A cost-effective, rapid point of care diagnostic platform for resistance guided and patient tailored treatment of bacterial and viral infections.

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Abstract

Sexually transmitted infections (STIs), especially among young adults, are surging, leading to long-term health complications and antimicrobial resistance. Current barriers, such as slow testing and stigma, result in inadequate treatment and antibiotic misuse. Sefunda is developing a rapid diagnostic platform that delivers comprehensive results, including antimicrobial resistance information, within 30 minutes at the point of care. Designed to meet U.S. CLIA waiver guidelines, this platform aims to address these challenges in emergency rooms, urgent care centers, pharmacies, and outreach centers, starting with a market launch in the USA. This innovative solution promises to transform STI diagnosis and management, promoting evidence-based, patient-tailored treatment while curbing antibiotic overuse and preserving treatment options.

Swiss precision meets American usability

Sefunda, founded 2019 in Switzerland, is an in-vitro diagnostics (IVD) company with a lean core-team with backgrounds in medical device(MD)/IVD development and microbiology/virology research. Together with an experienced MD/IVD developing partner, Sefunda developed a **fully automated, rapid, qualitative in-vitro diagnostic testing platform** intended for the use in a near patient/point-of-care setting. **A wide network of scientific, medical, and regulatory advisers** from Switzerland, and the U.S. is guiding the development of the first assay detecting nucleic acids from ***Neisseria gonorrhoeae*** **(NG) and *Chlamydia trachomatis* (CT) in physician-collected and self-collected clinical specimens from symptomatic and asymptomatic female and male patients**. The diagnostic platform is easy to use for an untrained person and displays a clear result in **less than 30 minutes** to guide immediate treatment decision in a busy emergency department, urgent care center, private practice or neighborhood pharmacy.

Urgent need for low barrier diagnostics

Sexually transmitted infections (STIs) remain prevalent and a major burden of morbidity and mortality globally. Left untreated they can cause serious reproductive health sequelae like infertility, chronic pelvic pain, ectopic pregnancy, or pelvic inflammatory disease. More than half of new STI diagnoses in the U.S. affect **youth below the age of 24** [1]. STIs also facilitate the sexual transmission of human immunodeficiency virus (HIV). ***Chlamydia trachomatis* (CT) remains the most prevalent bacterial STI**, is often asymptomatic in the acute phase of infection, and is commonly regarded as **the most frequent cause of female infertility** in developed countries. In addition, certain chlamydial serovars (LGV) can cause an invasive infection of the lymph system and require a more rigorous antimicrobial treatment. LGV infections are especially common among men who have sex with men (MSM); anorectal infections are significantly more frequent than urogenital infections [2] and **LGV is substantially underdiagnosed** [3]. Gonorrhea, caused by *Neisseria gonorrhoeae* (NG), has overlapping symptoms and long-term consequences with chlamydia, but the treatment differs, which makes it necessary to distinguish the infections for adequate treatment.

Molecular tests are recommended for the diagnosis of CT and NG, but the **results can take numerous days**. Studies have found that around 40% of patients with a positive test result that left untreated were lost to follow-up and never received treatment [4],[5],[6],[7]. Alternatively, many **physicians prescribe antibiotics before receiving a test result**, which can lead to significant overuse of antimicrobials (up to half of case where CT or NG infection is suspected but not confirmed[8],[9]) which ultimately increase side effects and promotes the spread of antimicrobial resistance.

Support the fight against antimicrobial resistance

Increasing antimicrobial drug resistance of NG has become a global burden. **NG is on WHO's list of priority pathogens**, whose drug resistance pose a **global health threat** [10]. As only few new drugs or a vaccine for gonorrhea are in development, and given the timeline to market introduction, it is essential to use the existing drugs as effectively as possible. Since 2021 only one last resort monotherapy is recommended for empiric treatment of gonorrhea by the CDC – intramuscular injection of the third generation cephalosporin ceftriaxone (CEF). The fluoroquinolone ciprofloxacin (CIP) is no longer endorsed as first line treatment since over 30% of registered cases in 2018 showed elevated tolerance. **Treatment guidelines are based on surveillance data** and cease to recommend drugs when the rate of resistant infections on a population-level increases. While on average about half of gonorrhea infections in the U.S. are estimated to be resistant to at least one antibiotic [11] **well above 60% of infections remained susceptible to CIP** over the last decade [12]. Irresponsibly using last resort treatments for these infections inadvertently shortens the time we can successfully used CEF for treatment.

CIP resistance is almost 100% correlated with a single nucleotide polymorphism (SNP) in the NG genome, which can be detected via PCR. The absence of this so called genetic antimicrobial resistance marker can predict susceptibility [13]. High-level azithromycin (AZM) resistance is mostly caused by two mutations in NG. AZM like CIP is not recommeded for the treatment of NG anymore because of increasing resistance rates but can cure both, uncomplicated CT and NG infections. CIP and AZM are orally administered, equally safe and widely available drugs and could replace the last resort CEF in susceptible cases – if diagnostic evidence including antimicrobial suseptibility data was available before treatment onset. Experts suggest combination therapies of CEF and AZM or CEF and CIP to help increase longevity of CEF by ensuring eradication of emerging CEF resistant strains if test results are available in time [14],[15].

There is clearly a **need for low barrier, affordable and rapid testing of CT and NG** along with an increasing **need for evidence based and resistance guided treatment** of gonorrhea to preserve existing treatment options and **prevent the spread of untreatable infections**. So far no test on the market can provide CT, NG and LGV results including antimicrobial resistance information in only one step at the point-of-care for genital and extragenital patient specimen.

Bringing the microbiology lab to the bedside

Sefunda is developing an infectious disease diagnostics platform that delivers actionable test results right **where counseling and treatment takes place** and **in time before treatment** decisions are made - **in under 30 minutes**. The platform is designed according to U.S. CSM CLIA waiver guidelines and intended to be used in emergency rooms, urgent care centers as well as non-clinical settings like pharmacies and outreach centers. The goal is an initial market launch in the USA.

The first assay detects CT and NG, discriminates between two types of CT and delivers information for 3 genetic resistance markers for NG together with the positive test result. The test enables healthcare professionals to immediately tailor antibiotic treatment for each patient based upon the infecting microorganism and susceptibility profile without the delays and costs for a comprehensive microbiological lab work-up.

The multiplex assay is based on real time quantitative PCR using fluorescent hydrolysis probes. Sample preparation, target amplification and detection are **automatically performed and contained** inside the cartridge, reagents for sample lysis are supplied in a liquid form, enzymes and reagents for detection are supplied lyophilized and **do not require refrigeration**. After nucleic acid extraction the system distributes the patient specimen into 5 parallel detection chambers for **multiplex amplification and detection**, 25 targets can be detected from one patient specimen. Currently 15 parallel targets are realized for the CT/NG assay for **dual target detection**, corresponding detection of the susceptible and resistant variant and an internal process control.

The design of the **single use cartridge is optimized for mass production** featuring a largely reduced complexity of only 3 functional layers (Figure 1). Proprietary reagent containers are used to reduce dead volumes and avoid the use of expensive blisters. The cost of goods is estimated to be **below $6**, including cartridge casing, chemistry, and packaging (semi-automated production $>$ 500 thousand cartridges/year).

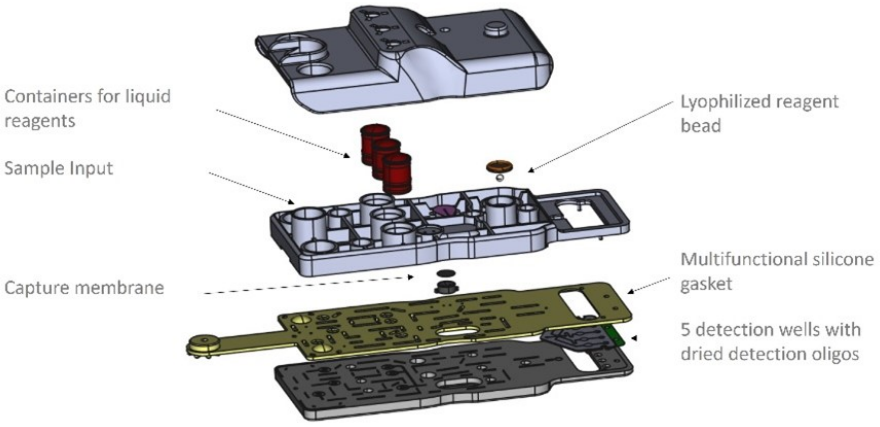

Figure 1: Representation of the cartridge structure

A second assay is currently under development. The combined SARS-CoV-2/Influenza A,B/RSV viral RNA test is designed for the same cartridge architecture, driving down manufacturing cost further. To optimize usage rates of the instrument in general health care settings the test menu will ultimately include bacterial and viral tests for sexually transmitted diseases, respiratory tract infections, nosocomial infection (hospital aquired infections) and emerging tropical diseases.

Integrated prototype in alpha-testing

Sefundas product development process features rapid iterations of prototype instruments and cartridges. Figure 2 displays the main development stages. Prototype v1.0 consists of a re-usable and screwable cartridge and two separate instruments for sample preparation and target amplification/detection. Prototype v2.0 is an integrated feasability demonstrator with pre-filled single use cartridges. Prototype v3.0 will be built to final functional design and footprint, note that the cartridge design is already close to final in prototype v2.0.

Protoype v1.0 was used to optimize the assay, roughly 1000 clinical samples were tested in house during that process. Prototype v2.0 has been alpha tested in a clinical laboratory by independent clinical personnel. The test demonstrated a sensitivity of over 90% and a specificity of 100% for both targets. TTR in this study was 32 minutes from sample input to result display for all targets combined (NG and CT species detection, AMR markers and LGV discrimination). A to date identified and currently addressed issue in the cartridge v2.0 built significantly compromised sensitivity compared to in house testing using prototype v1.0. It is expected that the overall sensitivity will increase with the next cartridge iteration (v2.1) when the built issue is solved. Initial usability feedback was solicited from clinical personnel operating the instrument during testing, respect data have been analyzed and will be used to optimize design features and streamline the workflow.

Figure 2: Development process instrument & cartridge

Next steps: Design finalization and analytical and clinical validation

As soon as analytical performance of the improved cartridge permits, design of the next instrument iteration (prototype 3.0) will be finalized. These units will be equivalent to the final product. About 6 units will be manufactured for further in-house pre-validation to gain in depth confidence in mechanical, optical and biochemical performance.

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