Rapid Analysis & Diagnostics

FOOD
FEED
WATER
PLANT
ANIMAL
HUMAN
FORENSICS

THE RME CONFERENCE SERIES

15TH CONFERENCE

RME2023

ABSTRACTS

OF LECTURES AND POSTERS

6-8 NOVEMBER 2023

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CONTENTS

Key to the abstracts of lectures and posters:

- abstracts of lectures and posters are grouped separately
- lectures are grouped according to the daily programme
- posters are grouped in an alphabetical order according to the presenting author

ADVISORY COMMITTEE

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RME2023
RAPID ANALYSIS & DIAGNOSTICS

RME2023 – the 15th conference in the Rapid Methods Europe series – is dedicated to innovations and breakthroughs in rapid analysis & diagnostics across the agri-food, plant science, water (environment), healthcare (human, animal), and forensic science sectors.

food/feed | plant | water *(environment)* | health *(human, animal)* | forensics | general

Technologies include, but are not limited to (bio)sensors (electrochemical, optical, photonic), DNA/RNA-based detection methods, paper-based detection methods, microfluidic/nano/quantum devices, spectrometry, and spectroscopy for rapid analysis & diagnostics of pathogenic and spoilage bacteria, viruses, chemical contaminants, allergens, antimicrobial resistance, substances of emerging concern, etc. From concept to product, from analytical methods to systems, and from laboratory to on-site testing are the main themes of the conference.

RME2023 is aimed at a wide range of scientists, technologists, and professionals wishing to gain insight into to innovations and breakthroughs in rapid analysis & diagnostics. Attending the conference is an invaluable opportunity for laboratory managers and technicians, food safety and quality assurance/ quality control managers, technical managers, healthcare professionals, veterinary diagnosticians, plant science and agronomy scientists, environmental (water) scientists, forensic researchers, and others interested in rapid analysis & diagnostics.

NEW SET-UP: HARNESS THE POWER OF NETWORKING

RME2023 is a networking event par excellence, the common theme being:
HOW CAN WE LEARN FROM EACH OTHER?

The conference will focus on the technology rather than the sector, aiming to bring together scientists with different expertise with the possibility of cross-fertilizing each other and developing new views and applications. **RME2023** aims to further strengthen the academia-industry relations and disseminate advanced research towards practical applications. Take part and experience the power of networking.

AT A GLANCE

MONDAY 6 NOVEMBER 2023

12:45 – 13:00	Opening of **RME2023**	*Exhibition & Innovation playground*
13:00 – 15:00	PLENARY SESSION *Rapid analysis & diagnostics – What's up, Doc?*	
15:00 – 15:30	Networking break	
15:30 – 16:00	PLENARY SESSION *From innovative research to a biotech company*	
16:00 – 16:35	SPEED PRESENTATIONS AND COMPANY PITCHES *short presentation by selected poster presenters and by sponsors*	
16:35 – 18:00	Happy Hour	
17:00 – 18:00	WORKSHOP DIAGNOSTICS AT WUR	

TUESDAY 7 NOVEMBER 2023

08:45 – 10:05	SESSION 1 *DNA/RNA-based detection methods – PART 1*	SESSION 2 *Spectrometry & spectroscopy – PART 1*	*Exhibition & Innovation playground*
10:05 – 10:30	POSTER TOUR		
10:30 – 11:00	Networking break		
11:00 – 12:45	SESSION 3 *Paper-based methods – PART 1*	SESSION 4 *Microfluidic devices*	
12:45 – 14:00	Lunch break		
14:00 – 15:20	SESSION 5 *DNA/RNA-based detection methods – PART 2*	SESSION 6 *Spectroscopy: NIR and Raman applications*	
15:20 – 15:45	POSTER TOUR		
15:45 – 16:15	Networking break		
16:15 – 17:35	SESSION 7 *Paper-based DNA testing*	SESSION 8 *Electrochemical-based detection*	

WEDNESDAY 8 NOVEMBER 2023

09:00 – 10:45	SESSION 9 *Paper-based methods – PART 2*	SESSION 10 *Spectrometry & spectroscopy – PART 2*	*Exhibition & Innovation playground*
10:45 – 11:15	Networking break		
11:15 – 12:35	FINAL PLENARY SESSION *Rapid analysis & diagnostics – What further?*		
12:35 – 12:45	POSTER AWARD CEREMONY		
12:45 – 13:00	LESSONS LEARNED		
13:00	Closing of **RME2023**		

PROGRAMME

12:45 OPENING AND RAPID OVERVIEW OF **RME2023**
Dr Aart van Amerongen, *BioSensing & Diagnostics, Wageningen University & Research, the Netherlands*

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food/feed | plant | water *(environment)* | health *(human, animal)* | forensics | general

RME2023 will focus on the technology rather than the sector, aiming to bring together scientists with different expertise with the possibility of cross-fertilizing each other and developing new views and applications. 'How can we learn from each other?' is the common theme of the conference.

PLENARY SESSION

Rapid analysis and diagnostics – What's up, Doc?

Chair: Dr Aart van Amerongen, *BioSensing & Diagnostics, Wageningen University & Research, the Netherlands*

13:00 **Pairs of aptamer-based biosensors for POCT application**
Prof. Man Bock Gu, *Department of Biotechnology, Korea University, Korea*

13:25 **Empowering test strips for rapid, highly sensitive and multiplexed analysis of small molecule analytes at a point-of-need**
Dr Knut Rurack, *Chemical and Optical Sensing, Bundesanstalt für Materialforschung und -prüfung (BAM), Germany*

13:50 **NIRS 4 food integrity, processing, and sustainable production**
Dr Silvia Grassi, *Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy*

14:10 **Technologies for criminal investigations at the crime scene**
Prof. Jaap Knotter, *Saxion University of Applied Sciences, the Netherlands*

14:30 **Harness the power of networking**
Michiel Hesseling, *MBTM, the Netherlands*

15:00 NETWORKING BREAK

PLENARY SESSION
From innovative research to a biotech company

Research results only have an impact once they find their way into practical application in society. Bring value to your results!

Chair: Dr Bert Popping, FOCOS, *Germany*

15:30 **Breaking our heads on brain injury: challenge accepted!**
Team from SensUs, the international student competition in the field of rapid sensors
Marrit Bosch *and* Gertjan Spakman, *Wageningen University & Research, the Netherlands*

15:45 **Technology Transfer, the 'Scope Bioscience' case**
Dr Maurits Burgering, *Corporate Value Creation, Wageningen University & Research, the Netherlands*

PLENARY SESSION
Company pitches and speed presentations

Chair: Dr Bert Popping, FOCOS, *Germany*

16:00 **Company pitches**
Short presentations (5-minutes) by sponsors/exhibitors to inspire the audience to visit their booths

- **New food trends – new challenges in food safety? Rapid methods for a rapidly changing market** – Ronald Niemeijer, *R-Biopharm AG, Germany*
- **Diagnostic reagent and lateral flow services** – Lisa Mansell, *BBI Solutions, UK*
- **Analyzer for rapid tests** – Philipp Jungmann, *Chembio Diagnostics, Germany*
- **Cytiva, providing leading life sciences brands** – Julie Dhaese, *Cytiva, Belgium*

16:20 **Speed presentations**
Selected poster presenters are given 5 minutes to present an overview of their research

- **A lateral flow test for rapid identification of paediatric viral infection based on host response mRNA transcripts**
  Dr Ruth Reid, *Imperial College London, UK*
- **Novel disposable LAMP platform for detection of Yersinia pestis in the field**
  Dr Kilian Stoecker, *Bundeswehr Institute of Microbiology, Germany*
- **Chemically profiling bile-acids in human sera with a sensing array to detect and distinguish severe alcoholic hepatitis**
  Liam T. Wilson, *University of Glasgow, UK*

16:35 – 18:00
Happy Hour

17:00 – 18:00
Workshop DIAGNOSTICS@WUR presented by diagnostic research groups from Wageningen University & Research
In addition to university departments, each of the five sciences groups from Wageningen University & Research (WUR) has an application-directed research institute. These institutes carry out application-oriented and field-based research. They are commissioned by the national and international government, commercial businesses, and non-profit organisations and operate in the three WUR core areas: Food, feed & biobased production, Natural resources & living environment, and Society & well-being. In 2018, four institutes joined their diagnostic forces and, if possible and desired, collaborate to increase the research value for their customers. In the workshop, examples of diagnostic platforms, research projects, and new innovations will be shown.

18:00 END OF DAY 1

SESSION 1
DNA/RNA-based detection methods — PART 1

There are many methods available for DNA/RNA detection.The choice of method depends on the specific application, the sensitivity required, and the availability of resources.

Chair: Dr Helen Bridle, *Heriot-Watt University, UK*

08:45 ***In situ* metagenomics: know all from the target microbiome in less than one day**
Dr Javier Tamames, *Microbiome Analysis Laboratory, Systems Biology Department, CNB-CSIC, Spain*

09:05 **Rapid DNA at the crime scene – a unique field experiment**
Rosanne de Roo, *Amsterdam University of Applied Sciences, the Netherlands*

09:25 **Nanoelectrochemical sensors for sustainable agrifood and the environment**
Dr Alan O'Riordan, *Nanotechnology Group, Tyndall National Institute, Ireland*

09:45 **Small RNA sequencing and multiplex RT-PCR for diagnostics of grapevine viruses and virus-like organisms**
Prof. Nataša Štajner, *Department of Agronomy, University of Ljubljana, Slovenia*

10:05 **Poster Tour**
Presenting authors are requested to be available at their posters during the Poster Tour. A Best Poster Award is given to the best poster presented at the conference. It rewards a combination of excellent research, innovation, and presentation. The conference participants will vote for the best poster. The winner will be recognized publicly at the final plenary session.

10:30 NETWORKING BREAK

SESSION 2
Spectrometry and spectroscopy — PART 1

Spectrometric and spectroscopic methods are powerful tools for analysis & diagnostics in a wide variety of fields.

Chair: Prof. Michele Suman, *Barilla SpA and Università Cattolica del Sacro Cuore, Italy*

08:45 **Lossy mode resonance-based optical immunosensor towards detecting gliadin in aqueous solutions**
Melanys Benítez, *Department of Electrical, Electronic and Communications Engineering, Public University of Navarra, Spain*

09:05 **Optical aptasensor for endotoxin detection with single-molecule resolution**
Dr Alina Rwei, *Department of Chemical Engineering, Delft University of Technology, the Netherlands*

09:25 **Towards the development of a portable biosensor for fingermark profiling**
Dr Annemieke van Dam, *Department of Biomedical Engineering & Physics, Amsterdam UMC, the Netherlands*

09:45 **Detect and react – exploiting volatile-based stress communication for plant protection**
Dr Andrea Ficke, *Department of Fungal Pathogens, Norwegian Institute of Bioeconomy Research (NIBIO), Norway*

10:05 **Poster Tour**

10:30 NETWORKING BREAK

SESSION 3
Paper-based methods — PART 1

Paper-based detection methods can be inexpensive, easy to use, and do not require sophisticated laboratory equipment.

Chair: Dr Peter Bonants, *Wageningen University & Research, the Netherlands*

11:00 **Farm to fork strategy: new appetite for low-cost and sustainable paper-based optical sensors**
Dr Elisa Michelini, *Department of Chemistry 'Giacomo Ciamician', University of Bologna, Italy*

11:20 **Electrochemical quantification of lateral flow rapid tests**
Dr Thomas Maier, *Molecular Diagnostics, AIT Austrian Institute of Technology GmbH, Austria*

11:40 **Origami-paper device for rapid wastewater surveillance at low-resource settings**
Qingxin Hui, *Centre for Water, Environment and Development, Cranfield University, UK*

12:00 **Using LFD assays to assess the occurrence of potato blight in an automated system**
Prof. Roy Kennedy, *Agri-Tech Research Centre, Warwickshire College University Centre, UK*

12:20 **Kinetic reading of lateral flow test lines or spots to quantify results**
Dr Heleen van den Bosch, *BioSensing & Diagnostics, Wageningen University & Research, the Netherlands*

12:45 LUNCH BREAK

SESSION 4
Microfluidic devices

Microfluidic devices offer several advantages, including faster analysis times, reduced sample volumes and reagent consumption, and higher sensitivity and accuracy.

Chair: Dr Andrew Ward, *University of Strathclyde, UK*

11:00 **Emerging measurement techniques for micro-particulate water contaminants: protozoa and microplastics**
Prof. Tarik Bourouina, *IZONICS and Université Gustav Eiffel, France*

11:20 **Plant pathogen detection at point-of-use using centrifugal microfluidics**
Dr David Kinahan, *School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland*

11:40 **Blink and you miss it – microfluidics for ultra fast medical and environmental sample processing**
Nicole Hall, *Department of Biomedical Engineering, University of Strathclyde, UK*

12:00 **Micro- and nanofluidics for analysis of extracellular vesicles**
Dr Alar Ainla, *Medical Devices Group, International Iberian Nanotechnology Laboratory (INL), Portugal*

12:20 **Development of a microfluidic-inspired soft robotic sensor array for detecting cardiac arrhythmias**
Dr Bobak Mosadegh, *Department of Radiology, Weill Cornell Medicine, USA*

12:45 LUNCH BREAK

SESSION 5
DNA/RNA-based detection methods — PART 2

Chair: Dr Annemieke van Dam, *Amsterdam UMC, the Netherlands*

14:00 **Drills, heat and osmosis: making more (DNA extractions) with less (funds)**
Dr Manousos E. Kambouris, *Department of Pharmacy, School of Health Sciences, University of Patras, Greece*

14:20 **Isothermal amplification mediated detection of the 'big 5' carbapenemase genes**
Dr Chris Johnson, *Diagnostic and Therapeutic Technologies, Translational and Clinical Research Institute, Newcastle University, UK*

14:40 **Moving from the lab to the field: applying CRISPR-Cas technology to environmental DNA for species specific monitoring**
Prof. Anne Parle-Mcdermott, *School of Biotechnology, Dublin City University, Ireland*

15:00 **Tracking down the counterfeiters: Identification of *Crocus sativus* (saffron) adulterants by loop-mediated isothermal amplification and lateral flow assay**
Prof. Markus Fischer, *Department of Chemistry, University of Hamburg, Germany*

15:20 **Microfluidic silicon chip technology for ultra-fast PCR detection**
Dr Vanessa Bonnard, *miDiagnostics, Belgium*

15:45 NETWORKING BREAK

SESSION 6
Spectroscopy: NIR and Raman applications

NIR and Raman spectroscopy are powerful analytical techniques being non-destructive, requiring minimal sample preparation, and providing rapid results.

Chair: Prof. Zoltán Kovács, *Hungarian University of Agriculture and Life Sciences, Hungary*

14:00 **Recent Results of near infrared spectroscopy in food quality evaluation**
Prof. Zoltán Kovács, *Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Hungary*

14:20 **Non-targeted authentication of black pepper by NIR spectroscopy and LASSO method using a local web platform**
Dr Alessandra Tata, *Laboratorio di Chimica Sperimentale, Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

14:40 **Surface Enhanced Raman Spectroscopy – applications in agriculture and food safety**
Dr Pierre Lovera, *Nanotechnology Group, Tyndall National Institute, Ireland*

15:00 **Spectral techniques for rapid analysis of food and biological materials**
Dr Mohammed Kamruzzaman,*Department of Agricultural and Biological Engineering, University of Illinois Urbana-Champaign, USA*

15:20 **Poster Tour**
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15:45 NETWORKING BREAK

SESSION 7
Paper-based DNA testing

Benefits of paper-based DNA methods include their simplicity, low cost, portability, and suitability for field settings or resource-limited environments.

Chair: Prof. Sarah De Saeger, *Ghent University, Belgium*

16.15 **Using paper and textile microfluidics towards democratizing molecular diagnostics**
Dr Mahiar Max Hamedi, *Division of Fibre Technology, KTH Royal Institute of Technology, Sweden*

16:35 **In search of parasites: bringing PCR diagnosis of parasitic diseases into the field**
Norbert van Dijk, *Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, the Netherlands*

16:55 **Challenges and opportunities to deliver paper-based nucleic rapid tests at the point of need**
Dr Alison Wakeham, *Global Access Diagnostics, UK*

17:15 **Point-of-care technologies for healthcare and wellbeing applications**
Dr Aurel Ymeti, *NanoBio, Saxion University of Applied Sciences, the Netherlands*

17:35 END OF DAY 2

SESSION 8
Electrochemical methods

Electrochemical sensing has a wide range of applications. It is a sensitive, selective, and cost-effective method for detecting a variety of analytes.

Chair: Dr Firat Guder, *Imperial College London, UK*

16:15 **Towards an electrochemical biosensor for antimicrobial detection in milk: low-cost and rapid on-site analysis**
Magdalena Raykova, *Civil and Environmental Engineering, University of Strathclyde, UK*

16:35 **Hydrophobin-based chimerae: the fusion proteins boosting electrochemical and optical biosensor evolution**
Prof. Alessandra Piscitelli, *Department of Chemical Sciences, University of Naples 'Frederico II', Italy*

16:55 **TETRIS – A new tool for accelerated chemical phenotyping of whole plants**
Dr Firat Guder, *Department of Bioengineering, Imperial College London, UK*

17:15 **Development of sample-to-result miniaturized electrochemical sensor platforms for the detection of chemical contaminants: analysis when and where you want**
Mabel Torrens, *Instituto de Microelectrónica de Barcelona (IMB-CNM), CSIC, Spain*

17:35 END OF DAY 2

SESSION 9
Paper-based methods — PART 2

Chair: Dr Andrew Ward, *University of Strathclyde, UK*

09:00 **Paper-based lateral flow test for rapid detection of antiparasitics in manure**
Dr Jeroen Peters, *Wageningen Food Safety Research, the Netherlands*

09:20 **Improving paper-based microfluidic devices for *in situ* analysis as real-time diagnosis tools**
Dr Raquel B.R. Mesquita, *Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Portugal*

09:40 **Characterization and selection of antibodies for lateral flow diagnostics using SPR technology**
Dr Klaus Hochleitner, *Cytiva, Germany*

10:00 **Mitigating the Hook effect in a hCG-specific lateral flow test and extending the dynamic range of the assay**
Dr Aart van Amerongen, *BioSensing & Diagnostics, Wageningen University & Research, the Netherlands*

10:20 **Can rapid methods be sustainable? Environmental concerns and solutions in the point-of-need sector**
Prof. Maïwenn Kersaudy-Kerhoas, *School of Engineering and Physical Sciences, Heriot-Watt University, UK*

10:45 NETWORKING BREAK

SESSION 10
Spectrometry and spectroscopy — PART 2

Chair: Prof. Michele Suman, *Barilla SpA and Università Cattolica del Sacro Cuore, Italy*

09:00 **Gas chromatography-ion mobility spectrometry approach for the geographical origin evaluation of dehydrated apples**
Prof. Michele Suman, *Barilla SpA and Università Cattolica del Sacro Cuore, Italy*

09:20 **Aptamer-based biosensors for real-time detection of protein binding**
Dr Anna-Kristina Marel, *Department of Food Technology and Bioprocess Engineering, Max Rubner Institute, Germany*

09:40 **Homogeneous non-competitive immunoassays for *Staphylococcus aureus* enterotoxin A**
Dr Tarja Nevanen, *VTT Technical Research Centre of Finland, Finland*

10:00 **Homogeneous immunoassay for cyclopiazonic acid based upon mimotopes and upconversion-resonance energy transfer**
Fernando Pradanas González, *Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Spain*

10:20 **Simplifying bioanalytical assays by light diffraction**
Dr Miquel Avella-Oliver, *Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Spain*

10:45 NETWORKING BREAK

FINAL PLENARY SESSION

Rapid analysis & diagnostics – What further?

Chair: Dr Bert Popping, FOCOS, *Germany*

11:15 **From water to whisky: golden opportunities in analysis with plasmonic particles**
Dr William J Peveler, *School of Chemistry, University of Glasgow, UK*

11:35 **Point-of-care colorimetric nanobiosensors: health, safety, food, environment**
Dr Tania Pomili, *Nanobiointeractions & Nanodiagnostics, Istituto Italiano di Technologia, Italy*

11:55 **Use of alternative methods in the frame of official controls in Europe: the case of *Listeria monocytogenes***
Nathalie Gnanou-Besse, *French Agency for Food, Environmental and Occupational Health & Safety* ANSES, *France*

12:15 **From self-reporting to behavioural markers: lifestyle monitoring through wearables and smartphone sensors**
Dr Bram Steenwinckel, *Internet Technology and Data Science Lab (IDLab), Ghent University, Belgium*

12:35 **Poster Award Ceremony**

12:45 **Lessons learned**

13:00 CLOSING OF **RME2023**

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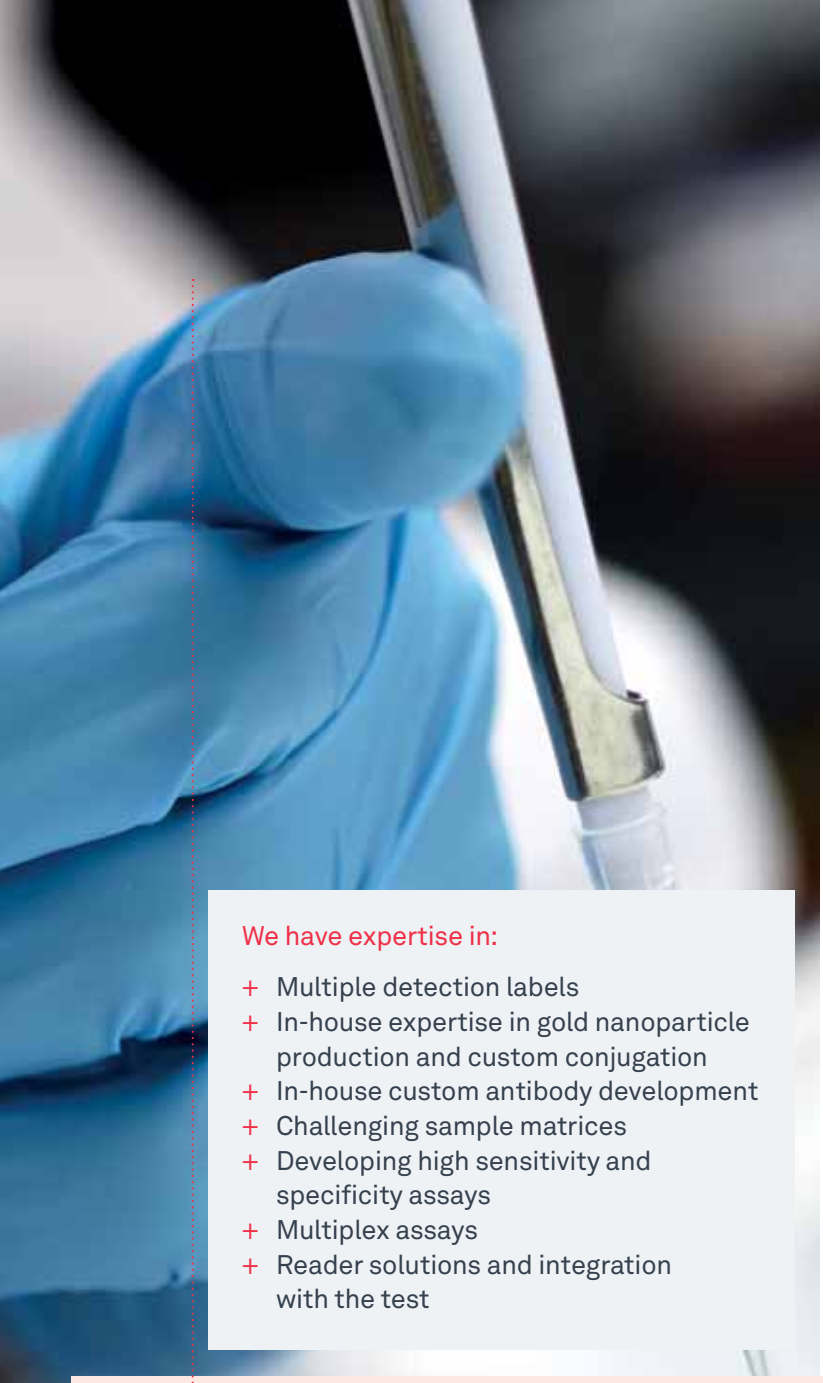

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We have over 25 years' experience developing qualitative, semi-quantitative and quantitative assays.

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Working with you to achieve your goals.

LECTURE ABSTRACTS

PLENARY SESSION
RAPID ANALYSIS & DIAGNOSTICS – WHAT'S UP DOC?

PAIRS OF APTAMER-BASED BIOSENSORS FOR POCT APPLICATION

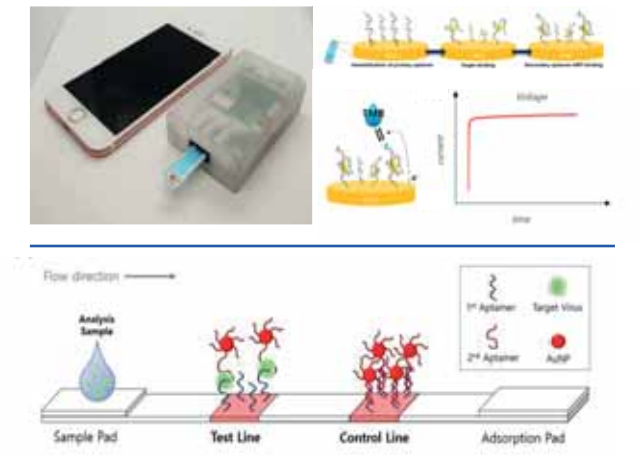
Man Bock Gu

Department of Biotechnology, Korea University, Korea

mbgu@korea.ac.kr

Only few of sandwich-type binding pairs of aptamers have been reported until recently, probably due to some technical limitations of the original SELEX protocol requiring target immobilization, which cause limited surface of the target accessible by nucleic acid library. An immobilization-free screening of aptamers by using graphene-oxide (GO-SELEX) for the first time in the world has been developed [1-3], and has widely and successfully been implemented to develop a few simultaneous binding pairs of aptamers successfully. A pair of aptamers simultaneous binding to the same target led to the sandwich-type binding in a form of aptamer$_1$-target-aptamer$_2$.

A few examples of sandwich-type binding pairs of aptamers and their biosensing applications in a POCT, such as lateral flow strip biosensors and electrochemical biosensors along with a mini-potentiostat, will be presented for protein biomarkers (type 2 diabetes and periodontal disease), pandemic viruses, and harmful bacteria. In addition, along with a new approach using non-essential sequences guided truncation to shorten aptamer size with enhanced affinity highlighted in this presentation, highly amplified signal generated on the electrochemical detection of *Staphylococcus aureus* will also be presented [4].



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2. US Patent 9,322,024 (2016).
3. US Patent 9,562,907 (2017).
4. Nguyen, T.T.-Q. and Gu, M.B., 2023. Biosensors and Bioelectronics 223: 115199.

EMPOWERING TEST STRIPS FOR RAPID, HIGHLY SENSITIVE AND MULTIPLEXED ANALYSIS OF SMALL MOLECULE ANALYTES AT A POINT-OF-NEED

Knut Rurack

Bundesanstalt für Materialforschung und -prüfung (BAM), Germany

knut.rurack@bam.de

In particular, the rapid development of lateral flow assays as indispensable tools for everyone to contain the SARS-CoV-2 pandemic has fuelled the global demand for analytical tests that can be used outside dedicated laboratories. In addition to their use in medical diagnostics, rapid tests and assays have become increasingly important in various fields such as food safety, security, forensics, and environmental management. The advantage is obvious: taking the assay directly to the sample minimizes the time between suspicion and decision-making, allowing faster action. Especially today, when mobile communication devices with powerful computing capabilities and built-in cameras are ubiquitous, more people than ever before around the world have the basic skills to operate a powerful detector at their fingertips. This sets the stage for a much wider use of analytical measurements in terms of prognosis and prevention, enabling professional laypersons in particular. However, current strip-based systems are primarily focused on single parameter analysis, whether it is SARS-CoV-2 biomarkers, blood glucose levels, or lead concentrations in water samples. Industrial applications of such methods also often still rely on single-parameter assays, requiring multiple runs even for a limited number of key parameters. Overcoming these limitations depends on developing low-number multiplexing strategies that ensure robustness, reliability, speed, ease of use, and sensitivity.

This lecture will give an overview of several generic approaches developed in recent years to address these challenges. It will highlight how the synergy of supramolecular (bio)chemistry, luminescence detection, hybrid (nano)materials and device miniaturization can result in powerful (bio)analytical assays that can be used at a point-of-need [1-5]. Selected examples will introduce key aspects of such systems that include tailored signalling mechanisms and recognition elements, materials functionalization and device integration, including hybrid nanomaterials, gated indicator release systems, strip modification, and smartphone-based analysis.

References

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5. Climent, E. *et al.*, 2020. Angewandte Chemie International Edition 59: 23862-23869.

NIRS 4 FOOD INTEGRITY, PROCESSING, AND SUSTAINABLE PRODUCTION

Silvia Grassi

Department of Food, Environmental and Nutritional Sciences (DeFENS),
University of Milan, Italy

silvia.grassi@unimi.it

The stakeholders of modern food production chain, aware of their pivotal role in facing Sustainable Development Goals, are envisioning the reorganization of their systems to reach optimized sustainable productions, guaranteeing product safety and quality while minimizing food wastage. Therefore, the scientific community must look for innovative approaches, answering the industrial needs in a holistic vision. The lecture aims at presenting few key insights into the application of near infrared spectroscopy (NIRS), combined with chemometrics, to preserve food integrity, monitor and optimize production processes and food quality, thus answering to loss reduction and sustainable production challenges. Pivotal applications will be presented providing the food sector with fast, green, and non-destructive methods in line with the Industry 4.0 vision.

Fish sector – To face fish integrity the implementation of NIRS has a relevant in common economic frauds such as the substitution of valuable species with cheaper ones. A successful case of NIR application demonstrates the reliability of a portable device in the assessment of fraudulent behaviours even at the marketplace [1].
Dairy sector - A NIRS Process Analytical Technology solution presents the usefulness for the monitoring of rennet-coagulation during cheesemaking. The approach allows the identification of profiles describing the three main coagulation phases. The obtained control charts provide the dairy sector with reliable solutions to describe the coagulation phases and to detect possible failures from the very beginning, thus improving product sustainability [2].
Milling sector –An integrated solution for process monitoring and control based on the real-time data collection by MicroNIR sensors, installed directly on-line, is described. The developed approach allows to predict relevant common wheat grain and flour characteristics from NIR data collected at industrial level, thus guaranteeing the desired quality while minimizing losses [3].
Extra Virgin Olive Oil sector – NIRS and image analysis could be successfully used for the assessment of the ripening stage of intact olives. By a robust sampling approach - 13 olive cultivars at different maturation stages along three harvesting years- classification models are able to predict ripening degree. The promising results pave the way for the development of dedicated devices to be used directly in the field for a more sustainable production and transformation [4].

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TECHNOLOGIES FOR CRIMINAL INVESTIGATIONS AT THE CRIME SCENE

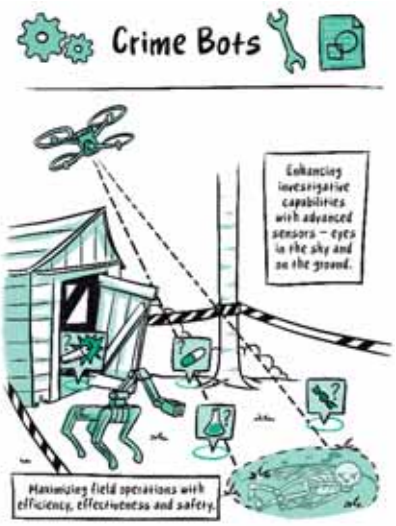
Jaap Knotter

Saxion University of Applied Sciences, the Netherlands

j.c.knotter@saxion.nl

Technologies for Criminal Investigations (TCI) is a joint research group of Saxion UAS and the Police Academy, led by Dr Jaap Knotter MEd. TCI focuses on innovative technology to be applied within three research lines: *Nano for Crime*, *CrimeBots* and *Data Science & Crime*. Our goal is to answer essential questions about crimes; not only regarding who committed the crime but also how and when the crime took place, which are of great importance to forensic investigators. Research, development, and application are intertwined within the research lines. This investigation does not have to be solely classically reactive and reconstructive in nature (investigation at a crime scene) but can also be more proactive in nature (aimed at detection and prevention). During this presentation, various projects within these three research lines will be discussed.

In *Data Science & Crime*, we observe that the volume of data collected today exceeds our imagination. Often, hidden patterns within this data are challenging for the human eye to detect. Data science applies technologies and expertise from Big Data, data analysis, and AI algorithms to get a grasp of all that is around us, combining human intelligence with computer precision.



CrimeBots, including forensic robotics like drones and the Spot platform (robot dog), are smart applications of combining robotics and mechatronics. These inno-vative platforms enhance the efficiency and effectiveness of investigations. By leveraging both existing and new detection techniques, investigators can swiftly and safely conduct their research at crime scenes.

Nano for Crime merges nanotechnology and forensics for applied and in-depth research. This research line includes quantum dots, hyper-spectral imaging, sensors for the detection of explosives and illicit drugs, single cell analysis and microfluidic devices. Smaller and more sensitive sensors and other detection techniques are making rapid analysis at the crime scene possible.Within this research line we recently got the ‘Criminal Investigation DX’ proposal granted. The aim of this project is developing rapid and reliable microfluidic devices for DNA analysis at the crime scene.

HARNESS THE POWER OF NETWORKING

Michiel Hesseling

MBTM, the Netherlands

michiel@mindpepper.nl

In today's world, networking is a necessity. A mountain of research shows that professional networks lead to more business opportunities, broader and deeper knowledge, improved capacity to innovate, and faster advancement. Are you open to build and nurture professional relationships?

This presentation will challenge you to create your own networking environment.

PLENARY SESSION
FROM INNOVATIVE RESEARCH TO A BIOTECH COMPANY

BREAKING OUR HEADS ON BRAIN INJURY: CHALLENGE ACCEPTED!

Team from SensUs, the international student competition inf the field of rapid sensors

Marrit Bosch and **Gertjan Spakman**

Wageningen University & Research, the Netherlands

marrit.bosch@wur.nl; gertjan.spakman@wur.nl

The SensUs student competition challenges students worldwide to develop innovative biosensing systems to make an impact in the field of health care. This year's theme comprised the detection of traumatic brain injury (TBI) by means of quantifying the glial fibrillary acidic protein (GFAP) in blood plasma.

The student team SenseWURk'23 consisting of nine students from Wageningen University & Research developed a lateral flow assay capable to quantify GFAP with use of fluorescent europium nanoparticles. Besides the development of a biosensor and determining its analytical performance, SensUs challenges students to look into the development of a business plan to discover the wishes of stakeholders and the value of their proposed sensor on the market.

During the presentation, two students will introduce the SensUs Challenge, tell about the development and application of the biosensor, and the journey it took to reach the end goals. We are happy to announce that six months of hard work in between lectures and writing theses resulted in a prestigious second placement in the analytical performance.

TECHNOLOGY TRANSFER, THE ‘SCOPE BIOSCIENCE’ CASE

Maurits Burgering

Corporate Value Creation, Wageningen University & Research, the Netherlands

maurtis.burgering@wur.nl

In the light of critical societal challenges of today, from affordable healthcare to ecological food production, there is a crucial need for fast, easy to use, and accurate analytics and diagnostics. Point-of-care medical diagnostics helps general practitioners in accurate and well pointed advice to their patients. Likewise, point-of-care testing in agriculture empowers farmers with timely data, enabling them to make informed decisions on treating plant pathogens and ensuring healthy crop yields to boost sustainability and limit, for instance, the use of pesticide. In academia, the latest insights in analytics and diagnostics are researched. However, it is a challenge to bring these new innovations into practice. For this purpose, an RVO subsidised instrument was put into action, the Thematic Technology Transfer program(s). The four technical Universities in the Netherlands (Delft, Eindhoven, Twente, and Wageningen) as well as the applied research organization TNO joined hands in 3 of such national TTT programs. The themes of these 3 TTT programs are MedTech, Smart Industries, and Circular Technologies. With our joint approach, we efficiently and effectively expedite the practical application of promising research. Our combined strengths make us an interesting partner for investors and businesses alike. As a result, communication with potential follow-up financiers is established at an early stage. These are important ingredients for (potential) spin-off businesses making a promising start and successfully develop into organizations with real impact.

The TTT programs have delivered successful spin-offs in diagnostics, such as Predica Diagnostics, cervical (pre)cancer test (MedTech), MantiSpectra, the next-generation spectral sensors (Smart Industries), and Scope Bioscience, CRISPR-Cas based molecular diagnostics (Circular Technology). During this presentation at RME2023, the latter case will be elaborated in more detail and how TTT has helped Scope Bioscience to mature its business. But this is not all, there is more to come. The new NGF program Biotech Booster has embarked with a dedicated Thematic Cluster on Diagnostics/Services that supports commercially driven projects focussing on collecting and interpreting biological information by measuring and detecting and monitoring of physiological, biochemical and genetic characteristics and services related to measurement, detection and monitoring.

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PLENARY SESSION
SPEED PRESENTATIONS

Short presentations (5-minutes) by selected poster presenters to provide the rationale of their research and inspire the audience to visit their posters

The abstracts can be found in the section ‘Poster abstracts’ (pages 71-103).

P21
A lateral flow test for rapid identification of paediatric viral infection based on host response mRNA transcripts
Ruth Reid
Paediatric Infectious Disease, Imperial College London, UK

P25
Novel disposable LAMP platform for detection of *Yersinia pestis* in the field
Kilian Stoecker
Bundeswehr Institute of Microbiology, Germany

P28
Chemically profiling bile-acids in human sera with a sensing array to detect and distinguish severe alcoholic hepatitis
Liam T. Wilson,
School of Chemistry, University of Glasgow, UK

SESSION 1
DNA/RNA-BASED DETECTION METHODS – PART 1

IN SITU METAGENOMICS: KNOW ALL FROM THE TARGET MICROBIOME IN LESS THAN ONE DAY

Javier Tamames[1], D. Jiménez[1], Á. Redondo[1], S. Martínez-García[2] and A. de los Rios[3]

[1] Microbiome Analysis Laboratory, Systems Biology Department, CNB-CSIC, Spain
[2] Centro de Investigación Mariña, Universidade de Vigo, Spain
[3] Department of Biogeochemistry and Microbial Ecology, National Museum of Natural Sciences (MNCN-CSIC), Spain

jtamames@cnb.csic.es

We present here a complete system for metagenomic analysis that allows performing the sequencing and analysis of a medium-size metagenome in less than one day, directly in the sampling location. This unprecedented development was possible due to the conjunction of state-of-the art experimental and computational advances: a portable laboratory suitable for DNA extraction and sequencing with nanopore technology; the powerful metagenomic analysis pipeline SqueezeMeta, capable to provide a complete analysis in a few hours and using scarce computational resources; and tools for the automatic inspection of the results via a graphical user interface, that can be coupled to a web server to allow remote visualization of data (SQMtools and SQMxplore).

We have tested the feasibility of our approach in several *in situ* scenarios: sequencing the microbiota associated to volcanic rocks in both Canary Islands and Antarctica. Also, we did a two-day sampling campaign of marine waters in which the results obtained the first day guided the experimental design of the second day. Since this system is portable, powered by batteries, and independent of internet connections, it offers the possibility of prospecting any habitat directly *in situ* and in a quick way, for obtaining both taxonomic and functional information about the target microbiome.

RAPID DNA AT THE CRIME SCENE – A UNIQUE FIELD EXPERIMENT

Rosanne de Roo

Amsterdam University of Applied Sciences, the Netherlands

r.h.d.de.roo@hva.nl

Technological innovations enable rapid DNA analysis implementation possibilities. Concordantly, rapid DNA devices are being used in practice. However, the effects of implementing rapid DNA technologies in the crime scene investigation procedure have only been evaluated to a limited extent.

In this study a field experiment was set up comparing 47 real crime scene cases following a rapid DNA analysis procedure outside of the laboratory (decentral), with 50 cases following the regular DNA analysis procedure at the forensic laboratory. The impact on duration of the investigative process, and on the quality of the 97 blood and 38 saliva trace results was measured. The results of the study show that the duration of the investigation process has been significantly reduced in cases where the decentral rapid DNA procedure was deployed, compared to cases where the regular procedure was used. Most of the delay in the regular process lays in the procedural steps during the police investigation, not in the DNA analysis, which highlights the importance of an effective work process and having sufficient capacity available.

This study also shows that rapid DNA techniques are less sensitive than regular DNA analysis equipment. The device used in this study was only to a limited extend suitable for the analysis of saliva traces secured at the crime scene and can mainly be used for the analysis of visible blood traces with an expected high DNA quantity of a single donor.

NANOELECTROCHEMICAL SENSORS FOR SUSTAINABLE AGRIFOOD AND THE ENVIRONMENT

Alan O'Riordan

Nanotechnology Group, Tyndall National Institute, Ireland

alan.oriordan@tyndall.ie

With the global population expected to grow to over 9.6 billion by 2050 it is a projected that a 50-60% increase in food production will be required. A key challenge then, going forward, will be to sustainably close the food gap. This must be achieved against the backdrop of climate change and desertification, labour shortages, and competition for energy, land and resources. It is clear then, that addressing this challenge will require the development of more efficient and sustainable food production techniques and processes.

To this end, new digital technologies that are fit for purpose, are urgently required to digitise the entire food chain. This convergence between the Internet of Things (IoT) and the agri-food industry requires sensor systems and technologies that provide real time data to producers and processers; required for rapid, but informed, decision making. This talk will provide an overview on current problems in agriculture and demonstrate application of digital technologies developed within our group can address the sustainability issues currently being experienced in this sector.

SMALL RNA SEQUENCING AND MULTIPLEX RT-PCR FOR DIAGNOSTICS OF GRAPEVINE VIRUSES AND VIRUS-LIKE ORGANISMS

V. Miljanić[1], J. Jakše[1], D. Rusjan[1], A. Škvarč[2] and **Nataša Štajner**[1]

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia
[2] Chamber of Agriculture and Forestry of Slovenia, Agriculture and Forestry Institute Nova Gorica, Slovenia

natasa.stajner@bf.uni-lj.si

Slovenia is a traditional wine-growing country with 15,075 hectares of vineyards in 2021. The area under vines is divided into three regions (Primorska, Podravje and Posavje) with a total of nine smaller districts. The Primorska wine-growing region accounts for 40.6% of the total Slovenian vineyard area, where a successful program of clonal selection has been carried out for decades. Viruses represent one of the biggest problems for certification and clonal selection. The first objective of the presented work was to investigate the virome of Slovenian grapevine clonal candidates using small RNA sequencing technology (sRNA-seq) and to study the genetic diversity of viruses and viroids as well as co-infections. The second goal of the presented work was to establish successful protocol to produce virus-free plants.

A total of 82 samples of six grapevine varieties, pre-clonal candidates (four white and two red) were taken for analysis. The selected samples were pooled into twelve pools. Small RNAs (sRNAs) were extracted by an enrichment procedure using a mirVana™ miRNA Isolation Kit (Ambion, Life Technologies, Waltham, MA, USA). The sRNA libraries were constructed using the Ion Total RNA-Seq Kit v2 (Ion Torrent™, Waltham, MA, USA) and barcoded using the Xpress™ RNA-Seq Barcode 1-16 Kit (Ion Torrent™, Waltham, MA, USA). Libraries were pooled at equimolar concentrations and prepared for sequencing using the Ion PI™ Hi-Q™ OT2 200 Kit and Ion PI™ Hi-Q™ Sequencing 200 Kit (Ion Torrent™, Waltham, MA, USA). Sequencing was performed on Ion PI™ chips v3 using an Ion Proton™ System (Ion Torrent™, Waltham, MA, USA). The sRNA-seq data were analyzed using the VirusDetect pipeline [1] and validated with RT-PCR and Sanger sequencing. Twenty-eight samples were randomly selected for the virus/viroid elimination study combining *in vivo* thermotherapy (36-38°C) with in vitro micrografting of meristem tips (0.1-0.2 mm) onto *in vitro* growing seedling rootstocks of Vialla (*Vitis labrusca* x *Vitis riparia*).

sRNA-seq revealed the presence of: grapevine fanleaf virus (GFLV), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine fleck virus (GFkV), grapevine red globe virus (GRGV), grapevine rupestris vein feathering virus (GRVFV), grapevine Syrah virus-1 (GSyV-1), grapevine Pinot gris virus (GPGV), raspberry bushy dwarf virus (RBDV), hop stunt viroid (HSVd), and grapevine yellow speckle viroid 1 (GYSVd-1). All in silico predicted viruses and viroids were validated with RT-PCR and Sanger sequencing. The highest number of viral entities in one plant was eight. In the second part of our study, using the described biotechnological approach to eliminate viruses and viroids, the overall regeneration rate of the plants was low (8.5%), but sufficient to obtain one virus-free regenerated plant per candidate that can be further micropropagated. The sanitation status of the *in vitro* regenerated plants was checked with RT-PCR. All viruses were eliminated, while the elimination of viroids was less successful (39.2% for HSVd and 42.6% for GYSVd-1). Multiplex reverse transcription-polymerase chain reaction (mRT-PCR) was developed for validation of HTS predicted infections. mRT-PCR provides rapid, time-saving and cost-effective molecular diagnosis including widespread, emerging, and seemingly rare viruses, as well as viroids.

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SESSION 2
SPECTROMETRY AND SPECTROSCOPY – PART 1

LOSSY MODE RESONANCE-BASED OPTICAL IMMUNOSENSOR TOWARDS DETECTING GLIADIN

Melanys Benítez

Department of Electrical, Electronic and Communications Engineering, Public University of Navarra, Spain

melanys.benitez@unavarra.es

The development of accurate, intuitive, and easy-to-handle devices to detect different types of allergens is on the rise, as these are useful tools to guarantee consumer safety, which should be a priority for any food industry. Gliadin, one of the main proteins present in gluten, is the one responsible for triggering the immune system to produce autoantibodies in celiac disease, the most dangerous pathology related to gluten. Lossy Mode Resonance (LMR) based biosensors are lately known as a promising sensing technology and its implementation on planar waveguides has been shown to result in manageable, sustainable and robust structures.

In this work, an LMR-based microfluidic biosensor for gliadin detection is proposed, by coating a coverslip with titanium dioxide (TiO_2) by atomic layer deposition (ALD) to generate the resonance phenomena and functionalizing the sensor surface with anti-gliadin antibody (AGA) through covalent bond. The sensor was exposed to different gliadin concentrations, in the range of 0.1-100 ppm. The calibration curve was obtained from the experimental data corresponding to three repetitions of the assay and a limit of detection (LOD) of 0.05 ppm was achieved. Moreover, the sensor was exposed to commercial flour samples, some of them labelled as gluten free (GF) and the response agreed with the expected results according to product label. Biosensor specificity to gliadin was demonstrated by injecting chicken egg white albumin without obtaining any significant response.

OPTICAL APTASENSOR FOR ENDOTOXIN DETECTION WITH SINGLE-MOLECULE RESOLUTION

P. Zhu[1,3], V.A. Papadimitriou[1], J.E. van Dongen[2], L.I. Segerink[2] and **Alina Y. Rwei**[1]

[1] Department of Chemical Engineering, Delft University of Technology, the Netherlands
[2] BIOS Lab on a Chip group, MESA+ Institute for Nanotechnology, Technical Medical Centre, Max Planck Institute for Complex Fluid Dynamics, University of Twente, the Netherlands
[3] State Key Lab of Mechanics and Control of Mechanical Structures, Nanjing University of Aeronautics and Astronautics, China

a.y.rwei@tudelft.nl

Endotoxin is a deadly pyrogen and a main trigger of sepsis, a disease that accounts for 1 out of 5 deaths in intensive care units and a mortality rate of 42%. Its accurate monitoring is therefore crucial. Current endotoxin detection relies on batch-based processes that are labour-intensive, time-consuming, and unsustainable. Here, we report an aptamer-based biosensor for the efficient, real-time, optical detection of endotoxin. The sensor transduces the conformational change of the endotoxin-specific aptamer upon target binding to a colour change of scattered light, via the plasmonic resonance of gold nanoparticles (AuNP). The sensor can either be used in ensemble or single molecule detection mode. In the ensemble mode, the sensor exhibits high reliability and specificity against endotoxin. We demonstrate the regeneration and reusability of the sensor suitable for repeated endotoxin measurement. The single molecule detection mode is enabled by monitoring the colour of individual AuNP via dark field microscopy. This platform has the potential to advance endotoxin detection to safeguard products in the medical, food and pharmaceutical industries.

TOWARDS THE DEVELOPMENT OF A PORTABLE BIOSENSOR FOR FINGERMARK PROFILING

Annemieke van Dam[1,2], M. Dede[1], N. Achetib[1], B. de Boer[3], S. el Bestawi[1], F. de Leeuw[1], P. van der Drift[1] and M. Aalders[1,4]

[1] Department of Biomedical Engineering and Physics, Amsterdam UMC, University of Amsterdam, the Netherlands
[2] Forensic Science, Amsterdam University of Applied Sciences, the Netherlands
[3] Delta Diagnostics, the Netherlands
[4] Co van Ledden Hulsebosch Center (CLHC), University of Amsterdam, the Netherlands

annemiekevandam@amsterdamumc.nl

During a forensic investigation, fingermarks found at the crime scene are vital pieces of evidence, since the ridge pattern is characteristic for each individual and can be used for individualization. However, individualization is not always possible, since the fingermarks can be smudged, distorted and/or because no reference fingerprint is present in the fingerprint database. In such cases, any additional information that can be retrieved from a fingermark is highly relevant to extract important investigative leads from the fingermarks.

Fingermarks contain much more information than solely a ridge pattern since it consists of a complex mixture of endogenous and exogenous components. Producing a chemical profile of the fingermark can thus provide more information about the donor, such as the sex or blood type, or relate them to certain activities. Creating a chemical profile is not only relevant for identification purposes when standard methods fail, but also for evidential purposes as this profile can potentially provide new investigative leads and be utilized in court to link a donor to certain activities or to prove or disprove testimonies.

Here, we present the first steps towards the development of a novel tool to create a chemical profile from fingermarks. Micro-ring resonators (MRR) are used to facilitate the detection of important biomarkers in a miniaturized portable and highly sensitive and specific system using antibody-antigen interactions. A first toolkit should allow the extraction of three types of information from the chemical fingermark: the sex of the donor, the blood type of the donor, and the time of deposition of the fingermark. In this presentation an overview of the project will be given, including the first findings towards the development of this new tool.

DETECT AND REACT – EXPLOITING VOLATILE-BASED STRESS COMMUNICATION FOR PLANT PROTECTION

Andrea Ficke

Department of Fungal Pathogens, Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research (NIBIO), Norway

andrea.ficke@nibio.no

Crops, insect pests and pathogens communicate their physio-chemical status to their environment via volatile organic compounds (VOCs). These VOCs can be either constitutively expressed or induced in response to certain changes in the environment, ontogeny or interactions with other organisms. The emitted VOC blends are often highly specific and can be used to track these changes in real time. VOCs are used in active chemical communication or passive signal exchanges between crops and their environment, including pests and pathogens, while insects are known to emit VOCs to locate their plant hosts and find their mates.

Exploiting VOCs for crop pest or pathogen detection requires identification of VOCs that are closely associated with the pest or pathogen attack, uniqueness of the target VOCs for this particular pest or pathogen and the ability to employ sensors that are able to detect these VOCs in very low concentrations. Sensor technology is a rapidly evolving field allowing detection of specific compounds at extremely low concentrations. Suitability of the different sensors to detect specific VOCs depends on the size, physical and chemical property of the target molecules.

An estimated crop loss of 10-15% is attributed to pest insects and pathogens globally despite the current use of pesticides. In order to reduce these losses and increase the economic and ecological sustainability of food production, early pest detection, pest risk models and site-specific pesticide applications are critical. Precision agriculture currently relies on visual detection of biotic or abiotic stress factors based on changes in light reflection. However, these changes often lack the specificity needed to identify the agent causing the particular stress. Volatile organic compounds emitted by pests or pathogens themselves or by the plants in response to the attack, are specific enough to detect and identify the attacking organisms. Early detection and accurate identification of the causal stress agent greatly facilitates its management in precision agriculture.

In our current Horizon Europe project ‘PurPest: Plant pest prevention through technology-guided monitoring and site-specific control’, we are determining the VOC blends emitted by 5 different target pests and pathogens (fall army worm, marmorated stink bug, cotton bollworm, *Phytophthora ramorum* and the pinewood nematode) and from host plants attacked by them. This information is then used to select suitable sensor components and to design a sensor platform prototype that can detect and identify these invasive plant health threats on imported plant material crossing borders into the EU. Excluding plant material contaminated with invasive pests and pathogens is the first and most effective step to reduce crop losses and pesticide applications. The next step is to detect pests and pathogens in the field and apply any control measure target-specific to those areas that are affected by the pest or pathogen.

SESSION 3
PAPER-BASED METHODS – PART 1

FARM TO FORK STRATEGY: NEW APPETITE FOR LOW-COST AND SUSTAINABLE PAPER-BASED OPTICAL SENSORS

Elisa Michelini

Department of Chemistry 'Giacomo Ciamician', University of Bologna, Italy

elisa.michelini8@unibo.it

Ensuring food safety, security, and sustainability is one of the current challenges that have been recognized as a priority by the United Nations. At a European level, the Farm to Fork Strategy of the European Green Deal, aims at speeding the transition to a sustainable food system, also considering that the global food demand will increase by 56% with a population reaching 10 billion persons by 2050. About 420,000 deaths per year are caused by unsafe food, with children under 5 years of age carrying the highest foodborne disease burden (40%). In this scenario, the availability of affordable tools to guarantee food quality and safety is vital.

Thanks to their numerous advantages, including low cost, low carbon footprint and greenness, paper biosensors garnered increased attention in the past ten years. Paper is an inexpensive, biocompatible support with a network of capillaries enabling self-pumping of fluids without the need of external pumps. The origami approach can be also exploited enabling reagents' addition or enzymatic reactions to be triggered sequentially. In addition, the availability of new bioluminescent proteins (i.e., highly stable luciferase mutants and new nanobiocomposites) [1], the use of smartphones and 3D printing are important factors that improve analytical performance, lower cost, promote sustainability, and democratize access to these tools.

Reflectance and bio-chemiluminescence detections have been implemented in paper biosensors to obtain user-friendly analytical tools for monitoring food safety and quality. Here we report a portfolio of paper biosensors designed with the 'Keep it simple' philosophy using coupled enzymatic reactions, living cells, and plant-derived molecules which were integrated into smartphone-based devices for rapid detection of food and water contaminants including organophosphorus pesticides, heavy metals and markers of food spoilage [2]. Paper-based sensors were also developed integrating different biorecognition elements and detections (i.e., colorimetric and bioluminescent detection) to obtain all-in-one devices which do not require additional steps such as substrate addition, thus simplifying the assay procedure and enabling use by non-skilled personnel.

Real-life applications are presented together with potential transferability to other fields (i.e., diagnostics), main limitations, and current challenges to turn them into marketable biosensors.

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ELECTROCHEMICAL QUANTIFICATION OF LATERAL FLOW RAPID TESTS

Thomas Maier

Molecular diagnostics, AIT Austrian Institute of Technology GmbH, Austria

thomas.maier@ait.ac.at

Thanks to their ease of use and short time to result, lateral flow devices (LFDs) are indispensable diagnostic tools allowing users to perform tests themselves, eliminating the need of medically trained personnel or a special infrastructure. This self-testing capability is the main reason for the success of the two most prominent LFD-based products: the pregnancy test, which was introduced in the late 1980s, and the rapid test for the early detection of SARS-CoV-2 infections, which has been an essential control measure during the COVID-19 pandemic.

In the self-test setting, the evaluation of the test result is entirely left to the end user and, thus, prone to misinterpretations. While optical LFD readers for the quantification of the analyte concentration are on the market, they are restricted to laboratory use because of their high costs. A more widespread use could be achieved with mobile phone cameras, but reliability is hampered by differences in camera soft- and hardware used in different mobile phone models.

In our presentation we present recent progress in the quantification of LFD test results by means of electrochemical sensing using C-reactive protein (CRP) as a model analyte. We describe the working principle of our readout scheme, the modifications required to make conventional LDFs fit for electrochemistry and discuss the possibilities of multiplexing. A main advantage of electrochemical sensing is the possibility of device miniaturization and the incorporation of additional electrical functionalities such as wireless connectivity to peripheral devices (e.g., mobile phones, tablets) for data transfer. A microchip-based solution will be presented offering the possibility to transfer the measured electrochemical data via NFC to a mobile phone app. This data flow leads to an objective, user-independent test result with hardware-independent signal evaluation. This approach leaves no room for misinterpretation by the user and offers the possibility for an easy implementation of a further transfer of the test results to a health information system.

Acknowlegements
This project has received funding from the European Union under grant agreement No. 101073982.

ORIGAMI-PAPER DEVICE FOR RAPID WASTEWATER SURVEILLANCE AT LOW-RESOURCE SETTINGS

Z. Yang, Y. Pan, **Qingxin Hu** and K. Caetano Souza

Centre for Water, Environment and Development, Cranfield University, UK

qingxin.hui@cranfield.ac.uk; zhugen.yang@cranfield.ac.uk

Pathogen detection is of significant importance for both biomedical diagnostics (e.g., infectious disease) and environmental analysis (e.g., pathogen contamination in drinking water; SARS-CoV-2 in wastewater for early warning of pandemic). This talk will present a low-cost, deployable paper-based biosensor device for rapid analysis of pathogens for a wide range of applications. I will show you the capability of a paper-origami device for field testing for veterinary diagnosis in India and for malaria testing in a local primary school in Uganda, Africa. I will present this device for the rapid analysis of pathogens in water and wastewater in low resource settings. Finally, this device has been developed o trace the source of SARS-CoV-2 for wastewater-based epidemiology for early warning of pandemics and demonstrated for field testing of wastewater at a quarantine hotel in London over the pandemics. Compared to the gold standard PCR assay, our platform provides similar or higher specificity and sensitivity for pathogen detection at a much cheaper and faster way, providing a high-resolution data set for highly responsive measurement during the pandemic and showing great potential for rapid and on-site wastewater surveillance at community settings in both developing and developed countries.

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USING LFD ASSAYS TO ASSESS THE OCCURRENCE OF POTATO BLIGHT IN AN AUTOMATED SYSTEM

Roy Kennedy

Warwickshire College University Centre, UK

rkennedy@warwickshire.ac.uk

Lateral flow assays, which detect sporangia of the potato blight pathogen *Phytophthora infestans* were developed based on recombinant IgG antibody fragments. The specificity of these lateral flow devices (LFD) was tested under laboratory and field conditions using a range of pathogens commonly found in the air in potato crops. LFD devices were used within an auto analyser trap specifically designed for early detection of crop disease pathogens within cropping environments. The autoanalyzer uses a high-volume cyclone sampler to trap particulates and micro-organisms in the air. One analyser can monitor a wide geographic area of around 100 Ha (dependant on local environmental conditions). The system has been tested using potato blight (*P. infestans*) as the target organism. The automated function enables samples to be routinely analysed for potato blight sporangial presence as detected using a specific LFD device. The analyser transmits 4G mobile data for storage in the cloud. Local pathogen data is analysed alongside local weather data within disease risk models to determine risks of crop disease infection. Analysis outputs at a local, regional or even national level can be viewed on multiple display devices with automatic alerts set at predetermined levels.

The daily LFD estimates of potato blight sporangia were compared to molecular estimates using similar daily samples obtained by PCR techniques. There was a high correlation between LFD and molecular estimates of the presence of *P. infestans* sporangia in air samples over a 2-month sampling period. Fragmentation of samples using beads increased the sensitivity of the assay. This could be used in the early stages of crop grow to identify initial development the *P. infestans* in the crop.

The project brings together necessary components of a disease management system and delivers user-friendly, in-field tests to growers and agronomists. Estimates of disease within the crop could be obtained in the absence of potato blight symptoms. A capability for both automated LFD assays and molecular assay is possible using the autoanalyzer estimated disease levels in air samples.

KINETIC READING OF LATERAL FLOW TEST LINES OR SPOTS TO QUANTIFY RESULTS

Heleen van den Bosch, H. Heuer and A. van Amerongen

BioSensing & Diagnostics, Wageningen University & Research, the Netherlands

heleen.vandenbosch@wur.nl

The lateral flow diagnostic platform is a user-friendly Point-of-Care test that can be applied anywhere [1]. Often the result is a qualitative or semi-quantitative signal that is sufficiently useful to the user. During the last pandemic millions of people have tested for a Covid-19 infection and the result could be simply read by the absence (no infection) or presence (infection) of the test line. Now, improvements in lateral flow test parameters such as materials, reagents, and labels, enable the platform to be used for more advanced applications [2]. For example, applying an array of printed spots instead of sprayed lines allows for multi-analyte detection, a configuration that easily supports detection of five of more analytes simultaneously. The spots can be printed in such a way that supply of test reagents in the fluid flow will be identical or highly similar for each of the various spots. In contrast, a sprayed line covering the full width of the nitrocellulose membrane will always influence the performance at test lines more distal from the preceding line.

In collaboration with the HAN University of Applied Sciences, the group BioSensing & Diagnostics has developed a real-time video reader that records the increasing colour intensity of the spots or lines in a lateral flow test. The reader is wirelessly connected to a smartphone, tablet or laptop to exchange instructions, data and/or results. Since the reader records data from the start of the experiment kinetic analysis of interactions between target analytes and binding ligands is possible as well, which adds an important advantage over other endpoint-directed immunochemical platforms such as the ELISA. In principle, having kinetic data would also allow the calculation of quantitative levels of the target analytes.

We are working on the quantification of microarray lateral flow data by analysing the spot intensity curves of each spot. Known target analyte concentrations are used to compose an analyte dose-response-curve by performing on-the-run curve fitting and calculating fitted curve parameters. If applicable the formula describing the analyte dose-response-curve will be used in the reader's software to calculate the concentration of the target analyte in unknown samples. Ongoing research examples will be shown such as the assessment of insulin concentrations in blood and of neutralizing antibody titers against the SARS-CoV-2 virus that highly correlate with immune protection.

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SESSION 4
MICROFLUIDIC DEVICES

EMERGING MEASUREMENT TECHNIQUES FOR MICRO-PARTICULATE WATER CONTAMINANTS: PROTOZOA AND MICROPLASTICS

Tarik Bourouina

IZONICS and Université Gustave Eiffel, France

tarik.bourouina@esiee.fr

Water quality and related instrumentation is an increasing area of interest regarding the scarcity of fresh water resources and the increasing pollutants induced by man-induced industrial activity. Besides the dissolved chemical pollutants, another class of water contaminants is the solid micro-particles. This class of pollutants includes mainly microplastics and biological micro-organisms, such as bacteria and protozoa.

In this presentation, we introduce the use of fluidic platforms, including flow-cytometry and microfluidic chips, for assessing this category of contaminants, with a focus on protozoa and microplastics.

PLANT PATHOGEN DETECTION AT POINT-OF-USE USING CENTRIFUGAL ANTIBODIES

David Kinahan

School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland

david.kinahan@dcu.ie

Centrifugal microfluidics is highly applicable to point-of-use diagnostic testing due to its ruggedness, portability, rapid processing times, and ease-of-use. The lab-on-a-disc platform can automate laboratory steps such as metering, mixing, and washing using valves to control liquid movement. This ability to directly translate assays directly from the standard protocols to 'on chip' with minimal biological optimisation is a key cost-saving measure. A further saving is the lab-on-a-disc requires just a low-cost spindle motor rather than specialized and expensive microfluidic pumps. Additionally, in many cases valves can be opened on-disc using by changing the speed at which the disc is rotated; effectively opening the valves by increasing or decreasing the 'pseudo-gravity' seen by the liquid. This allows the lab-on-a-disc to potentially be a truly portable, low-cost and accessible platform. Current rotationally controlled valves are typically opened by sequentially increasing the disc spin-rate to a specific opening frequency. However, as the rotational speed must be increased again and again to open each valve, and most low-cost motors have a maximum spin-rate of 6,000 rpm (100 Hz), using this ‘analogue’ approach places a limitation on the number of valves. This in turn limits the complexity of assays which can be automated.

In this work, we present a new type of valve that circumvents these issues by opening using a digital pulse in disc spin-rate. We first describe the operational principle of these valves followed by a demonstration of the capability of these valves to automate complex assays by screening tomato leaf samples against plant pathogens. Reagents and lysed sample are loaded on-disc and then, in a fully autonomous fashion using only spindle-motor control, the complete assay is automated. Amplification and fluorescent acquisition take place on a custom spin-stand enabling the generation of real-time LAMP amplification curves using custom software. To prevent environmental contamination, the entire discs are sealed from atmosphere following loading with internal venting channels permitting easy movement of liquids about the disc. The disc was successfully used to detect the presence of thermally inactivated *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), a bacterial pathogen on tomato leaf samples.

BLINK AND YOU MISS IT – MICROFLUIDICS FOR ULTRA FAST MEDICAL AND ENVIRONMENTAL SAMPLE PROCESSING

Nicole Hall and M. Jimenez

Department of Biomedical Engineering, University of Strathclyde, UK

nicole.hall@strath.ac.uk; melanie.jimenez@strath.ac.uk

Cell separation techniques are essential in a wide range of research areas – from blood analysis to water treatment. Currently, the widely accepted methods of cell sorting are fluorescent activated cell sorting (FACS) or magnetic activated cell sorting (MACS), methods which require the use of tags and reagents which are capable of altering a sample in addition to being time-consuming and extremely costly [1]. Moreover, tagged cells remain fluorescent, or magnetic, which may impede downstream analysis. To circumvent these challenges, microfluidic technologies offer a new avenue for high-throughput cell sorting in a chemical- and label-free manner to further assist cell sorting [2]. Inertial microfluidics more specifically relies on fluid manipulation at the micrometre scale to position cells at specific equilibrium positions according to their morphological phenotype (i.e., cell size/shape). Inertial microfluidics has demonstrated its unique potential for cell soring of, e.g., larger cancer cells in blood [2], stem cells [3], waterborne pathogens [4] or microalgae [5] among other applications. Importantly, however, the dependence of inertial forces on cells' morphological phenotype can also make the separation of cells challenging when these have similar sizes and, hence focusing positions.

In this work, we explore the behaviour of inertial microfluidics at extremely high pressures as a new route to isolate – with higher efficiency and throughput – cells with similar morphological profiles. While inertial microfluidics typically relies on Reynolds numbers (dimensionless number proportional to the flowrate) up to ~100 [6,7], we will present during this talk flow behaviours for Reynolds numbers exceeding 300. Interestingly, it was found that working with microfluidic devices that can handle such high flowrates enable new regimes of particle focusing positions that enhance sorting. It was found for instance that particles with only 1 µm difference could gain around a 20% difference in their focusing position – significantly easing the separation process. Additionally, we will demonstrate that the aspect ratio of the microfluidic channel plays a critical role, dictating the size range for this enhanced sorting. These new flow regimes might open a new way to handle ultra-rapid sample processing, while remaining portable for point-of-need use.

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MICRO- AND NANOFLUIDICS FOR ANALYSIS OF EXTRACELLULAR VESICLES

Alar Ainla

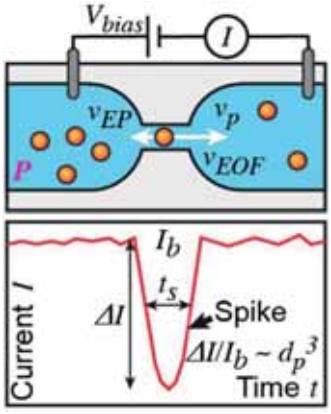
Medical Devices Group, International Iberian Nanotechnology Laboratory (INL), Portugal

alar.ainla@inl.int

Extracellular vesicles (EVs) are small vesicles released by many cell types. They mediate communication between the cells and can be categorized by their size, origin and molecular cargo, such as nucleic acids, metabolites and proteins. Since EVs end-up in different body fluids, they are also very attractive targets for minimally or non-invasive diagnostics (e.g., from blood and urine samples). This has contributed to the boost of the field of EVs research to analyse them, and to discover their role and relation to health and diseases. The EV analysis requires their isolation and purification from the biological samples and their detailed characterization. The most typical purification methods being ultracentrifugation (UC), filtration, size-exclusion chromatography (SEC) and precipitation. While analysis would usually target specific molecular markers of interest, almost always the first step routinely performed would be determination of their size distribution and concentration.

Our research group is working on development of both isolation and characterization methods for EVs using micro- and nanofluidic technologies. This presentation will specifically focus on our recent work on developing resistive pulse sensing (RPS) chips for determining EVs size distribution and concentration. Besides typical analytical merits, we have focused on the practical aspects of the technology, such as understanding failure modes, yields of successful experiments and their dependence on the chip designs and experimental setting, as well as required optimizations towards small and eventually low-cost instrumentation with hope that RPS technology will be able complement EVs analysis workflow, as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) do now.

Figure. Principle of resistive pulse sensing (RPS) with a nanochannel.



DEVELOPMENT OF A MICROFLUIDIC-INSPIRED SOFT ROBOTIC SENSOR ARRAY FOR DETECTING CARDIAC ARRHYTHMIAS

Bobak Mosadegh

Department of Radiology, Weill Cornell Medicine, USA

bom2008@med.cornell.edu

This work presents the design and fabrication of a novel cardiac mapping catheter that integrates microfluidic-inspired soft robotic actuators and stretchable electronics to enable whole-chamber mapping for the intended goal of diagnosis and treatment of atrial fibrillation and ventricular tachycardia. The novel catheter design aims to overcome the limitations of existing mapping technologies, such as speed of mapping time, increased sensor contact, and/or spatiotemporally resolved cardiac electrograms.

We describe the fabrication methods of both the soft robotic actuators and stretchable electronics using a laser cutting that can be done in any laboratory. To evaluate the device's performance, *in vitro* tests were performed using 3D printed heart models to assess the soft robotic design to achieve conformability. Subsequently, *in vivo* studies were conducted on live pigs to evaluate the device's performance in a beating heart model. Preliminary results demonstrate the successful integration of soft robotic actuators and stretchable electronics into a minimally invasive catheter that could be delivered percutaneously. The device exhibited excellent navigation capabilities, acquiring electrical signals from both the left atrium and left ventricle. These findings highlight the potential of this innovative catheter for improved diagnosis and treatment of cardiac arrhythmias. Future work will explore strategies to perform whole-chamber mapping with commercial mapping systems.

SESSION 5
DNA/RNA-BASED DETECTION METHODS – PART 2

DRILLS, HEAT AND OSMOSIS: MAKING MORE (DNA EXTRACTION) WITH LESS (FUNDS/FOOTPRINT)

S. Goudoudaki[1], **Manousos E. Kambouris**[2], S. Siamoglou[2], G.P. Patrinos[2,3] and Y. Manoussopoulos[1]

[1] Plant Protection Division of Patras, ELGO-Demeter, Greece
[2] Department of Pharmacy, School of Health Sciences, University of Patras, Greece
[3] Department of Genetics and Genomics and Zayed Center for Health Sciences, United Arab Emirates University, UAE

mekambouris@yahoo.com

DNA extraction systems in the form of ready-to-use kits provide standardized performance, optimized yield, simplicity in use and small footprint in stock reagents and material logistics in general. But their supply cannot be taken for granted in remote areas of interest or during times of crisis, where demand may surge and supply chains may deteriorate or collapse by a number of reasons, including restrictions in mobility/transportation due to lack of fuel or to contain easily transmissible pathogens. To this end, crude and simple DNA extraction protocols are needed for use both indoors, under supply chain strain, or outdoors, in dispersed field operations, regarding molecular surveillance and diagnostics of pathogens but also of non-microbial ailments.

The thermomotic protocol proposed in this presentation as a basis for adaptations uses only distilled water and heat to degrade the integrity of the cell envelope and then to rupture it, occasionally by some mechanical assistance [1], as suggested for human samples for SARS-CoV-2 molecular testing in the first massive outbreak of the disease [2]. Then, serial dilutions of the crude extract are expected to create at least one target DNA/inhibitor concentration combination suitable for immediate PCR. The extraction needs minimal amounts of equipment and expendables, few pieces of hardware and proved, up to now, workable for a number of samples (human mouth epithelia, human mouth bacteriome and fungal mycelia from plant tissue) when testing them for human congenital disease biomarkers and bacterial and fungal consensus sequences amenable to further testing by sequencing or restriction fragment analysis. More elaborate uses and better adapted protocols may be developed by interested parties, since flexibility is inherent in low-resource, low complexity approaches. Less crude but still plain and affordable methods have been tried beforehand, including a very basic but easy to modify and optimize alkaline protocol, which proved more versatile while of somewhat larger logistics footprint [3].

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ISOTHERMAL AMPLIFICATION MEDIATED DETECTION OF THE 'BIG 5' CARBAPENEMASE GENES

Christopher L. Johnson[1], W.A. Hassanain[2], K. Faulds[2], D. Graham[2] and N. Keegan[1]

[1] Diagnostic and Therapeutic Technologies, Translational and Clinical Research Institute, Newcastle University, UK
[2] Department of Pure and Applied Chemistry, Technology and Innovation Centre, University of Strathclyde, Glasgow, UK.

c.l.johnson@ncl.ac.uk

Antibiotic resistant bacteria pose a global threat to health and economics against a backdrop of an antibiotics supply pipeline which is drying up. Therefore, it is incumbent upon healthcare professionals not to overuse and prescribe the correct antibiotics in a timely manner in order to improve therapeutic outcomes for the patient and prevent the dissemination of antibiotic resistant bacteria. However, these goals are only truly achievable through the implementation of rapid and sensitive point-of-care (POC) diagnostics. Five carbapenemases, coined the 'big five', have been identified as the biggest threat to worldwide dissemination of antibiotic resistance based on their broad substrate affinity and global prevalence.

Here, we show the development of a penta-plex molecular detection method for the 'big five' utilising the isothermal amplification method of recombinase polymerase amplification (RPA). We demonstrate the successfully detection of each of the 'big 5' carbapenemase genes with sub-pM detection limits when carried out as a parallel multiplex using horseradish peroxidase (HRP) as the readout. In this solid phase RPA assay format, the forward primers for each of the five carbapenemase genes are immobilised to a streptavidin coated 96-well plate with the assay taking 2 h to result, including a 40 min RPA amplification time at 37°C. In order to further increase the sensitivity of the assay and to move from a parallel multiplex to a one-pot multiplex, we are modifying the readout from HRP to surface-enhanced Raman scattering (SERS) nanotags, in addition to utilising streptavidin coated magnetic beads as an alternative scaffold for solid phase RPA. Ultimately our goal is to develop a rapid and ultrasensitive POC test for the 'big 5' carbapenemase genes using a low-cost handheld SERS reader.

Acknowledgements
This research was funded by the EPSRC IRC in Early-Warning Sensing Systems for Infectious Diseases (i-sense) EP/ K031953/1, EPSRC IRC in Agile Early Warning Sensing Systems for Infectious Diseases & Antimicrobial Resistance EP/R00529X/1 and IRC Next Steps Plus: Ultra-Sensitive Enhanced Nano-Sensing of Anti-Microbial Resistance (uSense) EP/R018391/1.

MOVING FROM THE LAB TO THE FIELD: APPLYING CRISPR-CAS TECHNOLOGY TO ENVIRONMENTAL DNA FOR SPECIES SPECIFIC MONITORING

Anne Parle-McDermott

School of Biotechnology, Life Sciences Institute, Dublin City University, Ireland.

anne.parle-mcdermott@dcu.ie

The ability to monitor species in aquatic and other environments is becoming more urgent in the context of our biodiversity crisis and the increasing problem of invasive species across the globe. Environmental DNA (eDNA) has enormous potential as a non-invasive method to monitor species by taking water, air or soil samples from a given habitat without the requirement to have direct contact with the species themselves. While PCR based methods have been the most widely applied molecular method for the detection of single species from eDNA samples, they are increasingly being replaced by isothermal methodologies that do not require the energy intensity that thermal cycling typically requires. This bodes well for isothermal methods as the approach of choice for on-site monitoring.

The application of CRISPR-Cas technology coupled with recombinase polymerase amplification (RPA) to eDNA has numerous advantages over other isothermal methods, such as loop-mediated isothermal amplification (LAMP) or RPA on its own, including superior specificity and the ability to incubate the assays at 37°C.We originally applied RPA-CRISPR-Cas to eDNA for the detection of Atlantic salmon as a proof-of-concept. We have since demonstrated the applicability of this approach to other freshwater species in Ireland, including brown trout and Arctic char, and marine species, such as sea bass. More recently, we have taken the specificity of the assays a step further, enabling detection of different lineages of the same species. Our focus currently is to move these assays from the laboratory to the field using a variety of approaches. We have demonstrated that coupling RPA-CRISPR-Cas to lateral flow-based detection is a feasible approach for species specific monitoring. Moreover, the robustness of these assays allows their detection in an affordable portable fluorometer with incubating capabilities suitable for onsite monitoring (SensEDNA). The RPA-CRISPR-Cas assays are also suitable for drying down on a variety of different materials further highlighting their amenability for on-site detection including autonomous sensing.

In conclusion, coupling RPA-CRISPR-Cas technology with eDNA offers a versatile and sensitive and specific DNA-based detection system for rapid, on-site detection of species and sub-species lineages that can be adopted to a variety of detection formats.

TRACKING DOWN THE COUNTERFEITERS: IDENTIFICATION OF *CROCUS SATIVUS* ADULTERANTS BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AND LATERAL FLOW ASSAY

Markus Fischer

Hamburg School of Food Science, University of Hamburg, Germany

markus.fischer@uni-hamburg.de

Saffron (*Crocus sativus*) is one of the most valuable spices and therefore particularly vulnerable to fraudulent practices. The established methods for authenticity testing depend on the infrastructure of a laboratory and on qualified personnel. To circumvent these dependencies, there is a need for a cost-effective, easy-to-perform test system that can detect foreign plant material quickly and on-site.

The LAMP reaction is widely used for point-of-care diagnostics because of its robustness, reaction speed and independence from the laboratory environment. Both safflower (*Carthamus tinctorius*) and turmeric (*Curcuma longa*) are commonly used to stretch saffron. For the detection of unintentional impurities but also intentional stretches, a LAMP-based rapid test system for the detection of the adulterants turmeric and safflower in saffron samples was developed. The developed rapid test system can be performed within 25 min and consists of an isolation protocol, a LAMP assay and visualization of the test result with a lateral flow assay [1].

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MICROFLUIDIC SILICON CHIP TECHNOLOGY FOR ULTRA-FAST PCR DETECTION

Vanessa Bonnard

miDiagnostics, Belgium

vanessa.bonnard@midiagnostics.com

miDiagnostics is a medical devices company with the mission to provide a comprehensive range of health screening tools that will eventually grant diagnostic access for everyone, everywhere, and at any time. This is achieved by miniaturizing and simplifying the diagnostics process using silicon chips, developed by imec (the Belgian world-leading R&D and innovation hub in nanoelectronics and digital technologies) in collaboration with Johns Hopkins University (the leading US research and medical centre). The company is developing a molecular diagnostics pipeline, using its ultra-fast PCR technology for the diagnosis of SARS-CoV-2, with menu expansion possibilities towards sexual transmitted diseases and any other molecular test that could merit the benefits of decentralization, diagnostic accuracy, and ease of use.

In this presentation, we will explain how microfluidics is used to meter a well-defined sample volume and fill the silicon chip used as PCR detection chamber. The chip consists of an etched silicon meander bonded to glass. The good heat conductivity of the silicon allows for fast thermocycling while the glass allows for fluorescence detection to read-out the PCR amplification. Earlier work has shown for feasibility for using silicon chips also for sample preparation. Examples will be shown for metering, mixing, lysing cells, incubating….. These steps can be combined in a flexible way for sample preparation depending the application need. The reliability of the sequence of events is, however, very sensitive to the chip surface properties and functionalization.

SESSION 6
SPECTROSCOPY: NIR AND RAMAN APPLICATIONS

RECENT RESULTS OF NEAR INFRARED SPECTROSCOPY IN FOOD QUALITY EVALUATION

Zoltan Kovacs[1,2], F. Vitalis[1], M. Lukacs[1], J.P. Aguinaga Bósquez[1], M. Majadi[1], Z. Gillay[1] and G. Bazar[2]

[1] Department of Food Measurement and Process Control, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences (MATE), Hungary
[2] ADEXGO Ltd., Hungary

kovacs.zoltan.food@uni-mate.hu

Food raw materials undergo very complex journeys until they reach our table. The quality of our food and thus its influence on our health highly depends on the applied handling and processing technologies across the supply chain besides the origin of the raw materials. Modern digitalization tools provide ever-better opportunities to monitor and ensure the quality and authenticity of agri-food products. Development of digital fingerprinting approaches enable on-the-spot examinations providing comprehensive information about the product. This presentation aims to provide an overview of some of the recent studies of near infrared spectroscopy (NIRS) supporting the monitoring of food supply 'from farm to fork' – and beyond.

The quality of forage provided to cattle significantly affects milk yield and its composition, ultimately influencing the quality and storage potential of dairy products. This was demonstrated in a study where NIRS was used to monitor the shelf life of cheeses stored at different temperatures made from the milk of cows fed different diets. The liver of a fattened goose is a valuable luxury product, but the quality of foie gras is highly dependent on the blood content and its distribution, which are influenced by the breeding and slaughtering conditions. Our research findings confirmed the feasibility of precise liver blood content prediction and colour changes with the assistance of NIRS-based regression models. The extension of freshness is critical for food products, particularly for minimally processed leafy vegetables. The extension of iceberg lettuce's potential shelf life was examined using argon gas-modified atmosphere packaging compared to conventional packaging. NIRS proved to be useful to confirm that leaves of different stages of development and type of packaging react differently. Nutraceuticals, which are food products that have additional nutritional value, have seen significant growth recently. However, the legislative framework and established quality control measures have been lagging behind. Our studies have demonstrated that the identification and quantification of adulterants in grape seed extract as well as the extract-fortification of fruit juices are possible with high accuracy by means of NIRS.

Research has demonstrated the significant potential of NIRS for the rapid and comprehensive monitoring of the quality of agri-food products throughout the food supply chain. Recent discoveries of functional NIRS in the field of nutritional neuroscience suggest the potential of the technique to go beyond the assessment of food quality to a direct link between diet and health.

NON-TARGETED AUTHENTICATION OF BLACK PEPPER BY NIR SPECTROSCOPY AND LASSO METHOD USING A LOCAL WEB PLATFORM

Alessandra Tata[1], M. Suman[2,3], A. Massaro[1], M. Bragolusi[1], C. Zacometti[1], S. Lefevre[4], A. Fregiere-Salomon[4], J.-L. Lafeuille[5], G. Sammarco[2], I. Fiordaliso Candalino[6] and R. Piro[1]

[1] Laboratorio di Chimica Sperimentale, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
[2] Sensory and Analytical Food Science, Barilla G. e R. Fratelli S.p.A, Italy
[3] Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Italy
[4] Global Quality and Food Safety Center of Excellence, McCormick & Co, Inc., France
[5] Global Quality and Food Safety Center of Excellence, McCormick & Co., Inc., Italy

atata@izsvenezie.it

Black pepper is a valuable commodity susceptible to economically motivated adulterations. This research work describes the proper development, standardization, validation and late-stage validation of a non-targeted method for authentication of black pepper by near-infrared spectroscopy (NIR) coupled to a least absolute shrinkage and selection operator (LASSO). Note that this is the first successful attempt to apply the LASSO method to infrared spectroscopy data. We analysed 150 diverse samples of black pepper supplied by a well-recognized spice trader. A first batch of samples (n=116) were split into training and test sets and then normalized by multiplicative scatter correction (MSC). While the test set was withheld for further testing of the model, the training set was submitted to the LASSO method with the aim of retrieving the discriminant spectral features and classifying the samples as authentic, exogenously adulterated or endogenously adulterated.

The model was tested on the test set, achieving an overall accuracy of 94% with very high sensitivity and specificity rates. Furthermore, an R-based local web platform was created for immediate prediction of the samples from raw data. The NIR user simply uploads the raw data to the local web application and MSC normalization on the stored training set and interrogation of the LASSO classifier are performed. LASSO classifier was successfully challenged with samples from an international interlaboratory proficiency test. The online application facilitates the model testing and enables clear visualization of the outcomes. After the creation of the local web platform, the classifier was then validated with a new batch of independent samples, resulting in the correct prediction of 33/34 samples. Moreover, the robustness of this non-targeted method and its stability over time was further established by proficiency test. A late-stage validation of the classifier was successfully performed by inexperienced users.

SURFACE ENHANCED RAMAN SPECTROSCOPY – APPLICATIONS IN AGRICULTURE AND FOOD SAFETY

Pierre Lovera

Nanotechnology Group, Tyndall National Institute - University College Cork, Ireland

pierre.lovera@tyndall.ie

Raman spectroscopy is a powerful analytical technique capable of providing fingerprint of the samples under investigation. The main drawback of Raman spectroscopy is that it is not an efficient process, with only one out of 10^8 photons being Raman scattered – making detection of trace amounts of analytes challenging. Fortunately, however, these limitations can be overcome in surface enhanced raman scattering (SERS), where Raman signals are typically amplified by a factor of 10^6 owing to plasmonic effects taking place in electromagnetic hot spots found in gaps between nanostructured metallic surfaces. This considerable enhancement allows detection of trace of amounts of target analytes. With the recent advances in photonics and the wider availability of handheld Raman devices, this opens the way to onsite monitoring of targeted pollutants.

In this talk, we will present a brief overview of SERS and highlight SERS sensors – based on superhydrophobic substrates and electrochemically deposited silver nanodendrites – developed in our laboratories. We will show how those substrates can be used for rapid and sensitive detection of fungicide and herbicide as well as detection of adulterant and early indication of spoilage of dairy products.

SPECTRAL TECHNIQUES FOR RAPID ANALYSIS OF FOOD AND BIOLOGICAL MATERIALS

Mohammed Kamruzzaman

Department of Agricultural and Biological Engineering, University of Illinois Urbana-Champaign, USA

mkamruz1@illinois.edu

Conventional analytical tools are time-consuming, expensive, and destructive and require several chemical reagents. Moreover, it needs different analytical instruments/methods for each distinct attribute. Therefore, there's an urgent need for a cost-effective, chemical-free, and non-destructive analytical system that enables several constituents to be measured simultaneously, quickly, and accurately. One of the active areas of rapid analytical tools is spectroscopy or imaging spectroscopy. Currently, NIR spectroscopy or hyperspectral imaging is one of the most widespread modern analytical techniques with strong perspectives for further development due to the advancement in optics, computing power, and machine learning. It allows several constituents to be measured simultaneously from a single spectrum. Indeed, hyperspectral imaging is the only analytical technology that answers commonly asked analytical questions such as what chemical species are in the samples, how much, and most importantly, where they are located. While challenges remain in instrumentation, calibration transfer and data volume management, these spectral techniques hold immense potential for rapidly analysing food and biological materials.

SESSION 7
PAPER-BASED DNA TESTING

USING PAPER AND TEXTILE MICROFLUIDICS TOWARDS DEMOCRATIZING MOLECULAR DIAGNOSTICS

Mahiar Max Hamedi, A. Toldrà Filella, S. Khaliliazar, P. Réu Carvalho, G. Chondrogiannis and M. Hanze

School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Sweden

mahiar@kth.se

Early-stage testing of viral or bacterial infectious disease in modern healthcare is mostly done using molecular Nucleic-acid amplification testing (NAAT). The classical NAAT tests require several steps, and the current tests are mainly performed by highly trained personnel using advanced machinery in centralized laboratories. Future development could make NAATs available at low-cost for end-users revolutionizing food/environmental monitoring, and our local and global public health. In the past decade, the development of paper based analytical devices, has shown potential towards making molecular diagnostics and especially (NAATs) available for end-users. All steps of liquid handling [1], storage of reagents [2,3], biochemistry and electroanalytical detection [4-6], can be performed inside the paper and similar substrates. Importantly high throughput printing techniques can be used to fabricate these diagnostic devices making them very cheap and scalable [2,4].

This talk highlights three directions towards integrating NAATs in paper-microfluidics: (i) the use textiles and fibres as scalable platforms for biosensors; (ii) the use of paper as a powerful substrate for sample preparation to extract DNA and RNA from pathogens; and (iii) the fusion and use of printed paper electronics with industrial printed circuit boards and chip technology to bridge the gap between the bioanalytical and the digital domains [7].

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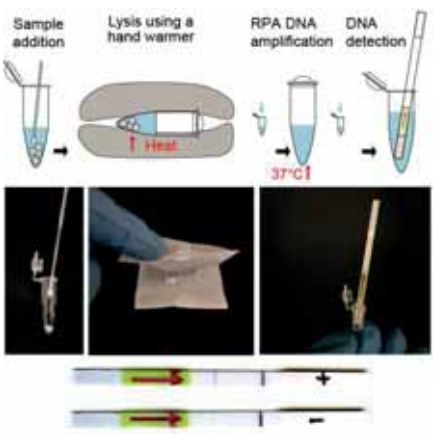
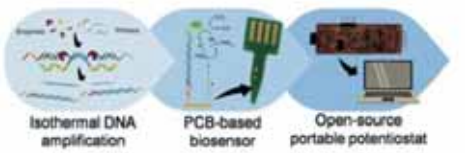
Paper-based kits towards DNA self-testing



Low-cost open source electroanalytical devices



IN SEARCH OF PARASITES: BRINGING PCR DIAGNOSIS OF PARASITIC DISEASES INTO THE FIELD

Norbert J. van Dijk

Department of Medical Microbiology and Infection Prevention – Experimental Parasitology, Amsterdam UMC, the Netherlands

n.j.vandijk@amsterdamumc.nl

Molecular techniques, such as polymerase chain reaction (PCR), have become a powerful tool for sensitive diagnosis of parasitic diseases. However, in many developmental countries, where the disease burden of parasite infections is often high, implementation of molecular diagnostics has proven to be a challenge. Reasons are the complexity of these techniques, with the need of well-trained personnel, requirement of sample processing steps such as DNA isolation, and expensive and sensitive equipment reliant on a stable power supply.

To overcome these challenges, we have developed the miniature direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA). This is an innovative, simplified molecular assay for the diagnosis of parasitic diseases in human blood in resource-limited settings. Unlike traditional molecular methods, mini-dbPCR-NALFIA does not require DNA extraction and makes use of a handheld, portable thermal cycler that can run on a solar-charged power pack. Result read-out is done using a rapid lateral flow strip that can simultaneously detect two PCR targets. Mini-dbPCR-NALFIA has been optimised for diagnosis of malaria, the most deadly parasitic disease in the world for which new diagnostic tools are warranted to improve case management and transmission control. The road towards introduction of mini-dbPCR-NALFIA as a new malaria diagnostic started with a laboratory evaluation. It showed a sensitivity of 96.6% and specificity of 98.3% in a set of samples from malaria-suspected returned travellers and healthy Dutch blood donors. The next step is currently underway: in the DIAGMAL study, the diagnostic performance, socio-economic aspects and road towards commercialisation of mini-dbPCR-NALFIA are evaluated in a field trial in five malaria-endemic African countries. Preliminary results from Burkina Faso show that mini-dbPCR-NALFIA has a high sensitivity of 99.3% and specificity of 81.0%, with blood smear microscopy as reference standard. Pending outcomes of the other diagnostic evaluations, cost-effectiveness analysis, health systems analysis and marketing research could pave the way for implementation of mini-dbPCR-NALFIA as sensitive tool for diagnosis and surveillance of malaria in endemic areas.

CHALLENGES AND OPPORTUNITIES TO DELIVER PAPER-BASED NUCLEIC RAPID TESTS AT THE POINT OF NEED

Alison Wakeham

Global Access Diagnostics, UK

alison.wakeham@globalaccessdx.com

To address the challenges and opportunities in the exciting and fast developing world of paper-based nucleic rapid tests, it is useful first to recap on the origins of this technology platform, and its development over the past 40 years. Immunochromatographic tests, commonly known as lateral flow tests, have been around since the 1980s and applied across multiple market segments to include human and animal health, crops, food authenticity and safety, the environment and biosecurity. Critical to test success is the sample, the target analyte(s) and the performance of the assay components.

A paper-based lateral flow packages both a laboratory and scientific expertise into a small affordable device which can be used at the point of need by an unskilled individual with results in minutes. Requires minimal or no sample preparation and is manufacturable at scale. A simple workflow of one or two manual steps is followed by a system of overlapping materials which enable liquid to travel across zones containing dried materials required to perform the assay. The choreography of which can vary depending on the sample type, the sensitivity and specificity required of the test, and whether a single or multiplex target analyte assay. The flexibility of the test enables opportunity for both qualitative and quantitative results. Recording of the test can be carried out visually by eye or may involve use of a portable reader such as a mobile phone App. Initial patents around these paper-based devices were filed in the 1980s and there remains an active patent landscape.

With the advent of nucleic acid-based diagnostics and assay development becoming commonplace in laboratories during the 1990s, the exquisite sensitivity and specificity which could be achieved, fuelled a desire to translate these tests out to the point of need. Initially lateral flow paper-based assays were deployed to capture labelled amplicons, e.g., biotin and FITC but the requirement of PCR thermal cycling presented a workflow challenge and affordability to deploy in the field. With the development of isothermal-based assays such as LAMP, RPA, NASBA, 3SR and SDA there is opportunity to circumvent thermal cycling and operate at a lower temperature. However, with sample matrices often requiring up-front processing, of which these can include extraction, concentration and clean up, the ability to provide an affordable, commercially available test with simple workflow that non-skilled operators can carry out in the clinic, field or home continues to challenge. As does the heating requirement, length of assay time, and for multiple samples the risk of amplicon sample contamination. Nevertheless, as isothermal patents fall away, there is an opportunity to overcome these challenges. With the Covid pandemic, increased activity in this field has led to the commercialization of a number of easy to use, simple ‘sample to answer’ qualitative isothermal nucleic acid-based lateral flow tests.

Next generation lateral flow platforms increasingly look towards molecular diagnostic technologies to perform at laboratory specificity and sensitivity. In addition, the ability to work at the single nucleotide level for discrimination of genetic mutation or to determine pathogen resistance. CRISPR-based diagnostics may provide the answer and provide a fast assay time with no heating requirement. By leveraging the adaptive immune system, guide RNA is programmed to recognize foreign nucleic ‘target’ sequences, and following complementary hybridization activate CRISPR-associated Cas enzyme to cleave nucleic targets. By harnessing these processes, assay variations have been demonstrated with results reported by lateral flow. CRISPR diagnostics have the potential for both simplex and multiplex target assays.

POINT-OF-CARE TECHNOLOGIES FOR HEALTHCARE AND WELLBEING APPLICATIONS

Aurel Ymeti, J. Jukema and M. Bennink

Center of Excellence for Point-of-Care Technologies (CoE4POCT), Applied Nanotechnology and Smart Health Research Groups, Saxion University of Applied Sciences, the Netherlands

a.ymeti@saxion.nl

Point-of-care technologies (POCT) allow end users, such as healthcare professionals and citizens, to accurately achieve real-time, lab-quality diagnostic results within minutes. POCT devices are based on various principles, including lateral flow immunoassays, (photonic) integrated biosensors and lab-on-a-chip microfluidic systems, which can be applied for analysing clinically relevant bodily fluids, such as urine, sputum, interstitial fluid, serum, plasma or whole blood. Some of the well-known POCT applications are blood glucose testing, rapid COVID-19 tests, rapid cardiac biomarker diagnostics and drugs of abuse screening tests.

The Center of Excellence for Point-of-Care Technologies (CoE4POCT) at the Saxion University of Applied Sciences is dedicated to supporting end users, including healthcare professionals and citizens, by facilitating the availability and accessibility of reliable, easy-to-use, and affordable Point-of-Care testing solutions. CoE4POCT's primary goal is to enable early detection and/or prevention of diseases, thereby contributing to the enhancement of the quality of life. This mission is realized through a range of activities, including:

- initiating and strengthening collaborations among knowledge institutions, technology development partners, healthcare professionals, and societal stakeholders;
- identifying key stakeholders and establishing a network of partners engaged in the field of POCT;
- forging connections with related initiatives, such as Lifestyle4Health;
- collaborating on joint national and international projects; and
- contributing to educational programs, including the M.Sc. Applied Nanotech.

CoE4POCT emerged as a collaborative endeavour between two strategic areas at Saxion University: Key Enabling Technologies and Health, Wellbeing & Technology. This collaboration brings together various research groups from Saxion University to engage in multidisciplinary projects.

SESSION 8
ELECTROCHEMICAL METHODS

TOWARDS AN ELECTROCHEMICAL BIOSENSOR FOR ANTIMICROBIAL DETECTION IN MILK: LOW-COST AND RAPID ON-SITE ANALYSIS

Magdalena Raykova[1], D. Corrigan[2], F. Henriquez[3] and A. Ward[1]

[1] Civil and Environmental Engineering, University of Strathclyde, UK
[2] Pure and Applied Chemistry, University of Strathclyde, UK
[3] School of Health and Life Sciences, University of the West of Scotland, UK

magdalena.raykova@strath.ac.uk

Antibiotic residues in food are strictly controlled by maximum residue limits (MRLs) and monitored by legislation in many countries and territories. In dairy farms, contamination of the farm bulk tank with milk containing residues (>MRL), such as beta-lactam antibiotics, presents a threat to confidence of supply, results in financial losses to the farmer and dairy industry and imposes a risk to the environment when disposed of. Ampicillin (AMP), a broad-spectrum antibiotic, is one of the leading choices for the treatment of dairy cows because it is active against a wide range of gram-positive and gram-negative bacteria.

In this work, a 19-base thiolated (5') aptamer labelled with a methylene blue (MB) tag (3') was employed as a biorecognition molecule for AMP. This was immobilised on a gold electrode surface to explore real-time quantification of AMP residues. The biosensor performance was interrogated in buffer before applying it on milk samples spiked with AMP. Following optimisation, this proposed internal redox reporter method exhibited reliable, rapid and sensitive performance with a limit of detection of 10nM AMP. Moreover, the aptamer was tested against other potential antibiotic targets and exhibited selectivity towards ampicillin only. The speed and sensitivity of this assay shows potential to introduce a more simplified and automated sampling process to detect the presence of AMP residues in milk.

HYDROPHOBIN-BASED CHIMERAE: THE FUSION PROTEINS BOOSTING ELECTROCHEMICAL AND OPTICAL BIOSENSOR EVOLUTION

Alessandra Piscitelli, P. Cicatiello, I. Stanzione, A. Pennacchio and P. Giardina

Department of Chemical Sciences, University of Naples Federico II, Italy

apiscite@unina.it

Immobilization procedures of biomolecules affect biological interfacing of materials and should ensure stable and spatial anchoring with a precise orientation. In this context, self-assembling amyloid proteins provide new and tangible opportunities as platforms to develop biohybrid functional materials. Indeed, adhesion to solid interfaces of amyloid protein layers can efficiently play the role of connecting bridges between organic and inorganic surfaces. Among self-assembling proteins, fungal Class I hydrophobins are characterized by high propensity to form amyloid fibrils and to efficiently and easily adhere to several conventional and nanostructured surfaces by direct deposition.

Our research group has exploited the layers of a Class I hydrophobin as an attractive platform to immobilize proteins and/or peptides on different surfaces. This adhesive ability has been further exploited by genetic fusion, designing and producing chimeric proteins built by fusing the self-assembling adhesive moiety of the fungal hydrophobin to various biotechnologically relevant proteins. Following this approach, different proteins and enzymes have been stably anchored on classical surfaces and 2D nanomaterials with a favourable orientation, thus forming a homogeneous biological layer on biodevices. The obtained results establish the effectiveness and versatility of both fungal hydrophobin and its chimeric variants in surface functionalization. Case studies assessing the exploitation of fusion proteins in the development of biosensing platforms for application to environmental monitoring, food safety and medical diagnostics will be presented and discussed.

TETRIS – A NEW TOOL FOR ACCELERATED CHEMICAL PHENOTYPING OF WHOLE PLANTS

Firat Güder

Department of Bioengineering, Imperial College London, UK

f.guder@imperial.ac.uk

Plants are non-equilibrium systems consisting of time-dependent biological processes. Phenotyping of chemical responses, however, is typically performed using plant tissues, which behave differently to whole plants, in one-off measurements. Single point measurements cannot capture the information rich time-resolved changes in chemical signals in plants associated with nutrient uptake, immunity or growth.

In this work, we report a high-throughput, modular, real-time chemical phenotyping platform for continuous monitoring of chemical signals in the often-neglected root environment of whole plants: TETRIS (Time-resolved Electrochemical Technology for plant Root *In situ* chemical Sensing). TETRIS consists of screen-printed electrochemical sensors for monitoring concentrations of salt, pH and H_2O_2 in the root environment of whole plants. TETRIS can detect time-sensitive chemical signals and be operated in parallel through multiplexing to elucidate the overall chemical behaviour of living plants. Using TETRIS, we determined the rates of uptake of a range of ions (including nutrients and heavy metals) in *Brassica oleracea* var. *acephala.* We also modulated ion uptake using the ion channel blocker $LaCl_3$, which we could monitor using TETRIS. We developed a machine learning model to predict the rates of uptake of salts, both harmful and beneficial, demonstrating that TETRIS can be used for rapid mapping of ion uptake for new plant varieties. TETRIS has the potential to overcome the urgent 'bottleneck' in high-throughput screening in producing high yielding plant varieties with improved resistance against stress.

DEVELOPMENT OF SAMPLE-TO-RESULT MINIATURIZED ELECTROCHEMICAL SENSOR PLATFORMS FOR THE DETECTION OF CHEMICAL CONTAMINANTS: ANALYSIS WHEN AND WHERE YOU WANT

Mabel Torrens[1], W. Duan[2], M. Gich[2] and C. Fernández-Sánchez[1,3]

[1] Instituto de Microelectrónica de Barcelona (IMB-CNM), CSIC, Spain
[2] Institut de Ciencia de Materials (ICMAB-CSIC), Spain
[3] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

mabel.torrens@imb-cnm.csic.es

Water pollution is an increasing global concern with strong implications in human health, aquatic ecosystems, and economic growth. Water monitoring with the frequency required to timely detect and control contamination outbreaks is seldom carried out because current applied analytical techniques require bulky and expensive instrumentation implemented in centralized laboratories and manipulated by highly qualified personnel. Nowadays, the development of novel cost-effective cutting-edge analytical technologies to early detect contaminants in water is highly demanded.

We have developed a technology for the production of low-cost electrochemical sensors for the rapid detection of contaminants in water, that could be used by non-specialized personnel and applied in field. The sensor main components are on the one hand a tailor-made carbon composite material containing metal nanoparticles used for the electrocatalysis of the target analytes and processed into an ink screen-printed on a plastic substrate. On the other hand, it incorporates an integrated filter paper disk loaded with an electrolyte that allows the simultaneous filtering and conditioning of the sample. The sensor approach developed so far has been applied to the detection of organic matter by measuring the chemical oxygen demand (COD) and to the detection of heavy metals.

The sensor architecture presented in this work and previously patent (Ref. EP21383025.0) responds to a clear environmental need and constitute an attractive market-oriented analytical platform that fulfils the requirements to be applied on-site. The sensor response can be recorded using compact low-power electronics connected to a mobile phone. Moreover, an analysis just requires the addition of a few microliters to get an analytical result in around 10 min. The material synthesis and fabrication technology can easily be upscaled for mass production of a high number of sensor units at a roughly estimated cost of 1 €/unit.

SESSION 9
PAPER-BASED METHODS – PART 2

PAPER-BASED LATERAL FLOW TEST FOR RAPID DETECTION OF ANTIPARASITICS IN MANURE

Jeroen Peters, X. Hong, R. van Hoof, T. Zuidema, T. Bovee and M. Pikkemaat

Wageningen Food Safety Research, the Netherlands

jeroen.peters@wur.nl

Antiparasitics are crucial drugs for treating parasitic infections in humans and animals. The importance of these antiparasitics becomes clear from the fact they comprise more than 20% of the animal health market. Once the antiparasitics are applied on, or ingested by, the animal, they will be submitted to the metabolic processes and the subsequent release from the body through skin, urine, and manure. Besides these excretion pathways, the antiparasitics may also end up in edible matrices like milk or meat. This raises a food safety concern as humans may be exposed unwillingly to residues of antiparasitics. To protect consumers from exposure to potentially harmful levels of these residues, maximum residue limits (MRLs) have been established and withdrawal periods apply for the veterinary products containing these substances.

To ensure compliance with residue legislation and facilitate point-of-need surveillance, an easy-to-perform on-site test for the detection of benzimidazoles, a major class of veterinary antiparasitics, was developed. The presented research shows the development of a lateral flow device (LFD) implementing a monoclonal antibody that targets albendazole. This LFD was able to detect nine benzimidazoles and their derivative metabolites at 10-100 ppb levels. The targeted matrix was manure, to facilitate a non-invasive approach of testing livestock animals for the presence of benzimidazoles. The developed LFD was applied to blank and naturally contaminated pig manure samples that were previously analysed with an in-house LC-MS/MS multi-method able to detect 45 antiparasitics and antiparasitic metabolites. Opting for an easy on-site extraction, in coherence with the easy on-site test, a straightforward procedure was developed by simply diluting manure in the assay buffer, excluding the necessity and safety of a laboratory environment. Currently, the LFD for broad benzimidazole screening is further developed into a multiplex test. A novel antibody directed against triclabendazole and 3 of its derived metabolites, has already been successfully tested in benchmark sensor technology. This means that the introduction of this new antibody in the existing albendazole LFD shows good prospects for the detection of 13 benzimidazoles at low ppb levels. This rapid test can be performed in an animal-friendly way by simply testing the manure at the point of need as an indicator of antiparasitics use, without directly involving the animal. Alternatively, further on-site simplification of the test is foreseen by introducing sample swaps. Besides testing living animals, the developed test can be introduced in the meat industry for testing edible tissues for safeguarding public health.

IMPROVING PAPER-BASED MICROFLUIDIC DEVICES FOR *IN SITU* ANALYSIS AS REAL-TIME DIAGNOSIS TOOLS

Raquel B.R. Mesquita

Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Portugal

rmesquita@ucp.pt

Nowadays, the interest in on-hand, easy-to-use devices has had exponential growth and, if used correctly, they can be an effective aid in both food safety and healthcare. So, the search for faster, user-friendly, ready-to-use, and still accurate monitoring techniques has been increasing. In this context, microfluidic paper-based analytical devices (μPADs) provide a novel approach for conducting low-cost *in situ* determinations posing as an attractive alternative to the conventional techniques which require specialized skills, laborious laboratory processes, or/and expensive equipment. The μPADs small dimensions, inexpensive materials, minimal consumption of both reagents and sample, and ease of operation, make them ideally suited for frequent analysis performed by unskilled operators.

Improving the analytical response represents a vital strategy to attain faster decisions on the course of action. An on-site accurate and reliable tool can be applied to environmental, food and health diagnosis, enabling to anticipate public health issues with water contamination, and food degradation in shelf life and to identify undiagnosed health issues, even with asymptomatic, pre-symptomatic or unrecognized symptomatic diseases. The use of digital scanning as a detection process has enabled to maintain the accuracy and reliability of the analysis based on colorimetric reactions, where the analyte concentration relates to the colour intensity. An overview of this emerging quantification method is presented, highlighting the versatility of μPADs with applications to natural waters, food samples and biological fluids of non-invasive collection.

CHARACTERIZATION AND SELECTION OF ANTIBODIES FOR LATERAL FLOW DIAGNOSTICS USING SPR TECHNOLOGY

Klaus Hochleitner

Cytiva, Germany

klaus.hochleitner@cytiva.com

Due to Covid-19, almost everybody has used lateral flow rapid tests for diagnosing an infection, and it is expected that we will see an increased demand for such rapid tests for home use to detect all kinds of analytes in human bodily fluids in the future. While lateral flow tests are easy to use, their development is far away from easy, and there are special requirements for the kinetic properties of the capture and detector reagents that are very different from the requirements for other test systems, such as ELISAs.

In a case study, we describe the process of a rapid down-selection of suitable binders for a lateral flow diagnostic test from a couple of commercially available antibodies, including the selection of the correct membrane surfactant to be used with the selected antibodies.

MITIGATING THE HOOK EFFECT IN A HCG-SPECIFIC LATERAL FLOW TEST AND EXTENDING THE DYNAMIC RANGE OF THE ASSAY

Aart van Amerongen[1], J. Veen[2] and J. Wichers[1]

[1] BioSensing & Diagnostics, Wageningen University & Research, the Netherlands
[2] HAN University of Applied Sciences, the Netherlands

aart.vanamerongen@wur.nl

The lateral flow (immuno)assay is a relatively old diagnostic platform that was developed in the 1980s. The first lateral flow test (LFT) that entered the market was the Clearblue 1-step test in 1988 (Unipath) as a home test for the detection of the pregnancy hormone [1]. The test was the first diagnostic platform that could be performed by the layman and is still very frequently used to assess pregnancy. It detects human Chorionic Gonadotropin (hCG) a hormone that is produced by the placenta. In early pregnancy, the hCG level in the blood doubles every two to three days [2]. By 6 to 7 weeks gestation doubling time decreases to roughly every 3 days and by reaching 8 to 10 weeks, the hCG level will have reached its peak. In pregnancy levels of hCG may vary between 10 and 300,000 mIU/ml. If hCG levels are dropping in the first trimester, this probably is a sign of impending miscarriage. On the other hand, slow-rising hCG levels that do not double every two or three days in early pregnancy may be a sign of problems as well but can also occur in a normal pregnancy. Of all positively assessed pregnancies 15-20% will end in a miscarriage [3]. Therefore, a test to assess hCG levels in the first trimester may be a valuable tool to assess low levels in order to timely address the potential underlying medical problem.

Generally lateral flow tests can measure hCG in a concentration range of 10 to 1000 mIU/ml (the dynamic range of the test). Higher concentrations will lead to the so-called hook effect; a decrease of the test signal up to a false-negative test upon increasing hCG concentrations. Although the dynamic range of the test can be shifted, this will result in only a limited change, by far insufficient to adequately measure higher hCG concentrations. Dilution of the sample would be needed to bring the concentration in the dynamic range of the test, but since the hCG concentration may vary between 10 and 300,000 mIU/ml it cannot be predicted which dilution factor should be used at a particular time. In addition, diluting a sample is not a user-friendly procedure and should be avoided in the performance of a point-of-care test.To overcome these limitations, we have investigated whether the lateral flow hCG test can be adapted such that it is applicable at higher concentrations as well. Thereto, we have sprayed additional test lines and a hCG-antigen line. The pixel grey values of the lines were recorded by a flatbed scanner. Combinations of data from at least one test line, the antigen line and the control line were used to train a linear regression model. The analysis resulted in a quite accurate algorithm that can be used to predict the hCG concentration of unknown samples up to a concentration of 300,000 mIU/ml.

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CAN RAPID METHODS BE SUSTAINABLE? ENVIRONMENTAL CONCERNS AND SOLUTIONS IN THE POINT-OF-NEED SECTOR

Maïwenn Kersaudy-Kerhoas

School of Engineering and Physical Sciences, Heriot-Watt University, UK

m.kersaudy-kerhoas@hw.ac.uk

Microfluidics is the underpinning technology behind many point-of-care diagnostic or point-of-need products, from complex integrated solutions to humble lateral flow tests. These tests are now ubiquitous tools in rapid methods for human diagnostics or environmental monitoring.

But how did we start to produce these tests? Where do they come from and who make them? Were they developed with enough considerations for environmental sustainability? Using my perspective as a microfluidic engineer, observations during travels and discussions with global health practitioners and anthropologists, I will share what I learned in recent years about the mishaps of point-of-need, single-use and disposable methods, and how researchers and practitioners can develop more sustainable solutions.

SESSION 10
SPECTROMETRY AND SPECTROSCOPY – PART 2

GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY APPROACH FOR THE GEOGRAPHICAL ORIGIN EVALUATION OF DEHYDRATED APPLES

Michele Suman[1,2], G. Sammarco[1,3] and C. Dall'Asta[3]

[1] Sensory and Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Italy
[2] Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Italy
[3] Department of Food and Drug, University of Parma, Italy

michele.suman@barilla.com

Gas chromatography-ion mobility spectrometry (GC-IMS) is an interesting candidate to face the geographical origin declaration fraud on dehydrated apple samples, as it represents a rapid, cost-effective, and sensitive solution for food authenticity issues. A design of experiment (DoE) led to a robust sampling, taking into account different factors, such as harvesting year, presence of peel, variety. The sample preparation was limited: GC-IMS analytical method permitted to obtain of a 3D graph in 11 min, and the multivariate statistical analysis returned a clear separation between Italian and non-Italian samples, considering both unsupervised and supervised approaches. The statistical model, created employing a training set, was applied on a further test set, with a good overall performance. GC-IMS could play a relevant role as an innovative tool to prevent/fight false origin declaration frauds, but also, potentially, for other kinds of food authenticity and safety frauds.

APTAMER-BASED BIOSENSORS FOR REAL-TIME DETECTION OF PROTEIN BINDING

A. Görner[1], T. Çanak-Ipek[2], M. Avci-Adali[2] and **Anna-Kristina Marel**[1]

[1] Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Germany
[2] Department of Thoracic and Cardiovascular Surgery, University Hospital Tübingen, Germany

anna-kristina.marel@mri.bund.de

Biosensors are applied in many research areas. Depending on the measurement principle, a distinction is made between optical, electrochemical and piezoelectric sensors. The quartz crystal microbalance (QCM) is a well-known system based on the piezoelectric effect that allows label-free detection of binding events. Furthermore, these interactions can be observed in real-time via the change of the resonance frequency. Different molecules can be used as recognition elements: antibodies, enzymes or aptamers. Aptamers are single-stranded RNA or DNA molecules with a length of 20 to 90 nucleotides.They are featured by high specificity and stability as well as consistent quality. When aptamers are combined with biosensors, they are referred to as aptasensors.

Generally, aptamers are immobilized on the biosensor surface via short linkers. The biological activity of proteins can be impaired at interfaces, especially at hard surfaces. Therefore, in this work, we established an alternative system for linking aptamers to the biosensor that is more close to physiological conditions. We used supported lipid bilayers (SLBs), which are used as artificial cell membranes and exhibit two-dimensional fluidity, which allows the self-organization of the involved molecules. The SLB was composed of POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine) and biotin-PE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl). The biotinylated aptamers were bound to the surface via biotin-streptavidin interactions. A well-studied aptamer against thrombin was applied as a model system. SLB formation was investigated in terms of temperature and biotin content. At temperatures of 25°C and below, the bilayer formation was incomplete while at higher temperatures a full layer was formed. Only a small influence of the content of biotinylated lipids in the mixture on SLB formation and the subsequent binding of streptavidin was observed. Different concentrations of aptamer were applied in order to assure full coverage of the sensor surface. The sensor was able to detect thrombin in the nM range even in complex matrices as human serum. Additionaly, reconstitution of the sensor surface was achieved by incubation with a 5 M urea solution which resulted in the release of the attached thrombin. Thereafter, it was possible to rebind thrombin.

The developed surface functionalization based on a SLB provides the advantage of a cell membrane-like interface that offers the possibility to study biological binding events under more physiological conditions. The described aptasensor design can be applied to further target analytes using a suitable aptamer.

HOMOGENEOUS NONCOMPETITIVE IMMUNOASSAYS FOR *STAPHYLOCOCCUS AUREUS* ENTEROTOXIN A

S. Jylhä[1], H. Arola[1,2], K. Iljin[1], P. Saviranta[1] and **Tarja K. Nevanen**[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] Centre for Military Medicine, Finland

tarja.nevanen@vtt.fi

Two rapid immunoassays developed for simple and sensitive detection of *Staphylococcus aureus* enterotoxin A (SEA) are introduced here and their performances are compared. Two recombinant anti-SEA antibodies (Fab fragments) were either labelled with fluorescence resonance energy transfer (FRET) compatible fluorescent labels [1] or fused with peptides complementing the ternary NanoLuc-luciferase enzyme [2]. The small size of the Fab fragments (48-50 kDa) enabled the distance-dependent FRET phenomenon. Well-known structures and dimensions of Fab fragments simplified the design of the linker between the antibody and luciferase completing peptides in case of Luminescent complement assay (LCA).

Staphylococcal enterotoxins are gastrointestinal toxins, also acting as superantigens causing non-specific T-cell proliferation. SEA is the most frequent toxin involved in food poisoning outbreaks by *Staphylococcus aureus*. It is a very potent toxin and stable for various methods of ravaging. Sensitive and specific detection methods integrated to user-friendly rapid diagnostics are needed for, e.g., food safety management.
Recombinant antibody fragments specific for SEA were discovered from the antibody phage display library. Biacore T200 was employed to determine their affinities, binding kinetics and epitopes. Two high affinity antibodies recognizing different epitopes of SEA (27 kDa) were chosen for assay development. Anti-SEA Fab fragments were functionalized with Europium chelates and Alexa Fluor 647 fluorophores for the FRET immunoassay. Recombinant fusion proteins containing of luciferase complementing peptides B9 and B10 fused either amino – or carboxy-terminally to the antibodies were constructed utilizing glycine-serine linkers in between. Furimazine substrate was applied to produce bright luminescent signal.

The sensitivity, repeatability and dynamic range of both FRET and LCA assays were compared both in fortified buffer and in full, non-homogenized milk. Performance, usability, time and costs of the assays were compared.

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HOMOGENEOUS IMMUNOASSAY FOR CYCLOPIAZONIC ACID BASED UPON MIMOTOPES AND UPCONVERSION-RESONANCE ENERGY TRANSFER

Fernando Pradanas González[1], R. Peltomaa[2], S. Lahtinen[2], Á. Luque-Uría[1], V. Más[3], R. Barderas[3], C.M. Maragos[4], T. Soukka[2], E. Benito-Peña[1] and María C. Moreno-Bondi[1]

[1] Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Spain
[2] Department of Life Technologies/Biotechnology, University of Turku, Finland
[3] Instituto de Salud Carlos III, Spain
[4] Mycotoxin Prevention and Applied Microbiology Research Unit, Agricultural Research Service, US Department of Agriculture, USA

ferprada@ucm.es

In this work, we describe a homogeneous upconversion-resonance energy transfer (UC-RET) immunoassay for detecting the neurotoxin α-cyclopiazonic acid (CPA) in food samples using a novel mimicking peptide, or mimotope, selected by phage display. Mouse anti-CPA monoclonal antibody was used to isolate mimotopes from a cyclic peptide library in successive selection series. Enrichment of antibody binding phages was observed after three panning rounds, and sequence analysis of randomly selected monoclonal phages revealed four conserved peptide sequences. For the development of a homogeneous assay, upconversion nanoparticles (UCNP, type $NaYF_4$:Yb^{3+}, Er^{3+}) were used as energy donors. These nanoparticles have a photophysical feature whereby they convert low-energy near-infrared (NIR) radiation into higher-energy radiation, typically in the ultraviolet-visible range, enabling the total elimination of autofluorescence [1] and reducing the possible optical interferences from the sample matrix [2]. UCNPs were coated with streptavidin to anchor the synthetic biotinylated mimotope. Alexa Fluor 555, used as an energy acceptor, was conjugated to the anti-CPA antibody fragment. The homogeneous single-step immunoassay could detect CPA in just 5 min and enabled a limit of detection (LOD) of 30 pg/ml (1.5 µg/kg) and an IC_{50} value of 0.36 ng/ml. No significant cross-reactivity was observed with other co-produced toxins. Finally, we applied the novel method for the detection of CPA in spiked maize samples using high-performance liquid chromatography coupled to diode array detection (HPLC-DAD) as a reference method [3].

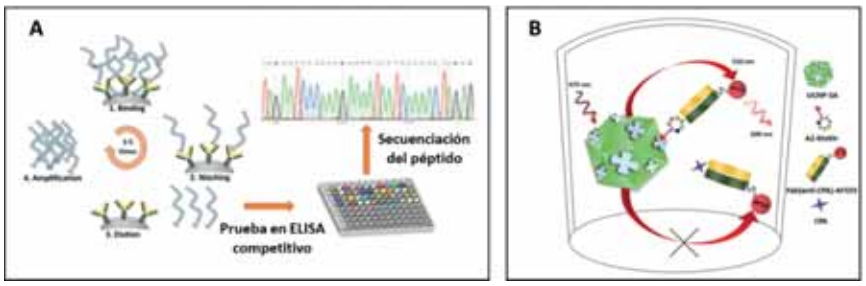

Figure. (A) Selection of CPA mimotopes by phage display and (B) scheme of the UC-RET immunoassay.

Acknowledgements
This work has been funded by the Ministry of Science, Innovation and Universities (MSIU) (RTI2018-096410-B-C21 and PDI2021-127457OB-C21). F.P. acknowledges the MSIU for an FPU contract.

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SIMPLIFYING BIOANALYTICAL ASSAYS BY LIGHT DIFFRACTION

Miquel Avella-Oliver[1,2], A. Juste-Dolz[1], E. Fernandez[1] and Á. Maquieira[1,2]

[1] Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, Spain
[2] Departamento de Química, Universitat Politècnica de València, Spain

miavol@upv.es

The simplicity of the analytical procedure for the end users is an important aspect that supports the transition from labs to on-site testing. Along these lines, the advances in nanoscience and nanotechnology offer new light-matter interaction phenomena that can be exploited for label-free detection, which reduces the number of incubations required to perform the assay, speeds up the analytical method, and minimizes the microfluidic needs.

Herein, we introduce a new photonic principle for biosensing, called Biogratings, that exploits light diffraction to quantify biorecognition assays. It relies on nanostructured gratings of bioreceptors (proteins) patterned on the surface of solid substrates and tailored to diffract incident laser beams, where the magnitude of this diffraction depends on the binding events of the assays [1,2]. Our results demonstrate that Biogratings enable to quantify, in label-free format, the concentration of biomolecular targets present in samples. The experimental dose-response curves with model immunoassays for bovine serum albumin, casein, and β-lactoglobulin exhibit good limits of detection for unlabelled specific antibodies in solution. To pattern the gratings of bioreceptors, we have developed different approaches based on micro-contact printing as well as the selective denaturation of biolayers by means of UV irradiation, reaching a favourable scalability and reproducibility.

Biogratings present a unique potential to avoid the common problems associated with the analysis of complex samples. Unlike other label-free technologies, the diffractive response of Biogratings is unaffected by non-specific binding processes taking place on the sensing surface. This is an important advantage that enables the direct analysis of complex samples, thus avoiding tedious sample pretreatment stages. Our results demonstrate this feature in the determination of specific antibodies present in pure human serum samples, reaching a limit of detection of 36 ngml in label-free format.
Finally, Biogratings introduce appealing perspectives to be materialized in different analytical devices, and this communication also summarizes our results towards the implementation of this technology in miniaturized photonic chips based on integrated waveguides. Besides, the scope of this technology can be expanded to multitude of different assays and applications, and our ongoing investigations are also exploring this aspect.

Acknowledgements
Funded by MCIN/AEI/10.13039/501100011033 and ERDF (PID2019-110713RB-I00), Generalitat Valenciana (PROMETEO/2020/094), and Universitat Politècnica de Valencia (PAID-06-22).

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FINAL PLENARY SESSION
RAPID ANALYSIS & DIAGNOSTICS – WHAT FURTHER?

FROM WATER TO WHISKY: GOLDEN OPPORTUNITIES IN ANALYSIS WITH PLASMONIC PARTICLES

William J. Peveler[1], J.R. Sperling[2], J. Gracie[1] and A.W. Clark[2]

[1] School of Chemistry, University of Glasgow, UK
[2] James Watt School of Engineering, University of Glasgow, UK

william.peveler@glasgow.ac.uk

Localised surface plasmon resonance, the strong interaction between electrons in nanoscale particles of metal, and light, results in the observed strong colour of nanoscale colloidal gold, silver etc. Such plasmonic colour is exquisitely sensitive to the material, size and shape of the colloidal particles as well as their local dielectric environment – what is bound to the particle surface. I will demonstrate that by using varied surface chemistries, inspired by mammalian taste or smell, we can control the interactions between gold nanomaterials and many different components in an analytical sample, and thus generate hyperspectral 'chemical tongues' as cross-reactive sensing units. By processing the combined colour change output of the plasmonic array a wide variety of samples can be studied or identified [1].

I will also discuss how we can utilise certain samples to actually generate colloidal gold nanoparticles in-situ. Through examination of the rate of formation, shape, size and surface chemistry of the resulting nanoparticles and correlating this with measured chemistry in a library of samples, we can generate a rapid and informative chemical test for quality control in a range of food and beverage products [2].

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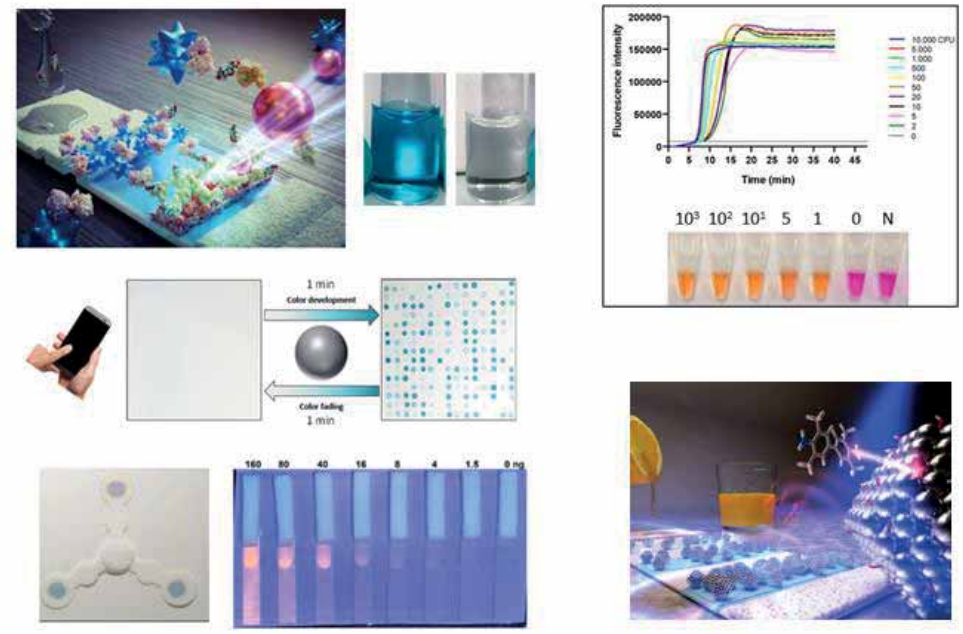
POINT-OF-CARE COLORIMETRIC NANOBIOSENSORS: HEALTH, SAFETY, FOOD, ENVIRONMENT

Tania Pomili[1], A. Scarsi[1,2], H. Torné-Morató[1,2], F. Gatto[1], P. Cecere[1] and P.P. Pompa[1]

[1] Nanobiointeractions & Nanodiagnostics, Istituto Italiano di Tecnologia, Italy
[2] Department of Chemistry and Industrial Chemistry, University of Genova, Italy

tania.pomili@iit.it; pierpaolo.pompa@iit.it

In this presentation, we show the development of our broad sensing platform for on-field and point-of-care diagnostics. Our technologies are based on hybrid detection strategies, exploiting the combination of plasmonic nanoparticles/nanozymes, molecular biology, biotechnology, and machine learning (ML), allowing the use of different matrices (such as water, saliva, urine, milk, food). The focus is on instrument-free portable assays, involving isothermal/room-temperature signal/target amplification, and naked-eye or smartphone-based colorimetric readout (powered by ML algorithms). Both in-solution and LFDs-like devices with multiplexing capabilities are developed. Specific areas of applications span from food safety and traceability to anticounterfeiting, non-invasive in-vitro diagnostics for healthcare and wellness, and environmental and on-farm veterinary controls.



ALTERNATIVE METHODS IN THE FRAME OF OFFICIAL CONTROLS IN EUROPE: THE CASE OF *LISTERIA MONOCYTOGENES*

Nathalie Gnanou Besse[1] , M. Mancier[1,2], L. Chikhi[1,2], L. Guillier[1], H. Brugère[2], L. Faouzi[2], A. Asséré[1], L. Barre[1] and B. Lombard[1]

[1] French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
[2] National School of Veterinary Medicine, France

nathalie.gnanou-besse@anses.fr

Listeriosis remains one of the most severe foodborne diseases regarding mortality rate. *Listeria monocytogenes* can grow under stressful conditions and contaminate various food categories. Here, we report the regulatory context of the use of alternative methods for the detection of *L. monocytogenes*, and current practice in Europe in the frame of official controls. We also report the comparison of alternative certified methods for the detection of *L. monocytogenes* in the frame of official controls, according to the criteria expressed by the National Reference Laboratories for *L. monocytogenes* network in Europe, through an enquiry conducted in 2022 by the European Union Reference Laboratory for *L. monocytogenes*.

Current practices in Europe reflect the successive development of complementary technologies in the field of food safety aiming at improving the efficiency, speed and automation of detection methods.
Emerging detection methods make it possible to diversify the panel of available detection methods. Each method has advantages and disadvantages, and the choice of a method depends on the needs, means and objectives of the laboratory. Our study focused on a comparison by category in order to provide an overview of the differences between the methods as complete and simple as possible, by examining mainly the certification files of the alternative methods, which constituted a reliable and publicly accessible source of information about specificity and performances of the method.

FROM SELF-REPORTING TO BEHAVIOURAL MARKERS: LIFESTYLE MONITORING THROUGH WEARABLES AND SMARTPHONE SENSORS

Bram Steenwinckel

Internet Technology and Data Science Lab (IDLab), Ghent University, Belgium

bram.steenwinckel@ugent.be

In the contemporary era marked by the usefulness of wearable technology and the omnipresence of smartphones, a profound transformation is underway in the realm of health and lifestyle monitoring. No longer are we limited solely on self-reported information to assess the behaviours and well-being of patients during studies. Instead, we now have the capability to gather objective, real-time data through sensors that we wear every day. They capture our daily activities, sleep patterns, stress levels, and even overall wellness. We see these captures as so-called behavioural markers, opening a treasure trove of insights into our lives and granting us the ability to make more informed decisions about our health or overall quality of life.

Nevertheless, this promising frontier is not devoid of challenges. Throughout our talk, we will shine a spotlight on the hurdles that must be tackled for widespread adoption and seamless analyses of such behavioural markers into our daily lives. These encompass concerns ranging from data privacy and security to issues surrounding the accuracy and reliability of the sensor-based insights. All these insights and challenges led to our real-world evidence framework, where wearable technology and sensor systems are used to perceive, monitor, and possibly manage lifestyles. The convergence of these technologies offers a panoramic view of possibilities, extending beyond mere data collection and analysis. We will also traverse the landscape of potential applications, spanning from personalized healthcare solutions and pioneering research avenues to enhanced well-being.

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[1]Instituto de Microelectrónica de Barcelona, CSIC, Spain; [2]Instituto de Química Avanzada de Cataluña, CSIC, Spain; [3]CIBER de Bioingeniería, Biomateriales y Nanomedicina, Spain; [4]Institut d'Investigació Germans Trias i Pujol, Spain; [5]\CIBER Enfermedades Respiratorias, Insituto de Salud Carlos III, Spain; [6]Departament de Bioquímica i Fisiologia, Facultad Farmàcia, Universitat de Barcelona, Spain

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[1]Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands; [2]Wageningen Plant Research, Wageningen University & Research, the Netherlands

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[1]iBET, Instituto de Biologia Experimental e Tecnológica, Portugal; [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal; [3]FFULisboa, Faculdade de Farmácia, Universidade de Lisboa, Portugal

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[1]Chemical and Optical Sensing Division, Bundesanstalt für Materialforschung und -prüfung, Germany; [2]Department of Organic Chemistry, University of Valencia, Spain; [3]Institute of Agricultural Chemistry and Food Technology, Spanish Council for Scientific Research, Spain

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[1]BIOSYST-MeBioS Biosensors Group, KU Leuven, Belgium; [2]BIOSYST-MeBioS Biostatistics Group, KU Leuven, Belgium

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[1]Department of Biophysics, All India Institute of Medical Sciences, India; [2]Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, India

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[1]Wageningen Plant Research, Wageningen University & Research, the Netherlands; [2]Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands; [3]Wageningen Food Safety Research, the Netherlands

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[1]School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, UK; [2]Department of Hepatology, St. Mary's Hospital, Imperial College Healthcare NHS Trust, UK; [3]Institute for Liver and Digestive Health, Division of Medicine, University College London, UK

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[1]Technology and Food Unit, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium; [2]Department of Physics and Astronomy, KU Leuven, Belgium; [3]Flanders' FOOD, Belgium; [4]Department of Pathobiology, Pharmacology and Zoological Medicine, Ghent University, Belgium

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C.-H. Chen and **Jitendra Patel**
Agricultural Research Service, US Department of Agriculture, USA

P1

RAPPID: A MIX-AND-MEASURE IMMUNOASSAY FOR ROBUST, SIMPLE AND FAST DIAGNOSTICS

Eva van Aalen[1] and M. Merkx[2]

[1] LUMABS B.V., the Netherlands
[2] Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, the Netherlands

e.v.aalen@lumabs.com

Immunosensors have transformed the field of diagnostics, facilitating the selective and sensitive detection of virtually any relevant biomarker. Heterogeneous immunoassays, such as ELISA or SPR, have become a valuable analytical tool that is routinely used both in clinical laboratories and academic environments. However, analytical assays such as ELISA require trained personnel, cumbersome workflows, sophisticated readout techniques, and often result in the generation of significant waste. Furthermore, these types of heterogeneous immunoassays have a long sample-to-result workflow, rendering them unsuitable for use outside of traditional laboratories. At LUMABS diagnostics, we employ the bioluminescent RAPPID technology to facilitate rapid diagnostics, accomplished through our homogeneous immunoassays that produce a blue bioluminescent signal upon the presence of analyte. RAPPID is based on the reconstitution of a split luciferase, which occurs when two analyte-specific antibodies bind to the target. The usage of antibodies for analyte detection makes RAPPID modular by design and allows the quantification of a wide range of (clinically) interesting analytes, such as (therapeutic) antibodies, inflammatory markers, viral proteins and cancer biomarkers. By introducing an internal calibrator luciferase, the RAPPID assay generates a ratiometric signal, ensuring signal stability over time and robust quantitative detection, also by non-expert users. Overall, RAPPID has been developed to replace complex, time-consuming and waste-intensive steps of traditional ELISA methods with a straightforward mix-and-measure approach, offering high sensitivities, large dynamic ranges and measurements in low volume samples (1 µl).

P2
DIMENSIONAL ESTIMATES OF OPTIMAL LATERAL FLOW TEST CONDITIONS

T. Beumer[1] and **Aart van Amerongen**[2]

[1] ELITechGroup B.V., the Netherlands
[2] BioSensing & Diagnostics, Wageningen University & Research, the Netherlands

aart.vanamerongen@wur.nl; tom.beumer@gmail.com

The lateral flow test (LFT) is an immunoassay format for rapid home and point-of-care testing [1]. LFTs have been on the market since 1989 when Unipath launched their home-use pregnancy hormone test. Today, this diagnostic platform is well-known to the general public, both from this pregnancy test and for being used for self-testing during the SARS-CoV-2 pandemic. For some decades the principle of this test has not been substantially changed, being used qualitatively (semi-quantitatively) to demonstrate, e.g., whether a person is infected with Covid-19. Over the last years however, the platform is frequently being adapted to increase sensitivity, robustness, number of simultaneously detectable analytes, and repeatability [2]. Especially repeatability is essential to transfer this diagnostic platform into a quantitative format. In this respect, a better understanding of and control over the physical principles underlying the performance of a lateral flow test are key. Thereto, we have analysed key materials' properties and their mutual interactions that govern test design and performance optimization: the capillary dimensions and porosity of the various porous carrier materials, the sample fluid's properties and volume, the label particle's dimensions and concentration and the signal and control capture zone's dimensions. We will present a very simple geometric method to rapidly achieve a reliable first estimate of the optimum combination of these properties and interactions.

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P3
SUSTAINABLE CASSETTES WITH OPTIMAL PRESSURE POINT DIMENSIONS FOR LATERAL FLOW TESTS

E. Busscher and **Aart van Amerongen**

BioSensing & Diagnostics, Wageningen University & Research, the Netherlands

aart.vanamerongen@wur.nl

The lateral flow (immuno)assay is a user-friendly diagnostic platform that can be performed by the layman. Ideally, LFTs should meet the REASSURED criteria, i.e., Real-time connectivity – Ease of specimen collection – Affordable by those at risk of infection/disease – Sensitive with very few false-negatives – Specific with very few false-positives – User-friendly tests that are simple to perform and require minimal training – Rapid, to enable treatment at first visit, and Robust, for example not requiring refrigerated storage – Equipment-free and Environmentally friendly – Delivered to those who need it. Today, LFTs are also well-known to the general public, as LFTs have been substantially used worldwide for self-testing during the SARS-CoV-2 pandemic [1]. By the end of October 2022, EU countries had issued nearly 365 million EU digital COVID certificates based on the results of antigen tests included in the EU common list [2]. Worldwide this number is even substantially higher. With respect to the REASSURED criterion 'Environmentally friendly' this huge number of tests also means that the environment has been contaminated with a similar number of plastic cassettes, all based on unsustainable fossil polymers. To lower the challenges to the environment and human health, sustainable and biodegradable polymers should be used as material for LFT cassettes [3]. To address this challenge BioSensing & Diagnostics in Wageningen is developing biodegradable LFT cassettes that are based on poly-lactic acid and other biopolymers. By rapid-prototyping cassettes have been designed that are fit-for-purpose as an adequate housing of lateral flow tests. Normally, cassettes are ordered from companies that produce bulk quantities and if different cassettes are available at all, the best performing is used for the final assay. However, generally cassette dimensions are not optimized with a view on improving test signals. A crucial cassette parameter is the placement and height of the pressure points that are used to guarantee a good overlap of the various membranes and pads applied in the test such as the sample and conjugate pads, the nitrocellulose membrane and the absorbent pad. To optimize pressure points, a device was designed and 3D-printed with which it is possible to objectively determine the pressure point dimensions. Initial results will be shown.

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P4

HYBRIDISATION-BASED DETECTION OF INFECTIOUS DISEASES FOLLOWING ISOTHERMAL DNA AMPLIFICATION

Madhura Bharati, H. Swinburne, C. Johnson and N. Keegan

Diagnostic and Therapeutic Technologies, Translational and Clinical research Institute, Newcastle University, UK

madhura.bharati@newcastle.ac.uk

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is an opportunistic pathogen and poses a significant health risk to neonates, immunocompromised individuals, and the elderly, potentially leading to severe conditions like sepsis, meningitis, and pneumonia. It is a leading cause of preventable neonatal and infant mortality globally, with long-term sequelae among survivors. Presently, screening strategies rely on microbiological methods (culture/molecular diagnostics) or risk-based strategies, wherein pregnant women are routinely screened at 35-37 weeks of gestation to ensure timely microbial culture results before labour. However, the rapid variability in S. agalactiae levels can render premature testing obsolete, resulting in antibiotic prescriptions based on outdated information. Thus, there is a need for a sensitive and rapid point-of-care (POC) diagnostic test that can detect all *S. agalactiae* strains to ensure appropriate and timely intrapartum antibiotic prophylaxis (IAP) for the right patients for achieving improved neonatal outcomes. Recombinase polymerase amplification (RPA) is an isothermal DNA amplification technique, which does not require the use of sophisticated laboratory equipment like a thermocycler. This gives it the potential to perform amplification in resource-limited settings, thereby enabling it to be deployed as a cost-effective and suitable POC test. A novel computer programme, IDRIS, was employed to identify unique DNA biomarkers present across all sequenced *S. agalactiae* strains. Using specific forward and reverse primers with single-stranded overhang sequences, the amplified product could hybridise with an immobilised complementary capture probe and a reporter-tagged detection probe. RPA was performed on DNA extracted from bacterial isolates. Enzyme-linked oligonucleotide assay (ELONA) was used to read out the results using a horseradish peroxidase tagged reporter probe. The ELONA format was converted into a lateral flow read-out to further reduce the turn-around-time (TAT). This system used a reporter probe conjugated with a gold nanoparticle (Au-NP) for visualization of a positive result. Magnetic beads with complementary sequences were used to remove excess primers to reduce false positive results. RPA was able to amplify DNA from all 10 strains of GBS and differentiate it from Group A *Streptococcus pyogenes* (GAS). Thus, RPA combined with hybridisation-based detection offers good sensitivity and specificity and has the ability to differentiate GBS from GAS. The possibility of translating the RPA assay to a paper-based lateral flow detection opens up the possibility to use it as an inexpensive intrapartum POC test for maternal screening.

P5

A SPECIES-INDEPENDENT LATERAL FLOW TEST TO DETECT RIFT VALLEY FEVER VIRUS ANTIBODIES IN SERA

Heleen van den Bosch[1], P. Wichgers Schreur[2], R. Massop[1], J. Harders-Westerveen[2], S. van de Water[2] and A. van Amerongen[1]

[1] BioSensing & Diagnostics, Wageningen University & Research, the Netherlands
[2] Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands

heleen.vandenbosch@wur.nl

Several arboviruses, viruses that spread by using arthropod vectors, pose an increasing risk for human and animal health and outbreaks have major consequences for the individual farmer, local communities, and local and national governments. Currently, PCR and ELISA are diagnostic methods of choice, however, pen-side tests would help the farmer and veterinarians to confirm or exclude presence of (arbo)virus pathogens at short notice. Therefore, a capillary flow based immunodiagnostic assay (lateral flow test; LFT) able to detect bunyavirus specific antibodies was developed, using Rift Valley fever virus (RVFV) as a model pathogen. Furthermore, the test was developed to be both immunoglobulin class- and species-independent by applying a double-antigen approach. Antigen involved in viral RNA encapsidation, the so called nucleocapsid protein (N), was produced in *E. coli* and the protein was purified using affinity chromatography. For the lateral flow assay, the antigen was coupled to carbon nanoparticles and also sprayed onto nitrocellulose membrane. This double-antigen approach was subsequently tested with sheep, calf, goat and human immune sera (150 in total) and compared with the commercially available competitive RVFV assay from IDvet (ID Screen® Rift Valley Fever Competition Multi-species). A species-independent double-antigen N-ELISA to detect antibodies against RVFV was developed as well and was compared to both assays using the same set of sera. Both the LFT and the ELISA showed a 100% correlation with the commercially available assay from IDvet. Importantly, in this setup no background signals were observed. Multi-analyte LFTs using a microarray of spots for detection of specific antibodies against several pathogenic viruses are being developed. The array platform also qualifies to develop a DIVA-LFT that differentiates infected from vaccinated animals.

P6

ELECTROCHEMICAL CHARACTERIZATION OF MAIN *PSEUDOMONAS AERUGINOSA* METABOLITES FOR DETECTION WITH AN INTEGRATED SENSOR SYSTEM

S. Schneider[1], J. Ettenauer[1,4], I.-J. Pap[2,3], C. Aspöck[2,3] and **Martin Brandl**[1]

[1] Department for Integrated Sensor Systems, University for Continuing Education Krems, Austria
[2] Clinical Institute for Hygiene and Microbiology, University Hospital St. Poelten, Austria
[3] Karl Landsteiner University of Health Sciences, Austria
[4] Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Austria

martin.brandl@donau-uni.ac.at

Pseudomonas aeruginosa is a ubiquitously distributed soil and water bacterium and considered an opportunistic pathogen in hospitals that can cause severe problems in persons with underlying diseases. Therefore, rapid detection and further identification of the bacterium are crucial to provide the appropriate treatment to patients or to take preventive measures. Such a fast detection may be achieved by electrochemical sensors, which have been increasingly used for biological purposes in recent decades and are proving to be reliable tools for potential bacterial identification outside of laboratories. This work focuses on the systematic electrochemical characterization of main metabolites of *P. aeruginosa* in the view as specific signals in an electrochemical detection system. Pure solutes of five metabolites known as part of the *P. aeruginosa* secretome – pyocyanin (PYO), Pseudomonas quinolone signal (PQS), pyochelin (PCH), 2-heptyl-4-hydroxyquinoline (HHQ) and 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) – were prepared in buffers of the whole pH range (pH 2 to 11), in LB or in Pseudomonas selective medium (containing 0.2 g/l cetrimide and 0.015 g/l nalidixic acid). Different electrochemical techniques (cyclic and square wave voltammetry) were applied with a Gamry Reference 600+ potentiostat using screen printed electrodes (SPEs) (DropSens DRP110; carbon working and counter electrodes, silver reference electrode) to gain information on signal specificity, limit of detection as well as pH dependency. In this study, we report the electrochemical behaviour of the five metabolites (PYO, PQS, PCH, HHQ, HQNO) considering the specific detection of the pathogen with a biosensor system. All the mentioned analytes were electrochemically inducible, with the appearance of well-separated oxidation and/or reduction peaks at defined peak potentials relative to the reference electrode (in mV). Moreover, all of them exhibited linear behaviour in concentration ranges classically observed in bacterial supernatants.

P7

A NUCLEIC ACID-BASED LATERAL FLOW DEVICE FOR RAPID DIAGNOSIS OF WEST NILE VIRUS INFECTION

Birgit Bräuer[1], T. Kothe[1], W. Pulverer[1], Y. Gillitschka[1], D. Cadar[2], M. Affara[2], F. Gehre[2] and J.R. Peham[1]

[1] Competence Unit Molecular Diagnostics, Center for Health and Bioresources, AIT Austrian Institute of Technology GmbH, Austria
[2] Bernhard-Nocht-Institut für Tropenmedizin (BNITM), Germany

birgit.braeuer@ait.ac.at

When epidemic outbreaks occur, fast screening of humans and/or animals as well as environmental monitoring of pathogens in the respective region is essential to locally contain the spread of the disease and prevent pandemics. Rapid diagnostic tests are a valuable tool in this context. Within our project MOBILISE, which is dedicated to establishing a mobile laboratory facility responding to disease outbreaks in remote areas, we are developing nucleic acid-based lateral flow devices (LFDs) as rapid tests for West Nile Virus (WNV). WNV has become more common in Europe in recent years due to increasing temperatures caused by climate change [1]. Nucleic acid-based LFDs directly target viral nucleic acids using DNA probes suitable for hybridization to the target sequence. Our approach involves immobilization of oligonucleotides complementary to a segment of the target WNV sequence on the test line as a capture probe. A gold nanoparticle-DNA-horseradish peroxidase conjugate containing oligonucleotides that bind a different section of the WNV target nucleic acid serves as detection probe to visualize the signal, with Au nanoparticles accumulating on the test line. The oligonucleotides immobilized on the gold nanoparticle and test line are derived from the forward primer and the reverse complement of the reverse primer of a published WNV PCR assay. In the rapid test developed herein, the optical signal is further enhanced by applying 3,3′,5,5′-tetramethylbenzidin, which is converted to a blue product by the horseradish peroxidase immobilized on the Au-nanoparticle-DNA-HRP conjugate. We varied the ratio of DNA loaded onto the Au nanoparticles to optimize sensitivity of our lateral flow device, which was lowest (1 nM WNV ssDNA) at a ratio of 63:1 of DNA:AuNP and 0.25 mg/ml HRP during conjugate synthesis. In conclusion, we have developed a nucleic-acid-based lateral flow device for WNV detection which directly detects viral nucleic acid without using antibodies. Targeting viral nucleic acids is beneficial for identifying pre-symptomatic and acute cases, where antibody titres are zero or low [2]. Furthermore, a nucleic-acid-based approach avoids frequently encountered problems of antibody-based rapid tests such as batch-to batch variations in antibody quality and enables rapid adaptation to new virus variants by simply changing the sequences of capture, detection and control probes. Future work will focus on improving sensitivity by optimizing the composition of the running buffer and implementing pre-amplification of the target into the LFD.

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P8

TOWARDS THE DEVELOPMENT OF A LATERAL FLOW IMMUNOASSAY FOR RAPID FORENSIC ANALYSIS OF FINGERMARKS

Maria Dede[1], M. Batus[1], M. Witmond[1], M. Aalders[1,2] and A. van Dam[1,3]

[1] Department of Biomedical Engineering and Physics, Amsterdam UMC, University of Amsterdam, the Netherlands
[2] Co van Ledden Hulsebosch Center (CLHC), University of Amsterdam, the Netherlands
[3] Forensic Science, Amsterdam University of Applied Sciences, the Netherlands

m.dede@amsterdamumc.nl

During crime scene investigations, fingermarks are valuable and non-transferable pieces of evidence for the evaluation of source and/or activity level propositions. The chemical analysis of a fingermark's composition can reveal donor profiling information among others, age, sex, drug abuse, blood type, and lifestyle. Such information can be used to provide new leads, exclude from, or restrict the list of possible suspects during the investigative phase. We conducted a literature search to determine the most suitable setup and labelling technique for implementing lateral flow assay (LFA) in forensic fingermark analysis, specifically for the purpose of donor profiling. We propose a simplistic one-step analysis using a noncompetitive LFA incorporating carbon-label nanoparticles for a sensitive, accurate, and reproducible fingermark analysis. The viability of this LFA setup in detecting substances (e.g., C-reactive protein) in spiked fingermark residues has been previously established. Our current efforts focus on optimizing the extraction protocol from fingermark residues and exploring the technique's feasibility for other biomarkers (e.g., albumin, dermicidin), present in body fluids and fingermark residues, of forensic and/or medical relevance. Ultimately, upon thorough validation, a well-designed LFA can be implemented at the crime scene for a time- and cost-efficient investigation to aid in extracting donor profiling information from fingermarks. Additionally, the obtained information may have implications for medical diagnostics, enabling point-of-care testing for disease biomarkers.

P9

APPLICATION OF WBE APPROACH TO MONITORING OF RESPIRATORY SYNCYTIAL VIRUS IN A SLOVENIAN WASTEWATER TREATMENT PLANT: A RETROSPECTIVE STUDY ON SAMPLES FROM THE NATIONAL SARS-COV-2 MONITORING

Ion Gutierrez-Aguirre, N. Prezelj, N. Matović, Ž. Lengar, M. Ferle, I. Bajde, M. Milavec, A. Bogožalec Košir, O. Maksimovic, A. Županic, M. Ravnikar and D. Kutnjak

National Institute of Biology, Slovenia

ion.gutierrez@nib.si

Wastewater-based epidemiology (WBE) has re-emerged during the COVID-19 pandemic as an important epidemiological surveillance tool supported by successful implementation of SARS-CoV-2 monitoring in many countries all over the world. The positive experience with the SARS-CoV-2 monitoring in wastewater opens doors for monitoring of other epidemiologically relevant respiratory and viruses of concern in wastewater. This would for example help epidemiologists to predict the seasonal disease peaks and to timely administer vaccines. Respiratory syncytial virus (RSV) is one of such important viral pathogens, causing respiratory tract infection (bronchiolitis) that can be critical in infants and other risk population. However, many RSV infections are not identified as people usually do not require medical help and testing is not always performed, which complicates the work of epidemiologists. Here we took advantage of stored RNA isolates from the Slovenian national SARS-CoV-2 wastewater monitoring, sampled in the period from January 2021 to March 2023 in one of the Slovenian wastewater treatment plants (Velenje-Šoštanj), and re-analysed them with a RSV specific quantitative PCR assay using pepper mild mottle virus (PMMoV) as faecal load corrector. In the first half of the year 2021, with strict precautions and governmental measurements still in place due to COVID-19, no RSV was detected in wastewater. After loosening the restrictions, we commenced to detect traces of RSV RNA below the LOQ of the method. From October 2022 to March 2023, we observed an autumn-winter peak in the RSV concentrations with values within the quantification range of the assay. In addition, we also assessed if another member of the *Tobamovirus* genus, tomato mosaic virus (ToMV), could be used as a potential faecal load indicator alternative to PMMoV.

P10
AMPLIFICATION-FREE DETECTION OF VIRAL RNA WITH A PORTABLE ELECTROCHEMICAL PLATFORM

Manuel Gutiérrez-Capitán[1], E. Balada[2], A. Aviñó[2], L. Vilaplana[2,3], A. Lacoma[4,5], A. Baldi[1], C.J. Ciudad[6], R. Eritja[2.3], M.-P. Marco[2,3] and C. Fernández-Sánchez[1.3]

[1] Instituto de Microelectrónica de Barcelona (IMB-CNM), CSIC, Spain
[2] Instituto de Química Avanzada de Cataluña (IQAC), CSIC, Spain
[3] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain
[4] Institut d'Investigació Germans Trias i Pujol (IGTP), Spain
[5] CIBER Enfermedades Respiratorias (CIBERES), Insituto de Salud Carlos III, Spain
[6] Departament de Bioquímica i Fisiologia, Facultad Farmàcia, Universitat de Barcelona, Spain

manuel.gutierrez@csic.es; cesar.fernandez@csic.es

The electrochemical biosensor platform combining three main components: (i) a reusable gold two-electrode electrochemical cell chip; (ii) A disposable paper single-channel fluidic component; and (iii) nucleic acid hybridization assay carried out on magnetic nanoparticles (MNPs). The device architecture comprises the two first components easily aligned into a tailor-made polymeric cartridge that facilitates the exchange of the paper component after every measurement. MNPs functionalized with a capture hairpin sequence are used for sample pre-treatment outside the device. A one-step incubation with the sample and a reporter sequence conjugated to a peroxidase enzyme (HRP) is carried out. Next, some 7 µl of the MNPs suspension are cast on the sample addition area of the electrochemical platform. The MNPs flow and are concentrated on the paper channel with the aid of a magnet inserted in the cartridge. The biosensor response is recorded by chronoamperometry following the HRP reaction in a buffered solution containing H_2O_2 substrate and ferrocenemethanol redox mediator, further added to the paper channel. The developed platform enabled the quantitative detection of specific sequences of SARS-CoV-2 RNA in nasopharyngeal swabs in around 40 min. The device readout was a cathodic current recorded by chronoamperometry and produced by the electrodic reduction of the oxidized form of the redox mediator produced by the HRP reaction. This current is directly proportional to the concentration of the target sequence in the sample. A carefully selected polypurine reverse Hoogsteen sequence formed a triplex with the target RNA oligonucleotide and provided very sensitive results. The lowest concentration that unambiguously produced a signal different from that of the blank was 0.01 nM (1 fmol). Some 58 nasopharyngeal swab samples were analysed in a retrospective study. Using the ROC curve, a sensitivity of 92% and a specificity of 86% were obtained, by comparison with RT-PCR gold standard. A lack of correlation between the Ct values and the RNA concentration estimated with the device was found. Such behaviour might be related to the secondary structure of the viral RNA and the intermolecular interactions taking place among RNA strands that may hinder the accessibility to the specific target sequence. A solution for correcting this effect was explored. In conclusion, an electrochemical biosensor platform for timely detecting specific SARS-CoV-2 RNA sequences in less than 1 h, showing good sensitivity and specificity, was developed. The electrochemical device could be made portable and of potential application for the point-of-need screening of the disease.

P11

DEVELOPMENT OF STABLE NANOBODIES FOR RAPID DETECTION AND NEUTRALIZATION OF STAPHYLOCOCCAL ENTEROTOXIN B IN MILK

A.C. Hughes, M. Kirkland and **Xiaohua He**

Western Regional Research Center, Agricultural Research Service, US Department of Agriculture, USA

xiaohua.he@usda.gov

In this study, sixteen unique staphylococcal enterotoxin B (SEB)-reactive nanobodies (nbs), including ten monovalent and six bivalent nbs, were developed. All characterized nbs were highly specific for SEB and did not cross-react with other staphylococcal enterotoxins (SE). Several formats of highly sensitive enzyme-linked immunosorbent assays (ELISAs) were established using SEB nbs and a polyclonal antibody (pAb). The lowest limit of detection (LOD) reached 50 pg/ml in PBS. When applied to an ELISA to detect SEB-spiked milk (a commonly contaminated foodstuff), a LOD as low as 190 pg/ml was obtained. The sensitivity of ELISA was found to increase concurrently with the valency of nbs used in the assay. In addition, a wide range of thermal tolerance was observed among the sixteen nbs, with a subset of nbs, SEB-5, SEB-9, and SEB-6^2, retaining activity even after exposure to 95°C for 10 min, whereas the conventional monoclonal and polyclonal antibodies exhibited heat-labile properties. Several nbs demonstrated a long shelf-life, with one nb (SEB-9) retaining 93% of its activity after two weeks of storage at room temperature. In addition to their usage in toxin detection, eleven out of fifteen nbs were capable of neutralizing SEB's super-antigenic activity, demonstrated by their inhibition on IL-2 expression in an ex vivo human PBMC assay. Compared to monoclonal and polyclonal antibodies, the nbs are much smaller, thermally stable, and easy to produce, making them useful in applications for sensitive, specific, and cost-effective detection and management of SEB contamination in food products.

P12
HOT TOPIC! WHAT'S IN THE CAN? DEVELOPING A FAST, ONSITE, PRESUMPTIVE TEST FOR PEPPER SPRAY AND TEAR GAS

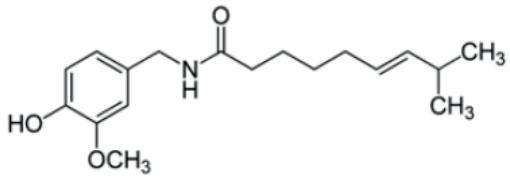
Jan-Hein Hooijschuur

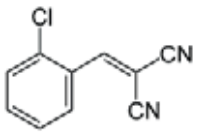
Amsterdam University of Applied Sciences, the Netherlands

j.h.hooijschuur@hva.nl

If the police finds a canister with an unknow substance on a person, the only way to find out whether it is pepper spray or not is an organoleptic analysis on him/herself or on a willing colleague. A fast andonsite presumptive colour test would be ideal for a quick and selective indication if a violation of the law ('Categorie II Wet Wapens en Munitie') applies. Different colour reactions for pepper spray have been selected for tests on different carriers, various designs of paper fluidic devices have been made and currently a student is designing a sampling system. In the end, all of these steps have to be combined for a small and easy presumptive test. And hopefully the same steps can be made for tear gas.

Figure. Pepper spray (left) and tear gas (right).





P13
ANTIGEN-BINDING FRAGMENTS (FABS) IN THE PRODUCTION OF RAPID LATERAL FLOW ASSAY PLATFORM FOR COVID-19 DIAGNOSTICS

Iida Jääskeläinen, A. Mursula, S. Pohjonen, S. Jylhä, J. Kotimaa, P. Saviranta and L. Hakalahti

VTT Technical Research Centre of Finland, Finland

iida.jaaskelainen@vtt.fi

COVID-19 pandemic has been one of the greatest challenges for the whole world in the recent years. Point-of-care (POC) testing against COVID-19 infection has become a common occurrence since the commercial lateral flow (LFA) rapid COVID-19 tests have entered the market. There is a high interest on effective and affordably produced alternatives to the conventional antibodies to utilize in LFA and biosensor development. Promising alternatives to the conventional antibodies are e.g., fragments of antigen-binding (Fabs), which can be produced recombinantly, bypassing the use of immunized animals. In this study, anti-SARS-CoV-2 Fab fragments developed and produced in *Escherichia coli* at VTT were applied on a rapid LFA platform. A sandwich assay was assembled at the test line, with both the capture and the detection particle complex employing Fab fragments. A visual detection system based on the cellulose nanobeads (CNBs) proved the most efficient, outperforming similar colloidal gold and fluorescent dye -based assays. CNB system allowed the detection of commercial SARS-CoV-2 Nucleocapsid N protein in concentrations as low as 2.5 ng/ml, without notable unspecific signal. The limit of detection (LOD) achieved is quite close to and comparable to the commercial LFA test kits. Moreover, the CNB-based system was further tested with clinical samples from COVID-19 positive and negative patients. The test platform could detect the SARS-CoV-2 positive PCR samples with low Ct-values quite reliably, even though the tested clinical samples were strongly diluted. Some issues were caused by the seemingly short shelf-life of the CNB conjugates in sample buffer, which should be addressed in the future by e.g., bringing the detection particles on a separate conjugate pad and/or adding an appropriate preservative agent to the samples. The buffer conditions and LFA materials appear to have a significant impact on the assay performance and require careful optimization to ensure optimal results. All in all, this work establishes the viability of VTT produced anti-SARS-CoV-2 Fab fragments as replacements of the conventional IgG antibodies on LFA platform and in the diagnostics of COVID-19 viral infection. In the future, recombinant Fab fragments could be considered a viable and affordable alternative for antibodies in rapid diagnostic test development in general.

P14
ELECTROCHEMICAL PROFILING OF INDOOR FUNGI FOR RAPID AIR QUALITY ASSESSMENT

Anja E. Knecht and M. Brandl

Center for Water and Environmental Sensors, Department for Integrated Sensor Systems, University for Continuing Education Krems, Austria

anja.knecht@donau-uni.ac.at

Fungi occur everywhere: in soil, water, air, materials, food, and ultimately in and on humans and animals. Typically, the diversity of fungal species found indoors reflects the range of species found outdoors, although indoor fungal concentrations are usually lower. The low life requirements of some fungi may make indoor spaces suitable habitats and mould growth can be the consequence. If indoor fungal concentrations are high and their composition is very different from outdoor air, indoor contamination can be assumed. Fungi can have negative effects. They can damage a building, they can make a building unpleasant by looking and smelling bad, and they can have negative health effects on susceptible individuals, such as children and immunocompromised persons. Conventional methods for evaluating indoor air quality are based on culturing methods and morphological examination. However, this approach is time-consuming, laborious, and often difficult and has been supplemented by methods based on DNA detection techniques. There is considerable interest in developing inexpensive and rapid methods for assessing indoor air quality. In this work, we propose an alternative way to identify fungal species based on recording the voltametric patterns of fungal microsamples (spores and mycelium) directly attached to disposable screen-printed carbon electrodes (SPCEs). Fungi of different genera, such as *Aspergillus*, *Penicillium*, *Wallemia*, *Alternaria*, *Cladosporium* and *Botrytis,* were grown on suitable culture media, then scraped off and extracted in ethanol. After microsamples of the fungi (approximately 100 µg fungal material) were applied to SPCEs in contact with aqueous acetate buffer, characteristic voltametric signals were recorded using square wave voltammetry (SWV). For almost each of the selected fungi, a characteristic electrochemical profile could be seen. Even small amounts, such as 40 µg of fungal material from *Aspergillus fumigatus* on the SPCE already clearly showed the typical electrochemical profile. These profiles can be used as basis for the development of a sensor that identifies indoor-relevant fungi by automatic pattern recognition and provides information about a possible mould problem when compared with the electrochemical patterns of samples from outdoor air.

P15

A METABOLOMICS APPROACH FOR THE EARLY DETECTION OF *CAMPYLOBACTER* SPP. IN BROILER HOUSES BY VOLATILE ORGANIC COMPOUNDS

Miriam G.J. Koene[1], M. van der Most[1], D. te Beest[2], C. van Solt[1], E. Pacholewicz[1] and R. Mumm[2]

[1] Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands
[2] Wageningen Plant Research, Wageningen University & Research, the Netherlands

miriam.koene@wur.nl

Campylobacter is the most reported foodborne pathogen in the European Union and chickens are the main reservoir for human campylobacteriosis [1]. Rapid on-site detection methods could be a powerful tool in the control of *Campylobacter* in the primary production sector. It would provide farmers with real-time information on the *Campylobacter* status of their flocks, leading to better insight into relevant introduction routes of *Campylobacter* into broiler houses. Such knowledge will allow implementation of more adequate control strategies. The potential of measuring volatile organic compounds (VOCs) to detect the presence of *Campylobacter* in broiler flocks was investigated. VOCs were captured from the air of 27 different broiler houses in the second half of the production cycle through dynamic headspace sampling on sorbent tubes. Thermodesorption was used to recover the trapped VOCs before analysing them by gas chromatography mass spectrometry (TD-GC-MS). Simultaneously with the air samples, faecal droppings were collected to determine the *Campylobacter* status of the flocks by culture and PCR. Using a metabolomics approach, a total of 162 VOCs were identified in the air of the broiler houses. Of the 27 flocks included in the study, 8 (29.6%) were *Campylobacter* positive, and differences in VOC profiles were detected between *Campylobacter* positive and negative flocks. Remarkably, many VOCs, including the most differential ones, were more abundant in the samples from negative flocks. In conclusion, the study resulted in a number of candidate VOCs. More field trials should be conducted to validate the robustness of VOC profiles as markers for the presence of *Campylobacter* in broiler houses.

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P16

A GC-MS OFF-FLAVOUR QUANTIFICATION APPROACH HELPS DECIPHER A NEW LAYER OF SPOILAGE POTENTIAL BY *ALICYCLOBACILLUS* SPP.

Inês Carvalho Leonardo[1,2], A. Ferreira[1], M. do Rosário Bronze[1,2,3], M.T. Barreto Crespo[1,2] and F. Bustos Gaspar[1,2]

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
[3] FFULisboa, Faculdade de Farmácia, Universidade de Lisboa, Portugal

ines.leonardo@ibet.pt

Industries that produce or use fruit-based products have faced several spoilage events, resulting in economic losses caused by product recalls and loss of consumer confidence. Some of these events correlate to the presence of Alicyclobacillus (ACB) in food products since they can produce off-flavours in the final products. Guaiacol (2-methoxyphenol) and halophenols (2,6-dichlorophenol and 2,6-dibromophenol) have been widely explored as the major culprits of off-flavour spoilage by ACB. These compounds are associated with medicinal, disinfectant, or smoky odours. In this work, relevant metabolites produced by ACB were identified and quantified by GC-MS using a Wax MS capillary column, while simultaneously investigating their potential as spoilage-related compounds. The ability of distinct ACB species (*Alicyclobacillus acidoterrestris*, *A. acidocaldarius*, and *A. cycloheptanicus*) to produce off-flavour volatile compounds was evaluated in different conditions (e.g., different time spans, off-flavour precursors added, matrix – growth medium, fruit juice). For the first time, isobutyric acid (2-methylpropanoic acid) and isovaleric acid (3-methylbutanoic acid) were reported, as soon as two days of incubation, as being produced in different conditions at concentrations which could surpass the described odour threshold. The sweaty, sour, and unpleasant profile of these newly reported compounds is often associated with certain metabolites produced during milk fermentation by lactic acid bacteria in cheese production. Most importantly, isobutyric acid and isovaleric acid were found to be produced by all three ACB species, regardless of their ability to produce guaiacol and/or halophenols. This work clearly shows that even ACB species previously identified as non-spoilage bacteria can also pose a threat to the fruit juice and beverage industries. Therefore, the risk assessment currently used in the industry for ACB control may need to be revised to accommodate these new findings.

Acknowledgements
ICL acknowledges Fundação para a Ciência e a Tecnologia (FCT) for the financial support received through PhD scholarship 2020.08210.BD. Funding by iNOVA4Health—UIDB/04462/2020 and UIDP/04462/2020 and LS4FUTURE (LA/P/0087/2020), programmes financially supported by FCT and Ministério da Ciência, Tecnologia e Ensino Superior, are also gratefully acknowledged.

P17

SUPERPARAMAGNETIC CORE-SHELL PARTICLES APPLICATION: FROM CYTOMETRY ASSAY TO SIMPLIFIED FLUIDIC SYSTEM

Daniel López-Puertollano[1,2], C. Tobias[1], J. Bell[1], A. Abad-Somovilla[2], A. Abad-Fuentes[3] and K. Rurack[1]

[1] Chemical and Optical Sensing Division, Bundesanstalt für Materialforschung und -prüfung (BAM), Germany
[2] Department of Organic Chemistry, University of Valencia, Spain
[3] Institute of Agricultural Chemistry and Food Technology (IATA), Spanish Council for Scientific Research (CSIC), Spain

daniel.lopez-puertollano@bam.de

Superparamagnetic hybrid polystyrene-core silica-shell beads have emerged as promising alternatives to traditional in flow cytometry-based competitive antibody assays [1]. These materials consist of a polystyrene core and a silica shell, in which magnetic nanoparticles are embedded, facilitating the handling and retention in tests. The outer silica surface allows for easy modification through silane chemistry, allowing the attachment of antibodies, or other molecules of interest. Ochratoxin A (OTA), a mycotoxin that can be found in grain products, coffee, cacao, or grapes, was chosen as the main target analyte to detect [2]. In this study, previously in-house produced anti-OTA antibodies [3] were attached to the surface of the particles and the whole system was used as detection entity. In a first approach, the system was used for the development of a competitive cytometry assay using an OTA-fluorescein (OTA-F) adduct as competitor and marker. In this assay the fluorescence emitted by the OTA-F competitor on the surface of the particle was detected at a wavelength of 518 nm using a 533/30.H filter and was correlated to the forward scatter (FSC) to distinguish it from the excess of competitor still in solution. Under optimised conditions, the final assay showed a limit of detection of 0.03 nM. In a second approach, a simplified ready-to-inject fluidic system was built based on a laser (488 nm) and a photomultiplier detector to measure the signal of competitor still in solution. The competition step was carried out in a vial and the whole mixture was injected into the fluidic system. To avoid signal scattering, the particles were separated in-line using a magnet and only the OTA-F competitor still in solution was detected, reaching a limit of detection of 1.2 nM. With the aim to reduce user manipulation, the final assay is still under development for in-line incubation during the competitive step.

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P18
APTAMER-BASED ELECTROCHEMICAL BIOSENSOR FOR DETECTION OF *APHANIZOMENON* SPECIES

Somayeh Maghsoomi[1,2], M.L. Pham[1] and M. Brandl[1]

[1] Center for Water and Environmental Sensors, Department for Integrated Sensor Systems, University for Continuing Education Krems, Austria
[2] Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria

somayeh.maghsoomi.taramsari@donau-uni.ac.at

Aphanizomenon is a genus of cyanobacteria that is filamentous, nitrogen-fixing, and inhabits aquatic environments. This genus is known as one of the primary producers of cyanotoxins following its blooming, affecting water quality. Most conventional methods for the detection of cyanobacteria are based on enzyme-linked immunosorbent assays (ELISA) and liquid chromatography-mass spectrometry (LC-MS), which allow for the determination of cyanotoxins. However, these methods are time-consuming and costly. Therefore, biosensors have become alternative tools for rapid and accurate detection of not only these toxins, but also typical photosynthetic pigments produced by cyanobacteria. By employing a specific aptamer for recognition of *Aphanizomenon* sp. ULC602, we aim to develop an electrochemical apta-biosensor for rapid and sensitive detection of this bacterium. The principal working mechanism of the created apta-biosensor is based on the conformation change of the electrode-binding aptamer in the presence of the target bacterium, resulting in a decrease in the current peak measured by square wave voltammetry (SWV). This apta-biosensor has a limit of detection (LOD) value of OD_{750} ~0.3 with an extension up to OD_{750} ~ 1.2 and a sensitivity of 511µA.OD_{750}^{-1}.cm^{-2} with no interference from other cyanobacteria. This is the first studied apta-biosensor, which offers an alternative for rapidly and specifically monitoring the spreading of this bacterium.

P19
RAPID DETECTION METHODS FOR TRACEABILITY AND TRANSPARENCY

Bert Popping[1], I. Guelinckx[2] and E.L. Sliwinski[3]

[1] FOCOS, Germany
[2] ILSI Europe, Belgium
[3] The European Federation of Food Science & Technology, the Netherlands

bert.popping@focos-food.com; e.sliwinski@effost.org

The TITAN project will demonstrate the latest transparency-related solutions to help drive the formation of a demand-driven European economy predicted on the production and consumption of healthy, sustainable, and affordable food.Comprising 27 partners from 14 countries (10 MS and 2 associated countries), TITAN will showcase in total 21 innovations covering these themes with TRLs moving on average from TRL 5 to 7 within the lifetime of the project. For the RME2023 conference, we would like to mention four specific pilots which are concerned with the development and/or application of rapid detection methods: (i) demonstration of traceability and authenticity in the olive supply chain; (ii) development of a traceability system and food safety testing for the presence of undeclared food allergens; (iii) microbiology of fermented food products, safety demonstration of food cultures; and (iv) omics and molecular approaches for microbial and chemical quality of long shelf-life food products. The TITAN innovations, all transparency-related, address the following themes: enhancing transparency in agri-food businesses with a focus on SMEs; improving food choices by providing more transparent information to the consumer; using improved transparency to enhance food safety and authenticity of products; and providing improved information on the health and sustainability of food products. By bringing together business strategy, the latest technology innovations, policy and the consumer, TITAN will provide the blueprint of a demand driven economy that provides healthy, sustainable, and accessible food for its citizens.

P20

POINT OF CARE COMPATIBLE SAMPLE PREPARATION BUFFER FOR DIRECT SARS-COV-2 DETECTION IN GARGLE SAMPLES

Barbara Posch[1], L. Osinkina[2], J. Stehr[2] and J.R. Peham[1]

[1] Molecular Diagnostics, Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria
[2] HP Health Solutions GmbH, Germany

barbara.posch@ait.ac.at; johannes.peham@ait.ac.at

The SARS-CoV-2 pandemic increased public interest in fast sample analysis of saliva or gargle samples. The gold standard for saliva or gargle sample processing still includes expensive and time-consuming RNA extraction. Here we propose a fast and simple sample treatment buffer (RACE buffer) as alternative for gargling samples, which is PCR compatible and applicable for point of care detection methods such as loop-mediated isothermal amplification (LAMP). Different sample treatment buffer components were tested for their PCR compatibility, resulting in the determination of TCEP, Triton-X100 reduced, EDTA and Tris-HCl as suitable components and their maximal concentration tolerated in PCR. After adjusting to an optimal formulation of 2.5 mM TCEP, 1% Triton X-100 reduced, 0.25 mM EDTA and 5 mM Tris-HCl, the RACE buffer was shown to have RNase inhibiting effects. This will minimise RNA degradation in saliva and gargle samples. The sample treatment buffer was validated with gargle samples from the internal AIT SARS-CoV-2 screening covering SARS-CoV-2 positive and negative cases. The gargle samples were mixed with RACE buffer and incubated 5 min at room temperature. This mix was then directly analysed with RT-qPCR, resulting in Cq values correlating with the data from the reference method using time-consuming RNA purification protocols. Moreover, the RACE buffer performed well in combination with a SARS-CoV-2 LAMP assay obtaining reliable results after 30 min, where the distinction between positive and negative samples was possible without any readout equipment proofing its compatibility with point-of-care detection methods. The required time for sample preparation is reduced from 1.5 h to about 10 min and the material costs per sample are reduced from 3-5 EUR to 0.01 EUR (20 µl RACE buffer + 5 µl gargle sample). In future perspective, this buffer could be used either in laboratory routine analysis of gargle samples as fast and cheap alternative to RNA extraction or in combination with point-of-care molecular diagnostic platforms.

Acknowledgements
EU-funded DECISION project, Grant agreement ID: 101005111.

P21

A LATERAL FLOW TEST FOR RAPID IDENTIFICATION OF PAEDIATRIC VIRAL INFECTION BASED ON HOST RESPONSE MRNA TRANSCRIPTS

Ruth Reid[1], J. Cheema[1], R. Womersley[1], W. Bolton[1], M. Kaforou[1], M. Levin[1], P. Georgiou[2] and J. Herberg[1]

[1] Paediatric Infectious Disease, Imperial College London, UK
[2] Electrical and Electronic Engineering, Imperial College London, UK

rreid@imperial.ac.uk

The current diagnostic pathway for identifying the cause of infection has several limitations. The clinical features of infection overlap, and diagnostic tests are expensive and lack discriminatory power. Infections can be defined by their unique host-response transcriptomic profile but there are no clinically available cheap, quick, and accurate test platforms that leverage published mRNA host transcript signatures. We aimed to develop a lateral flow assay to detect and quantify the mRNA transcript IFI44L, a biomarker for viral infection. By modifying primers for lateral flow, an RT-LAMP nucleic acid lateral flow assay was developed to detect IFI44L and PPIB (housekeeping gene). Whole blood samples were collected from febrile children with viral (8) or bacterial (6) infections. mRNA was extracted using the Dynabeads mRNA direct kit from 300 µl of PAXgene stabilised blood (equating to <100 µl of whole blood). RT-LAMP was carried out for 30 min at 63°C and 1 µl of each product was added to a universal lateral flow strip. Bands were quantified using a lateral flow reader. IFI44L values were normalised to both the control and PPIB expression to produce Quantified IFI44L expression (QIE). The sensitivity and specificity of the assay were evaluated using QIE distributions grouped by gold-standard phenotypes and a prototype decision boundary was established based on the results. We evaluated the proposed algorithm using the Receiver Operator Characteristic (ROC) analysis and reported AUC statistics. The limit of detection was 100 copies/µl. The average QIE for bacterial infection was 0.855 while the average QIE for viral infection was 1.198. An unpaired T test showed a significant difference between bacterial and viral means (P=0.028). Both groups have similar variability (σM = 0.175 (bacterial), σM = 0.165 (viral)) despite their different sample sizes. There was a +19.8% overexpression of IFI44L in viral infection, compared to a -14.5% under-expression in bacterial infection. This model achieved an AUC of 0.9375. The final algorithm correctly identified 87.5% of viral infections and 83.3% of bacterial infections. In conclusion, these results suggest the feasibility of a lateral flow test to detect and quantify host-response transcripts from whole blood. Such an approach would enable low-cost, laboratory-free, rapid diagnosis of childhood febrile illness, based on a point-of-care technology that leverages host transcript diagnostic signatures and is suitable for use across a range of clinical settings.

P22

MILK ANALYSIS BY FT-IR – ENSURING CONSISTENCY AND RELIABILITY OF RESULTS WITHIN A NETWORK OF LABORATORIES

Giuseppe Sammarco[1], M. Verster[2], T. Miller[3], Y. Soler[4] and P. Tonini[1]

[1] Mérieux NutriSciences, Italy
[2] Mérieux NutriSciences, South Africa
[3] Mérieux NutriSciences, USA
[4] Mérieux NutriSciences, Australia

giuseppe.sammarco@mxns.com

FT-IR technology is nowadays widely used for the multi-component analysis of milk and liquid milk products, where typically fat, protein, lactose, as well as solid-non-fat and urea are tested. The technique is faster and more environmentally friendly than wet chemistry methods; it is a certainly reliable technique, provided that fundamentals measures are taken into account. In routine dairy laboratories, where thousands of samples per day may be tested, the robustness of the method, the equipment calibration and a structured QC are critical aspects in order to ensure the reliability of results and efficient batch management. Mérieux NutriSciences has established a harmonized QC system throughout its network of 6 milk FT-IR laboratories, made up of several pillars: (i) use of validated methods; (ii) equipment calibrations performed on a scheduled basis with external reference materials (RM); (iii) QC (pilot) samples run each batch, with multiple readings per batch, and control charts established; (iv) regular participation in PT schemes; and (v) implementation of internal audit programme. The monitoring of key parameters such as zero stability, carry-over, repeatability, within-lab reproducibility, calibration slope/intercept correction is done at scheduled frequencies. Acceptance criteria are established according to ISO guidelines; this enables compliance with customer requirements. The QC ensures the same level of results reliability among the sites though allowing flexibility (e.g., local RM are chosen due to the geographical variability of the milk) and it is applicable to a variety of equipment brand/models. In addition to the advantages in quality, this approach allows the reduction of the number of samples to be retested in the event of out of specs QC.

P23

RAPID *IN OVO* SEXING USING ISOTHERMAL AMPLIFICATION AND LATERAL FLOW STRIP FOR FEMALE-SPECIFIC GENE DETECTION

Simão Santos[1], M. Corion[1], B. De Ketaelere[2], J. Lammertyn[1] and D. Spasic[1]

[1] BIOSYST-MeBioS Biosensors Group, KU Leuven, Belgium
[2] BIOSYST-MeBioS Biostatistics Group, KU Leuven, Belgium

simao.monteirobelodossantos@kuleuven.be; jeroen.lammertyn@kuleuven.be

Several European countries have implemented new legislation to eliminate male chick culling practices and improve animal welfare in the poultry industry. However, the industry is still seeking solutions that can handle all egg colours with >98.5 % accuracy at low-cost and minimal disturbance while allowing sexing before day 7 of incubation, and processing >20,000 eggs per hour [1]. Although PCR (currently the most accurate method) offers 99 % accuracy using an allantoic fluid (AF) sample [2], it has insufficient throughput, high cost, and forces the embryos out of the incubators for a long period. Recombinase polymerase amplification (RPA) is an alternative method with accuracy and sensitivity similar to PCR. During RPA, DNA amplification is performed within 10-30 min at isothermal temperatures matching those in the incubators [3,4]. Moreover, the RPA can be combined with lateral flow strips (LFS) for an easy colorimetric readout within seconds [5]. In this work, we established for the first time an RPA on an LFS for sexing embryos on days 6 to 9 of incubation based on amplifying a female-specific gene (*HINTW*) in the W-chromosome. A total of 80 ISA brown eggs was incubated at 37.7°C, and AF was sampled with a needle from days 6 to 9 for subsequent DNA extraction. RPA optimized with extracted DNA was then validated directly on 0.5 µl of AF samples (without prior DNA extraction). The obtained LFS bands' intensities were measured using a tabletop scanner and analysed with ImageJ software. The RPA was completed in less than 30 minutes with the final colorimetric-based readout. The assay revealed an accuracy of 93.3% and a specificity of 96.6% in samples from all tested days. In our previous work, we detected *HINTW* from the same developmental stages using qPCR, achieving 99% accuracy. The observed difference in accuracy is possibly related to the higher sensitivity of RPA compared to PCR [3], leading to the detection of female gene contamination from the mother hen [6]. Further fine-tuning should be performed to increase the RPA accuracy. Importantly, RPA provides higher throughput compared to PCR due to the faster time-to-result and user-friendly sex identification by the presence of a colour band on the LFS. Furthermore, the RPA assay does not require bulky and expensive thermocyclers, thus decreasing the assay price and avoiding the need for laboratory settings, which are all important features for adopting this assay in industrial settings.

Acknowledgements
This work received funding from the Flemish environment department, the Foundation for Food and Agricultural Research [EggTech-0000000028], and the Research Foundation – Flanders [SB project 1SC7219N and SB project 1S54823N].

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P24
MULTIPLEX LATERAL FLOW IMMUNOASSAY FOR THE DETECTION OF MARINE BIOTOXINS AND CHEMICAL CONTAMINANTS

C. Mills[1], M.J. Dillon[2], P. Kumar Kulabhusan[1], **Diana Senovilla-Herrero**[1] and K. Campbell[1]

[1] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK
[2] Peninsula Medical School, University of Plymouth, UK

dsenovillaherrero01@qub.ac.uk

Multiplex lateral flow immunoassays (LFAs) offer numerous advantages compared to traditional methods of detection such as rapid results, efficiency, ease of use, sample conservation, cost savings, reduced human error, and versatile applications. These benefits make them a valuable tool for various industries that require efficient and accurate multiplex detection of target analytes for novice end users. Chemical contamination poses significant environmental and health risks due to their widespread distribution and persistence in various ecosystems. A multiplex LFA was designed to detect the three main groups of marine biotoxins, demonstrating the feasibility and utility of this approach. A rapid sample preparation method encompassing a straightforward process of single sample extraction and dilution was coupled with the design and development of a multiplex LFA, therefore enabling the simultaneous detection of okadaic acid (OA), domoic acid (DA) and saxitoxin (STX) marine biotoxins. OA and dinophysistoxins (DTXs) are the primary toxins causing diarrhetic shellfish poisoning (DSP), DA is representative for amnesic shellfish poisoning (ASP), and STX and its derivatives are linked to paralytic shellfish poisoning (PSP). The efficacy of the assay was evaluated as a screening tool for detecting marine biotoxins in point-of-site shellfish samples, where no cross-reactivity was observed among the three respective toxin classes. The specific assay could detect all three groups concurrently, adhering to the permissible regulatory limits set by the European Union. The application of a user-friendly, compact and lightweight Cube Reader device with USB connectivity was considered in the design to deliver precise quantitative results at any location. This achievement serves as a model example and inspiration for additional projects and the development of multiplex solutions for other chemical contaminants in the environment leveraging the principles of lateral flow technology, and integration of user-friendly visual or digital readout systems for result interpretation. The successful development of such a platform for additional chemicals will significantly enhance the cost-effective monitoring and assessment of multiple contaminations in soil, water, and biological matrices, thereby contributing to better-informed environmental management strategies and public health protection.

P25
NOVEL DISPOSABLE LAMP PLATFORM FOR DETECTION OF *YERSINIA PESTIS* IN THE FIELD

K. Zwirglmaier and **Kilian Stoecker**

Bundeswehr Institute of Microbiology, Germany

kilianstoecker@bundeswehr.org

Detection of biothreat agents such as *Yersinia pestis* in the field by first responders and SIBCRA teams currently relies mainly on lateral flow tests. These antigen-based tests are easy to perform under field conditions in full PPE and require no instrumentation. The drawback is their well-known limited sensitivity. On the other hand, nucleic acid-based tests such as PCR or LAMP (loop-mediated amplification) have a substantially higher sensitivity, but usually require some instrumentation and are therefore not suitable for field use. Here, in a proof-of-concept study, we adapted the Multitest (SelfDiagnostics GmbH, Leipzig) for detection of *Y. pestis* in the field. The Multitest is a novel disposable LAMP platform, which is as straightforward to use as an antigen lateral flow test. The palm-sized device contains lyophilized reagents and compartmentalized buffers and is powered by two AAA batteries. It performs a LAMP assay in ~30 min. The readout consists of a lateral flow strip that is sealed inside the cartridge, preventing any cross-contamination. We field-tested the device in a biothreat scenario at the NATO CBRN Exercise Precise Response in Suffield, Canada. It out-performed the concomitantly used antigen-based lateral flow assay in terms of sensitivity.

P26

SENSITIVE MAGNETIC NANOPARTICLE ASSISTED RAPID SANDWICH ASSAY (S-MARSA) APPROACH TO QUANTIFY BIOMARKERS FOR MONITORING PARKINSON'S DISEASE AND SCHIZOPHRENIA PHARMACOTHERAPY

Neelam Upadhyay[1], R. Elangovan[2] and G. Hariprasad[1]

[1] Department of Biophysics, All India Institute of Medical Sciences, India
[2] Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, India

neelam.aiims26@gmail.com; dr.hariprasadg@gmail.com; elangovan@dbeb.iitd.ac.in

Parkinson's disease and Schizophrenia fall under low dopamine neurodegenerative and high dopamine psychiatric disorders respectively. Pharmacological interventions to correct mid-brain dopamine concentrations sometimes overshoots the physiological dopamine levels leading to psychosis in Parkinson's disease patients and, extra-pyramidal symptoms in schizophrenia patients. Currently no validated method is available to monitor side effects in such patients. Apolipoprotein E and alpha-synuclein are protein biomarkers identified in the recent past that shows an inverse relation to mid-brain dopamine concentration. These biomarkers dictate the mid-brain dopamine levels. We have developed sensitive magnetic nanoparticle assisted rapid sandwich assay (s-MARSA) for the detection of apolipoprotein E in cerebrospinal fluid (n=53) and alpha-synuclein protein in both CSF and serum (n=33). The immunoassay works on the principle of magnetic nanoparticle functionalized antibody for target protein enrichment and enzyme mediated signal amplification. The values measured by s-MARSA strongly correlates with the values measured by ELISA. s-MARSA exhibits a broad detection range with lower limit of detection and could be performed within an hour, utilizing low volume sample. α-Synuclein levels clinically correlates with the disease scoring scale Unified Parkinson's Disease Rating Scale (UPDRS) and Brief Psychiatric Rating Scale (BPRS), and treatment duration of Parkinson's disease and schizophrenia patients. We have designed a microfluidics cartridge to quantify alpha-synuclein in serum, which could be used for routine detection and monitoring of side effects to benefit clinicians and patients.

P27
FAST SCALABLE DIAGNOSTICS FOR THE DETECTION OF AVIAN INFLUENZA

V. Kurm[1], Y. Griekspoor[1], P. Bonants[1], M. Engelsma[2], R. Heutink[2], B. Fronen[3], V. van der Vorst[3] and **Marleen Voorhuijzen**[3]

[1] Wageningen Plant Research, Wageningen University & Research, the Netherlands
[2] Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands
[3] Wageningen Food Safety Research, the Netherlands

marleen.voorhuijzen@wur.nl

Diagnostic tools for the early warning toolbox for pandemic preparedness and response are of paramount importance. These tools include, amongst others, diagnostic assays for the rapid, at-location and cost-effective detection of possible zoonotic viruses, to be used in or around the agri-food system for early identification of hotspots for spill-over. Within the ERRAZE (Early Recognition and Rapid Action in Zoonotic Emergencies) programme of Wageningen University & Research, we developed rapid diagnostic assays using reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) for the specific detection of genetic material of avian influenza (AI) subtypes in swab samples from infected birds. In addition, a diagnostic pipeline was designed that can be used for future rapid design and production of new molecular tools to monitor a broad range of zoonotic viruses.

P28

CHEMICALLY PROFILING BILE-ACIDS IN HUMAN SERA WITH A SENSING ARRAY TO DETECT AND DISTINGUISH SEVERE ALCOHOLIC HEPATITIS

Liam T. Wilson[1], L. Tyson[2], M. Thursz[2], W.M. Rosenberg[3] and W.J. Peveler[1]

[1]School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, UK
[2]Department of Hepatology, St. Mary's Hospital, Imperial College Healthcare NHS Trust, UK
[3]Institute for Liver and Digestive Health, Division of Medicine, University College London, UK

liam.wilson@glasgow.ac.uk

Severe alcoholic hepatitis (SAH) is an inflammatory liver condition caused by chronic, excessive alcohol consumption [1], which requires immediate clinical intervention to improve chances of short- and long-term survival [2]. However, distinguishing SAH from other causes of liver decompensation can be challenging – often requiring biopsy [3]. Collaborators (Tyson and Thursz) have identified distinct individual bile acid (IBA) profiles in serum samples from patients with SAH, suggesting that they may serve as a useful biomarker panel for diagnosis. Unfortunately, at present, IBA measurement relies on UPLC/MS (ultra-performance liquid chromatography/mass spectrometry), which requires extensive sample preparation and measurement time. A rapid, potentially near-patient, method for identifying SAH patients based on IBA profiles is, therefore, an unmet need. Array based sensing platforms hold promise for point of care (POC) diagnostics, capable of profiling complex mixtures of biological analytes rapidly, without requiring bespoke sensor elements for each analyte [4,5]. We present the development of a sensing array for the detection and discrimination of IBAs in human serum, and its application to distinguishing the SAH patient serum samples from related hepatic dysfunctions. The sensor array proved capable of discriminating four key IBAs at a range of clinically relevant concentrations in 'spiked' human serum samples. When applied to spiked serum samples reflecting the IBA profiles of profiled patients (n=39 across SAH=16, decompensated cirrhosis DC=5, compensated cirrhosis CC=11, heavy drinking control HDC=7) discriminant analysis of the array spectral profile proved capable of distinguishing between mock samples of SAH from non-SAH DC with each sample requiring only 7.5 µl of serum and <1 min measurement time.

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P29

SURFACE IMPRINTED POLYMERS TECHNOLOGY AND NANOPORE SEQUENCING FOR POINT-OF-CARE FOOD MICROBIAL ANALYSIS

Ellen Lambrecht[1], P. Wagner[2], D. Yongabi[2], C. Samoy[4], S. Van Campenhout[3], G. Antonissen[4] and M. Heyndrickx[1]

[1] Technology and Food Unit, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
[2] Department of Physics and Astronomy, KU Leuven, Belgium
[3] Flanders' FOOD, Belgium
[4] Department of Pathobiology, Pharmacology and Zoological Medicine, Ghent University, Belgium

ellen.lambrecht@ilvo.vlaanderen.be

Microbial analyses are key for every agrifood company. Their importance is increasing due to stringent EU food safety regulations and export regulations which are demanding for high quality and safe raw and processed materials. In addition, control agencies are gradually introducing high performance technologies, such as Whole Genome Sequencing to trace foodborne outbreaks. Novel developments in biosensor and DNA sequencing technology might offer agrifood companies opportunities to perform on-site microbial analyses on a novel level: detection, quantification and characterization. The SIPORE project [1] aims at exploring and testing such SIP-biosensor and Nanopore DNA sequencing opportunities for fast on-site microbial analysis, by demonstrating proof of concept, performance testing, improvements and protocol optimization. Surface imprinted polymer chip coatings were synthesized by (i) soft-lithographic stamping and (ii) electropolymerization for the selective detection of *Listeria monocytogenes* and *Salmonella* Enteritidis and Typhimurium. In addition, the use of the portable whole genome nanopore sequencing system for strain characterization on subspecies level (*Salmonella* and *L. monocytogenes*) was evaluated by benchmarking against Illumina sequencing. Finally, the potential of using SIP biosensors as initial screening and selection (with and without cultivation step) for downstream Nanopore sub-species typing will be assessed. Therefore, qualitative and quantitative recovery of bacterial cells from the biosensor, by use of several protocols, will be evaluated. There is a need for easy-to-use, rapid and accurate diagnostic devices for better in-field, on-farm and in-line detection of pathogens and tracing them throughout the food production cycles. The combination of the SIP biosensor technology with portable DNA sequencing devices can perform on-site food microbial analyses (detection, quantification & characterization). This will contribute to a more adequate monitoring and surveillance system.

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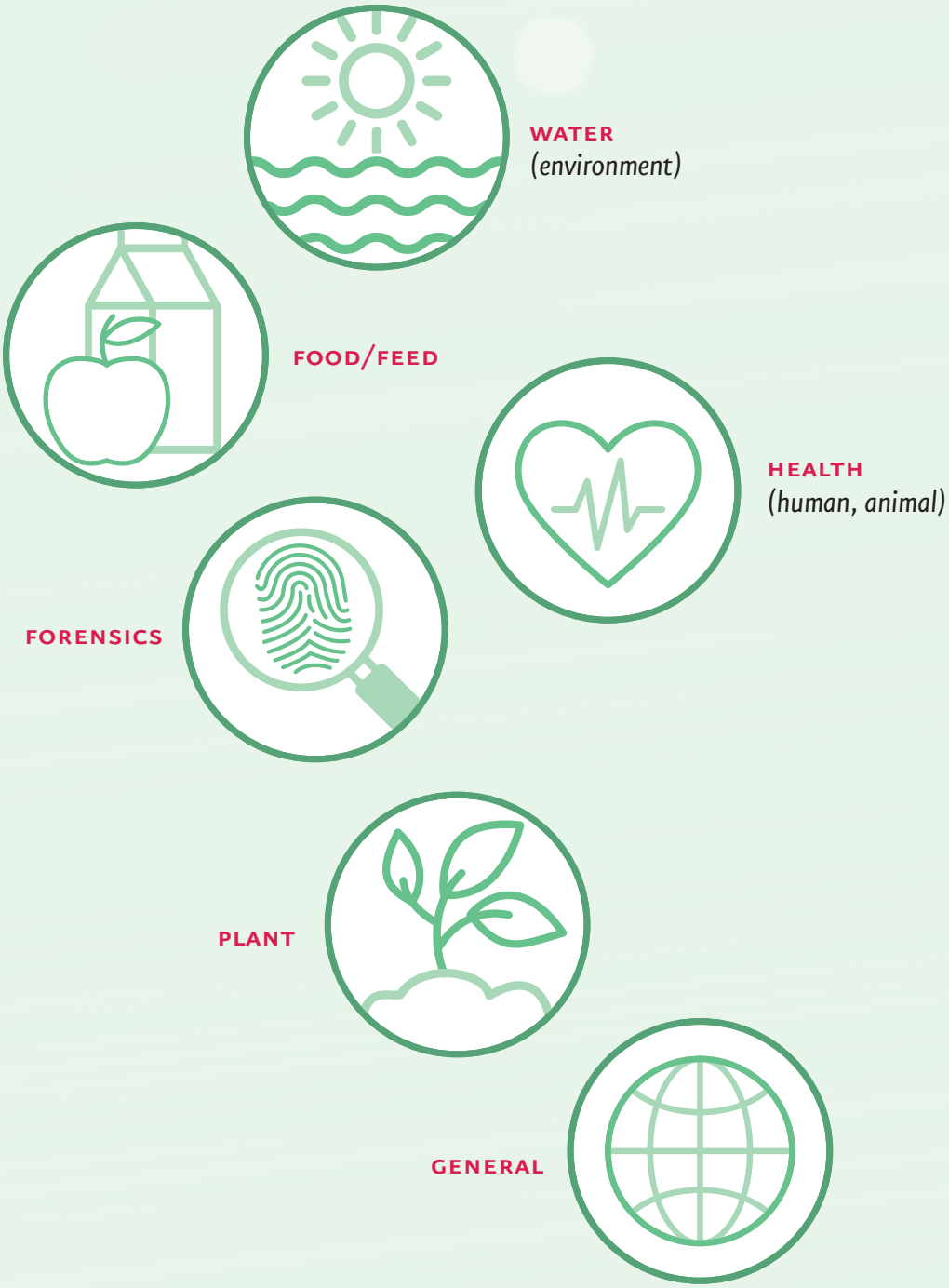
P30
RAPID DETECTION OF *LISTERIA* SPP. IN ENVIRONMENTAL SAMPLES BY A NOVEL MICROARRAY BASED PATHOGENDX SYSTEM

C.-H. Chen and **Jitendra Patel**

Agricultural Research Service, US Department of Agriculture, USA

jitu.patel@usda.gov

Listeria monocytogenes are ubiquitous in nature and can found in food processing environment. Inadequate sanitation can lead to transfer of this pathogen to food and cause foodborne illnesses as outbreaks linked to cross-contamination of food with *L. monocytogenes* has been documented. Rapid detection of pathogen on equipment surfaces is required, if any, to minimize cross-contamination of foods and prevent foodborne outbreaks. The purpose of the study was to determine the efficacy of PathogenDx microbial detection system in rapid detection of *Listeria* contamination on equipment surface. Stainless steel templates (4'x4') were inoculated with 200 CFU/inch^2 of *L. monocytogenes* and allowed to airdry overnight for bacterial attachment and biofilm formation. Inoculated surface area on templates were sampled with swab or sponge, transferred to 1 ml neutralizing broth followed by analyses with microarray based PathogenDx identification system. Briefly, the Enviro$^{X\text{-}F}$ assay consisted of DNA extraction, the two-step PCR, and hybridization to microarray which was imaged to yield a Cy3 hybridization pattern distributed among the probe spots. The PathogenDx software analysis tool, Augury©, automatically detected the hybridized spots in the image and then calculated the median Cy3 intensity of each hybridized spot. Swab and sponge samples were simultaneously spiral plated on MOX agar to confirm presence of pathogen. All surface swab/sponge samples spiked with 200 CFU of *Listeria* were detected by PathogenDx system. In a similar study, fresh produce samples inoculated with 5 CFU *Salmonella* in 25 g fresh leafy greens were detected following 3 h enrichment and concentration by Innovaprep pipette. The findings reveal that this novel approach may be used for rapid detection of pathogens in food and environmental samples.



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P.O. Box 179
NL-3720 AD Bilthoven
the Netherlands
T +31 30 2294247
RME@bastiaanse-communication.com
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