have debatable impact on clinical decisions (Figure 3).

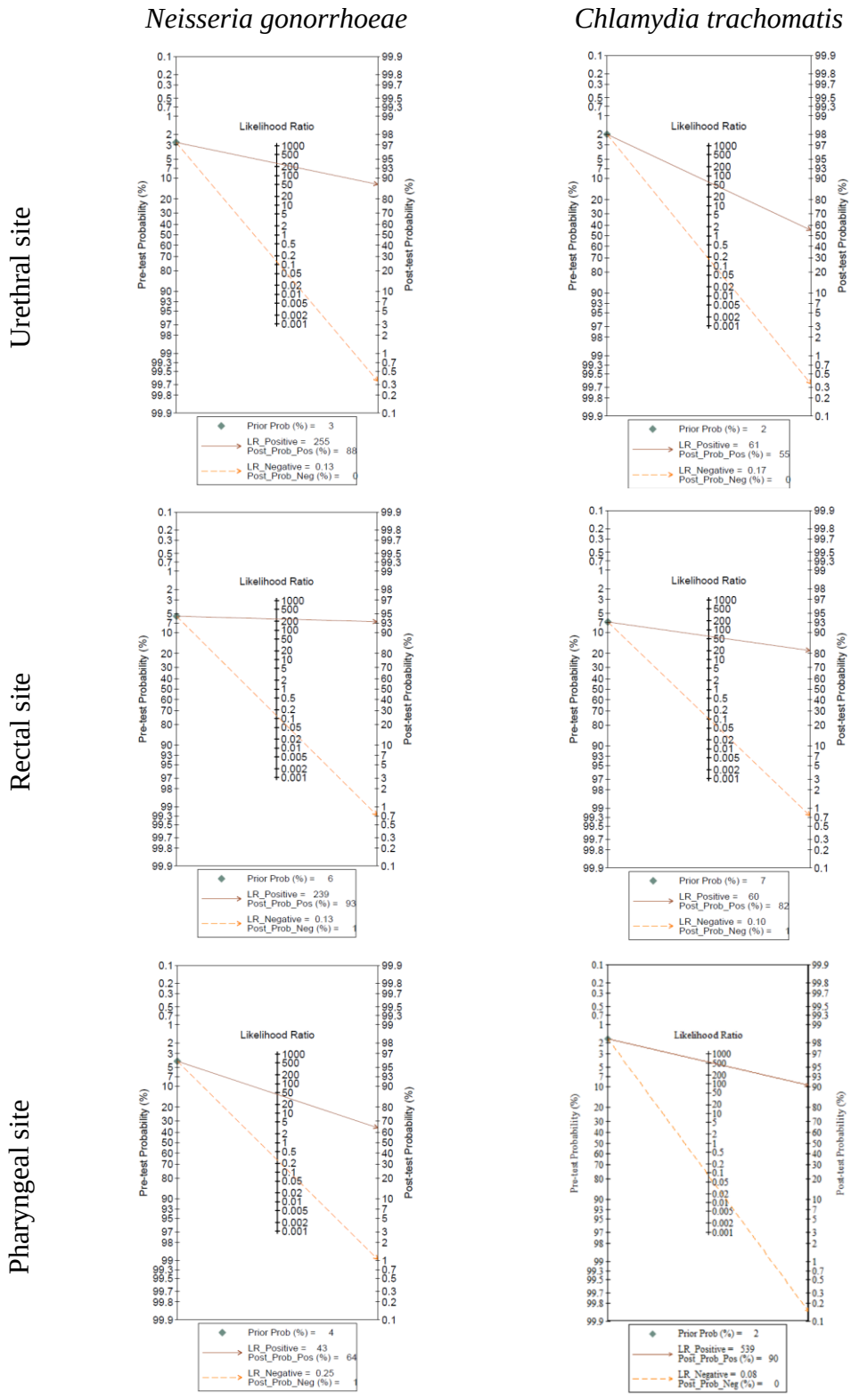

Figure 3: Fagan's nomograms per type of infection and anatomical site.

The second element that deserve to be considered by CT/NG POCT stakeholders is the establishment of the optimal compromise between test sensitivity and acceptability, accuracy and accessibility. As no test is perfect and it is scarcely likely that a POCT would be both as sensitive and specific as a laboratory test, a direct comparison of the diagnostic accuracy between the two tests might be deceptive. As shown by Gift et al in the paper on rapid test paradox, a POCT with a sensitivity much lower (63%) than that found in our study, may result in more infected people treated and more complications prevented when the laboratory test withdrawal rate is low (65-70%) [107]. In addition, Urdea et al stated that STIs rapid diagnostic tools with suboptimal performance, due to the chance, embedded in the test, of providing treatment within the same clinical assessment, could have maximal impact on people's quality of life, preventing long-term complications, averting stillbirths as well as congenital infections and reducing HIV transmission [85].

Conclusions

- *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection are common among MSM living in Verona. In most cases these infections are fully asymptomatic and are detected in extra-genital sites through screening practices using NAA tests. In settings where screening in both genital and extra-genital sites is not feasible, rectal and secondly pharyngeal screening should be prioritised.
- CT/NG POCT showed optimal specificity regardless of the type of infection and the anatomical site. In particular, our findings showed very low negative likelihood ratio and confirm NG/CT POCT ability in ruling out further assessment in case of negative result.
- In case of NG/CT POCT positive results, positive likelihood ratio varied largely across sites reaching values capable of guiding clinical decision for both infections only at rectal site. NG/CT POCT showed also good performance for gonorrhoeae at genital site and *C. trachomatis* infection on pharyngeal specimen.

General conclusions

- Independent STIs POCTs field evaluations should be a key component of the pathway of STIs diagnostic tools, from manufacturers to the market. This route should be outlined by public health international institutions as the studies' findings should represent the basis on which recommendations and guidelines for the use of the diagnostic tools should rely on.
- STIs POCT should be evaluated not only in terms of analytical characteristics but, above all, in terms of clinical utility. POCT stakeholders should put as much effort as is needed in finding the optimal threshold between accuracy and usefulness, acceptable risks and incremental clinical benefit. This cannot be disentangled from the features of the population we are considering and the socio-geographical context in which they live.
- Regardless the setting and the type of operator who perform the STIs POCTs, it would be mandatory to receive a proper training on how to perform and use at best that rapid tests. In addition, a quality management programme for STIs POCT should be set up. In that plan, quality assurance and control tests as well as the development of standard operational procedure manuals should play a major role in guaranteeing the availability of highly reliable POCT results despite the short turnaround time needed to produce them. Indeed, although easy to use and user friendly, POCTs should be managed with the same scrupulousness as laboratory tests as, ultimately, clinical decisions are based on their results.

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Annex 1

Maurine M Murtagh. “The Point-of-Care Diagnostic Landscape for Sexually Transmitted Infections (STIs)” 2019, The Murtagh Group, LLC.

Annex 2

SD Bioline and Chembio POCTs results among CLIA and TPPA-positive individuals: RPR-positive and RPR-negative individuals

	Laboratory test qualitative result			**Laboratory test Titres**		**Syphilis history**	**POCT – Treponemal component**		
ID	CLIA	TPPA	RPR	TPPA	RPR		Bioline Reader	Chembio Reader	Chembio MicroReader
RPR positive									
1	+	+	+	20480	128	Neg	R	R	R
2	+	+	+	20480	64	Pos	R	R	R
3	+	+	+	20480	32	Pos	R	R	R
4	+	+	+	20480	16	Pos	R	R	R
5	+	+	+	20480	16	Pos	R	R	R
6	+	+	+	20480	16	Pos	R	R	R
7	+	+	+	20480	16	Pos	R	R	R
8	+	+	+	20480	16	Pos	R	R	R
9	+	+	+	20480	8	Pos	R	R	R
10	+	+	+	20480	8	Pos	R	R	R
11	+	+	+	20480	8	Pos	R	R	R
12	+	+	+	20480	4	Pos	R	R	R
13	+	+	+	20480	4	Pos	R	R	NR
14	+	+	+	20480	4	Pos	R	R	R
15	+	+	+	20480	4	Pos	R	R	R
16	+	+	+	20480	4	Pos	R	R	R
17	+	+	+	20480	4	Pos	R	R	R
18	+	+	+	20480	4	Pos	R	R	R
19	+	+	+	20480	4	Pos	R	R	R
20	+	+	+	20480	4	Pos	R	R	R
21	+	+	+	20480	2	Pos	R	R	R
22	+	+	+	20480	2	Pos	R	R	R
23	+	+	+	20480	2	Pos	R	R	R
24	+	+	+	20480	2	Neg	R	R	R
25	+	+	+	20480	2	Pos	R	R	R
26	+	+	+	20480	2	Pos	R	R	R
27	+	+	+	20480	2	Pos	R	R	NR
28	+	+	+	20480	2	Neg	R	R	R

29	+	+	+	20480	2	Pos	R	R	R
30	+	+	+	20480	2	Pos	NR	NR	NR
31	+	+	+	20480	2	Pos	R	R	R
32	+	+	+	20480	2	Pos	R	R	R
33	+	+	+	20480	1	Pos	R	R	R
34	+	+	+	20480	1	Pos	R	R	R
35	+	+	+	10240	4	Neg	NR	NR	NR
36	+	+	+	10240	2	Pos	R	R	R
37	+	+	+	10240	2	Pos	R	R	R
38	+	+	+	10240	1	Neg	R	R	R
39	+	+	+	10240	1	Pos	R	R	R
40	+	+	+	10240	.	Pos	R	R	R
41	+	+	+	5120	8	Neg	R	R	R
42	+	+	+	5120	2	Pos	R	R	R
43	+	+	+	5120	2	Pos	R	R	R
44	+	+	+	5120	1	Pos	R	R	R
45	+	+	+	5120	1	Pos	R	R	R
46	+	+	+	2560	2	Pos	R	R	R
47	+	+	+	2560	1	Pos	R	R	R
48	+	+	+	2560	1	Neg	R	R	R
49	+	+	+	2560	1	Pos	R	R	R
50	+	+	+	1280	4	Pos	R	R	R
51	+	+	+	640	1	Pos	R	R	R
52	+	-	+	0	.	Pos	R	R	R
RPR negative									
53	+	+	-	20480	0	Pos	R	R	R
54	+	+	-	20480	0	Pos	R	R	R
55	+	+	-	20480	0	Pos	R	R	R
56	+	+	-	20480	0	Neg	R	R	R
57	+	+	-	20480	0	Pos	R	R	R
58	+	+	-	20480	0	Pos	R	R	R
59	+	+	-	20480	0	Pos	R	R	R
60	+	+	-	20480	0	Pos	R	R	R
61	+	+	-	20480	0	Pos	R	R	R
62	+	+	-	20480	0	Pos	R	R	R
63	+	+	-	10240	0	Pos	R	R	R
64	+	+	-	10240	0	Pos	R	R	R
65	+	+	-	10240	0	Pos	R	R	R
66	+	+	-	10240	0	Pos	NR	NR	NR

67	+	+	-	10240	0	Pos	R	R	R
68	+	+	-	10240	0	Pos	R	R	R
69	+	+	-	10240	0	Pos	R	R	R
70	+	+	-	10240	0	Pos	R	R	R
71	+	+	-	10240	0	Pos	R	R	NR
72	+	+	-	10240	0	Pos	R	R	R
73	+	+	-	10240	0	Pos	R	R	R
74	+	+	-	10240	0	Pos	NR	NR	NR
75	+	+	-	10240	0	Pos	R	R	R
76	+	+	-	10240	0	Pos	R	R	R
77	+	+	-	10240	0	Pos	R	R	R
78	+	+	-	10240	0	Pos	R	R	R
79	+	+	-	5120	0	Pos	R	R	R
80	+	+	-	5120	0	Pos	R	R	R
81	+	+	-	5120	0	Pos	R	R	R
82	+	+	-	5120	0	Pos	R	R	R
83	+	+	-	5120	0	Pos	NR	NR	NR
84	+	+	-	5120	0	Pos	R	R	R
85	+	+	-	5120	0	Pos	R	R	R
86	+	+	-	5120	0	Pos	R	R	R
87	+	+	-	5120	0	Pos	R	R	R
88	+	+	-	5120	0	Pos	R	R	R
89	+	+	-	5120	0	Pos	R	R	R
90	+	+	-	5120	0	Pos	R	R	R
91	+	+	-	5120	0	Pos	R	R	R
92	+	+	-	5120	0	Pos	R	R	R
93	+	+	-	5120	0	Pos	R	R	R
94	+	+	-	5120	0	Pos	R	R	R
95	+	+	-	5120	0	Pos	R	R	R
96	+	+	-	5120	0	Pos	R	R	R
97	+	+	-	5120	0	Pos	NR	NR	NR
98	+	+	-	5120	0	Pos	R	R	R
99	+	+	-	5120	0	Pos	R	R	R
100	+	+	-	5120	0	Pos	R	R	R
101	+	+	-	2560	0	Pos	R	R	R
102	+	+	-	2560	0	Pos	R	R	R
103	+	+	-	2560	0	Pos	R	R	R
104	+	+	-	2560	0	Pos	NR	NR	NR
105	+	+	-	2560	0	Pos	R	R	R

106	+	+	-	2560	0	Pos	R	R	R
107	+	+	-	2560	0	Pos	NR	R	R
108	+	+	-	2560	0	Pos	R	R	R
109	+	+	-	2560	0	Pos	R	R	R
110	+	+	-	2560	0	Pos	R	R	R
111	+	+	-	2560	0	Pos	R	R	R
112	+	+	-	2560	0	Pos	R	R	R
113	+	+	-	2560	0	Pos	R	R	R
114	+	+	-	2560	0	Pos	NR	NR	NR
115	+	+	-	2560	0	Pos	R	R	R
116	+	+	-	1280	0	Neg	R	R	R
117	+	+	-	1280	0	Pos	R	NR	NR
118	+	+	-	1280	0	Pos	R	R	R
119	+	+	-	1280	0	Pos	NR	R	R
120	+	+	-	1280	0	Pos	R	R	R
121	+	+	-	1280	0	Pos	R	R	R
122	+	+	-	1280	0	Neg	NR	NR	NR
123	+	+	-	1280	0	Pos	R	R	R
124	+	+	-	1280	0	Pos	R	R	R
125	+	+	-	1280	0	Pos	R	R	R
126	+	+	-	1280	0	Pos	R	R	R
127	+	-	-	1280	0	Pos	R	R	R
128	+	+	-	1280	0	Pos	R	R	R
129	+	+	-	1280	0	Pos	R	R	R
130	+	+	-	1280	0	Pos	R	R	R
131	+	+	-	1280	0	Pos	R	R	R
132	+	+	-	1280	0	Pos	NR	R	R
133	+	+	-	640	0	Pos	NR	R	R
134	+	+	-	640	0	Pos	R	R	R
135	+	+	-	640	0	Pos	R	R	R
136	+	+	-	640	0	Pos	R	R	R
137	+	+	-	640	0	Pos	NR	NR	NR
138	+	+	-	640	0	Pos	NR	NR	R
139	+	+	-	640	0	Pos	R	R	R
140	+	+	-	640	0	Pos	R	R	R
141	+	+	-	640	0	Pos	NR	NR	NR
142	+	+	-	640	0	Pos	NR	R	NR
143	+	+	-	640	0	Pos	NR	NR	NR
144	+	+	-	640	0	Pos	R	R	R

145	+	+	-	640	0	Neg	NR	R	R
146	+	+	-	320	0	Pos	NR	NR	NR
147	+	+	-	320	0	Pos	NR	NR	NR
148	+	+	-	320	0	Neg	NR	NR	NR
149	+	+	-	320	0	Neg	NR	NR	NR
150	+	+	-	320	0	Pos	NR	NR	R
151	+	+	-	320	0	Pos	NR	NR	NR
152	+	+	-	320	0	Pos	NR	NR	NR
153	+	+	-	320	0	Pos	NR	R	R
154	+	+	-	320	0	Neg	NR	NR	NR
155	+	+	-	320	0	Pos	NR	NR	NR
156	+	+	.	160	0	Pos	NR	NR	NR
157	+	+	-	160	0	Pos	NR	R	NR
158	+	+	-	160	0	Pos	NR	NR	NR
159	+	+	-	160	0	Pos	NR	NR	NR
160	+	+	-	80	0	Pos	NR	NR	NR
161	+	+	-	80	0	Pos	R	R	NR
162	+	+	-	80	0	Pos	NR	NR	NR
163	+	+	-	80	0	Pos	NR	NR	NR
164	+	+	-	80	0	Pos	NR	NR	NR
165	+	+	-	80	0	Pos	NR	R	R