Chair/bedside diagnosis of oral and respiratory tract infections, and identification of antibiotic resistances for personalised monitoring and treatment

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Abstract: Global healthcare systems are struggling with the enormous burden associated with infectious diseases, as well as the incessant rise of antimicrobial resistance. In order to adequately address these issues, there is an urgent need for rapid and accurate infectious disease diagnostics. The H2020 project DIAGORAS aims at diagnosing oral and respiratory tract infections using a fully integrated, automated and user-friendly platform for physicians' offices, schools, elderly care units, community settings, etc. Oral diseases (periodontitis, dental caries) will be detected via multiplexed, quantitative analysis of salivary markers (bacterial DNA and host response proteins) for early prevention and personalised monitoring. Respiratory Tract Infections will be diagnosed by means of DNA/RNA differentiation so as to identify their bacterial or viral nature. Together with antibiotic resistance screening on the same platform, a more efficient treatment management is expected at the point-of-care. At the heart of DIAGORAS lies a centrifugal microfluidic platform (LabDisk and associated processing device) integrating all components and assays for a fully automated analysis. The project involves an interface with a clinical algorithm for the comprehensive presentation of results to end-users, thereby increasing the platform's clinical utility. DIAGORAS' performance will be validated at clinical settings and compared with gold standards.

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Chair/bedside diagnosis of oral and respiratory tract infections, and identification of antibiotic resistances for personalised monitoring and treatment

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Abstract. Global healthcare systems are struggling with the enormous burden associated with infectious diseases, as well as the incessant rise of antimicrobial resistance. In order to adequately address these issues, there is an urgent need for rapid and accurate infectious disease diagnostics. The H2020 project DIAGORAS aims at diagnosing oral and respiratory tract infections using a fully integrated, automated and user-friendly platform for physicians' offices, schools, elderly care units, community settings, etc. Oral diseases (periodontitis, dental caries) will be detected via multiplexed, quantitative analysis of salivary markers (bacterial DNA and host response proteins) for early prevention and personalised monitoring. Respiratory Tract Infections will be diagnosed by means of DNA/RNA

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differentiation so as to identify their bacterial or viral nature. Together with antibiotic resistance screening on the same platform, a more efficient treatment management is expected at the point-of-care. At the heart of DIAGORAS lies a centrifugal microfluidic platform (LabDisk and associated processing device) integrating all components and assays for a fully automated analysis. The project involves an interface with a clinical algorithm for the comprehensive presentation of results to end-users, thereby increasing the platform's clinical utility. DIAGORAS' performance will be validated at clinical settings and compared with gold standards.

Keywords. Antibiotic resistance, dental caries, centrifugal microfluidics, diagnostics, periodontitis, point-of-care, respiratory tract infections

1. Introduction

Communicable infectious diseases and concomitant antimicrobial resistance represent one of the major causes of the 52.8 million (year 2010) global deaths in the world [1]. Detection of the correct etiological agents (bacteria, viruses) associated with such infections is crucial for the correct treatment of patients. Unfortunately, however, microbial diagnosis still requires centralised diagnostic laboratories, where current diagnostic procedures may take several days to complete, often forcing doctors to make empirical, rather than evidence-based, treatment decisions. For a more accurate decision-making process, disruptive changes in the current practice of infectious disease diagnostics are required, namely: (i) shifting the *diagnostic methodology* from an empirical approach (based on clinical symptoms) or traditional laboratory approach (e.g., bacterial/viral cultures) towards multiplex molecular-based analysis (involving q-PCR and antigen detection); (ii) shifting the *diagnostic location* from centralised laboratories and diagnostic centres to the patient chair/bedside, all via a compact, user-friendly, easy-to-interpret diagnostic system.

In this respect, the H2020 project DIAGORAS aims to develop a point-of-care device that will provide accurate detection and diagnosis for: (i) a large panel of viral and bacterial respiratory tract infections (RTIs), including their associated antibiotic resistances (ABR), and (ii) oral infections such as periodontitis and dental caries. These diseases were chosen due to: 1) their extremely high occurrence; 2) their morbidity (RTIs among both adults and children); 3) their age-related nature (oral infections especially among elderly people; RTIs among both elderly people and children); 4) their contagious nature and the possibility of epidemics (RTIs); and 5) the global increase in antibiotic resistance.

2. Current state-of-the-art and challenges in diagnosis of infectious diseases

2.1. Respiratory Tract Infections (RTIs) and Antibiotic Resistances (ABR)

Upper and lower RTIs, e.g., pneumonia, pharyngitis, rhinitis, sinusitis, COPD (chronic obstructive pulmonary disease), tonsillitis, otitis media, etc, are among the main reasons for visiting a general practitioner (GP) [2] and one of the major causes for the unnecessary prescribing of antibiotics. Further, there are annually 5.5 million GP consultations for RTIs in England [3], and 2 million cases in the Netherlands [4]. Respiratory tract infections tend to share common symptoms even though the causative agents may differ widely in their nature. Currently, the most frequently used protocol

for diagnosing patients visiting their GP is via a short physical examination and empirical experience, for example a stethoscopic inspection of the lungs, looking for coarse breathing or crackling sounds. Further, although rapid diagnostic tests (RDTs), such as antigen or antibody test strips, may be used at some GP practices, in general, these tests lack sensitivity and their specificity may be sub-optimal.

In the above context, empirical-based therapy tends to lead to the prescription of broad-spectrum antibiotics. Even in the case of a non-empirical examination, when samples (typically nasal/pharyngeal swabs or sputum) are sent to a central laboratory for analysis, the diagnostic result may take a few days before it is communicated to the GP or clinician, who, in the meantime, tend to prescribe broad-spectrum and expensive antibiotics in order to bridge the gap between the taking of a sample and eventually knowing which pathogen is associated with an infection. This delayed or incorrect treatment increases the risk of the patient experiencing unnecessary side-effects e.g., diarrhea and allergic reactions; increases the costs of hospitalisation; increases the prevalence of antibiotic resistance in the community [5]); and can lead to the development of antimicrobial resistance with severe, sometimes even lethal consequences for the patient [6], [7].

2.2. Oral infections

Oral infections represent the most prevalent chronic diseases worldwide, accounting for almost 5 billion cases globally [8]. The two most prevalent bacteria-borne oral diseases are periodontitis and dental caries. Dental caries affects the hard tissue of the teeth, causing tooth decay, while periodontitis affects the tissues that surround and support the teeth, leading to progressive loss of the bone and soft tissue attachment, and eventually tooth loss. More than 50% of the European population suffers from periodontitis and over 10% develop severe complications, with the prevalence increasing to 70-85% in the age-group of 60-65 [9]. Thus, oral infections pose a very high global prevalence, severely deteriorating people's quality of life (through pain, discomfort, and loss of tooth function) and placing an enormous cost burden on global healthcare systems (in 2012, the costs on all aspects of oral care and treatment among the EU member-states was estimated to be €79 billion [10]).

In contrast to the aforementioned RTIs, where each disease is caused by a specific bacterium or virus, periodontitis and dental caries are related to the co-influence of several oral bacteria. In addition, not only the microbial species but also the host response to their presence must be monitored, for example via the measurement of the production of specific protein biomarkers, particularly in the case of periodontitis. The critical diagnostic point is to assess the degree to which the disease has progressed, in order to decide on the suitable treatment. This is where the quantitation in analysis of the associated microbes and host proteins is of major importance.

Currently, there is no system that enables both quantitative protein and quantitative bacterial analysis in the same test and in a multiplexed manner. This means that, for a truly detailed and concrete diagnosis, assessment of status and frequent monitoring, a patient's sample must be analysed using several methods, such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and bacterial detection by culture, or by labour-intensive microscopic imaging. These current standard laboratory techniques are practically inefficient for regular dental check-ups because of their time-consuming nature (it may take several days from sample collection to result reporting) and resource-consumption.

3. Objectives and technologies

The project aims at the following specific technical objectives in order to tackle the aforementioned challenges (the overall diagnostic workflow is shown in Fig.1):

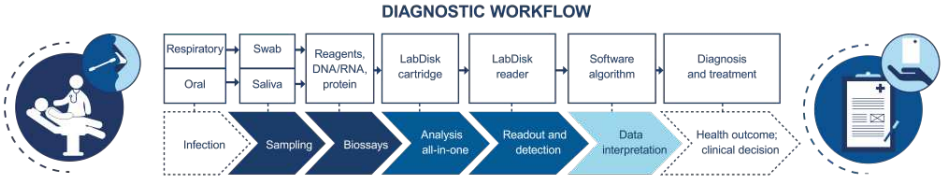

Figure 1. Diagnostic workflow of the DIAGORAS chair/bedside point-of-care device for the detection of RTIs, antimicrobial resistance and oral infections for use by clinicians, general practitioners and dentists.

Development of a simple interface between the sample collection tools, e.g., swabs, saliva that are used by the GPs, clinicians and dentists, and the diagnostic system.

Development of highly specific and sensitive bioassays: for RTIs and ABR, real-time RT (reverse transcriptase) PCR for a variety of viral RNA and bacterial DNA targets and antibiotic resistance genes; for oral infections, real-time quantitative PCR for the identification of bacteria associated with dental caries and periodontitis and immunoassays for the detection of elevated concentration of specific host response biomarkers, such as MMP-8, MMP-9, IL-1, IL-8, etc, associated with periodontitis.

Development of a centrifugal microfluidic [11] cartridge (LabDisk, Fig.2), which will integrate all biochemical components for nucleic acid purification (magnetic beads, buffers, etc) and amplification (amplification reagents, primers/probes, etc). All reagents will be pre-stored [12] for fully automated analysis. Two discs will be developed, one for RTIs (the "R-disc") and one for oral infections (the "O-disc").

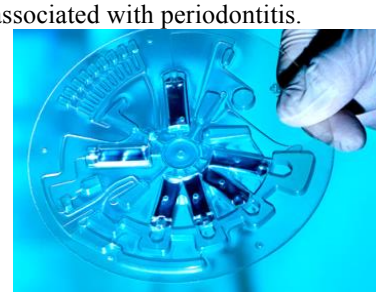

Figure 2. The LabDisk cartridge [13]

Upgrading the scale of disc fabrication from manual prototyping by developing industrially relevant manufacturing processes. This will be done via interfacing existing process modules and performing parametric investigation for process optimisation.

Development of the disc processing device, consisting of: (i) a mechanical unit (rotational protocols for fluid handling); a thermal unit (temperature profiles for rapid PCR); (iii) an optical unit (fluorescent readout and imaging); (iv) a graphical user interface.

Setting up a software platform for: (i) raw data analysis (biomarker concentrations, species identification, ABR, etc), and (ii) the interpretation and presentation of results to end-users for assistance in their decision making processes.

Validation of the final system using spiked samples (viruses and bacteria) and patient samples - initially in clinical settings and finally at selected dentists/GPs - and including comparison with gold standard/reference methods.

4. Expected outcome

4.1. Innovation potential

The innovation potential of DIAGORAS lies in the fact that it gathers technical features and applications that are unavailable in existing diagnostic systems. Further, DIAGORAS will ensure diagnostic reliability, simplicity in use, and robustness, as well as future product viability for end-users, through its following features: (i) full *system integration and automation* - due to disc-integrated *in situ* sample preparation - will lead to de-centralised patient diagnosis and disease management (a shift from hospital to chair/bedside diagnosis with minimum need for user intervention); (ii) culture-free, *molecular-based* pathogen detection with high sensitivity and specificity - will lead to personalised monitoring, for efficient and rapid patient management; (iii) *multiplexity* in diagnostic panels - nucleic acid (pathogen) and protein (host response) detection - will lead to increased reliability, minimised chances of misdiagnosis, and identification of the pathogen etiology and antibiotic resistance; (iv) *modularity* in the structural nature of the platform - based on interfaceable microfluidic unit operations incorporated as "building blocks" - will lead to system openness and adaptability with respect to the number and nature of detectable pathogens (bacteria, viruses) depending on application requirements or particular customer/end-user needs.

4.2. Impact

The impact of DIAGORAS on public health is expected at the following levels: (i) *better patient management* - via the rapid (<1 hour) and accurate differentiation of bacterial and viral infections in patients presenting with similar clinical symptoms; (ii) *prevention of antibiotic overprescribing* - via antimicrobial resistance screening and informed antibiotic prescribing practices; (iii) *increased accessibility* - by allowing more widespread and exhaustive epidemiological data to be gathered within hospitals, and especially in the community, regarding endemic and epidemic infectious disease-causing pathogens; (iv) *early diagnosis and determination of the disease level* - via quantitative bacterial DNA assays and host response protein biomarker detection; (v) *personalised monitoring* for status control during the entire period of the oral treatment.

The socioeconomic impact and reduced burden to healthcare systems is expected at the following levels: (i) *reduced expenditure* - on unnecessary and non-targeted antibiotic prescribing; (ii) *shortened stays in hospitals* - reduced disease complications during infections and reduced risk of nosocomial and epidemic infections; (iii) *reduced burden and costs associated with oral diseases* - e.g., lower insurance costs for oral diseases by the avoidance of severe complications due to a lack of correct treatment and patient monitoring; (iv) *increased accessibility to healthcare data* - by allowing more widespread collection of epidemiological data; and (v) *reduced time required for detecting infectious disease outbreaks in the community* - allowing rapid implementation of cost-effective vaccination campaigns and disease prevention actions.

5. The consortium

The DIAGORAS consortium brings together a multidisciplinary group of organisations that cover the entire value chain for the project. The partners and their roles are: (1) *Hahn-Schickard* (Germany) is a private non-profit research organisation responsible for

project coordination, microfluidic integration in the LabDisk, and optimisation of the manufacturing/processing chain; (2) *AIT Austrian Institute of Technology GmbH* (Austria) is a research organisation responsible for nucleic acid assay development for RTIs and antibiotic resistances; (3) *University of Zurich, Center of Dental Medicine* (Switzerland) participates with two divisions: (i) the Clinic for Preventive Dentistry, Periodontology and Cariology for the clinical validation regarding the oral infections; and (ii) the Section of Oral Microbiology and Immunology for the assay development for oral infections; (4) *Erasmus University Medical Center Rotterdam* (Netherlands) is responsible for choice of pathogens and antibiotic resistances, laboratory testing, clinical validation regarding the RTIs and associated resistances, and development of a central server for data processing; (5) *Askion GmbH* (Germany) is an SME, responsible for the development of the LabDisk processing device; (6) *MagnaMedics Diagnostics BV* (Netherlands) is an SME responsible for the development of the extraction kit for nucleic acid purification; (7) *ClinicaGeno Ltd* (UK) is an SME responsible for software algorithms for data analysis and interpretation, user interface and data storage; (8) *BioVendor Laboratorni medicina a.s.* (Czech Republic) is an SME responsible for the immunoassays and the post-project commercialization; and (9) *Sparks & Co* (France) is an SME responsible for the dissemination and communication of the project to the relevant stakeholders and the healthcare community.

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