Publishable Summary

Executive Summary

The Aquawarn Project (Deployable early warning pollution device for application in water) has been an FP7 cooperative project (2013-2015), following the structure of the Grant Agreement no. 605937. According to the title of the project, the aim of the project was to develop an innovative integrated deployment device for the detection of water pollution within freshwater and waste water using innovative microfluidic technology. The developed device can be used for monitoring key water quality parameters. The primary analytes include phosphate, nitrite, nitrate and pH with a secondary list also defined. The secondary list includes: conductivity, COD, ammonia, sulphate, chloride, metals and TOC in wastewater and environmental waters. Aquawarn developed a deployable multi-parametric monitoring device based on microfluidic technology using colorimetric detection methods. The monitoring device featuring communication and data logging systems enables multiple water analyses and low-cost real time monitoring. The prototype includes features such as ultra-low energy consumption, in-situ automatic calibration, low maintenance costs, advanced automation capabilities and wireless remote monitoring. The device is easy to install, low cost, robust and small in size. Under environmental legislation such as the Water Framework Directive (2000/60/EC), Groundwater (80/68/EEC), Drinking Water (80/778/EEC) and the Urban Wastewater Treatment Directive (91/271/EEC), there is a demand among Government monitoring agencies and legislative bodies throughout Europe for a water quality monitoring system with the ability to reliably monitor a large volume of water quality parameters at regular intervals but in a cost effective manner. The Aquawarn device can deliver cost-effective analysis in a transportable and deployable unit, providing high quality data during field deployment, which can be key in identifying pollution from agricultural sources or from urban areas or it can detect where waste water treatment is not functioning to its full capacity. The provision of clean water through regular monitoring and protection of water resources is important, not only to the government bodies/agencies or water utilities to have to provide these services but also to the general public. The provision of clean and safe water reduces diseases among the general population and also the aquatic life in the water systems.

Summary description of project context and objectives

The Aquawarn Project (Deployable early warning pollution device for application in water) was a 2 year EU FP7 project running from 2013-2015, following the structure of the Grant Agreement no. 605937 with the European Commission. It brought together 6 partners from research institutions and small and medium enterprises across the EU, as shown below.

Participant’s legal name Country Organisation type

1. (Coord) (TEL) T.E. Laboratories Ltd, Ireland (SME)
2. WIS - Williams Industrial Systems, UK (SME)
3. KSL - Kalite Sistem, Turkey (SME)
4. RTE - RT Environmental Srl, Italy (SME)
5. DCU - Dublin City University, Ireland (RTD)
6. NERC - National Environmental Research Council, UK (RTD)

The main context of the Aquawarn project is to produce a device for the detection of water pollution within freshwater and waste water using innovative microfluidic technology. The primary analytes include phosphate, nitrite, nitrate and pH. The device is designed for daily, on-site use by end-users in self regulating industries, government agencies responsible for policing environmental legislation as well as operators of wastewater treatment plants. It can be used by the food sector, the public health sector, water utilities and indeed any industry with discharges to water. Microfluidic based colorimetric and fluorometric analysers using low cost LEDs and photodiode detection are a feasible solution for widespread deployment of sensors for water chemistry but there are currently limitations that Aquawarn addresses. These include: developing a broad suite of analytes, instrument size and ease of use, minimising waste and reagent volumes, energy consumption, drift and reliability of in-situ testing.

Regarding the development of a broad suite of analytes, current analysers typically address one or two analytes only and cannot be easily changed to make additional measurements. For the instrument size, they are too large and complex to be considered for frequent and widespread deployment particularly on an operational basis: frequently competitor (non microfluidic) systems require a very skilled operator with intimate knowledge of the reagent based assays and the technology. Current systems with sufficient reliability are large (typically 50 to 100 litres in volume). Waste and reagent volumes: They use large volumes of reagent and generate large volumes of waste: this is problematic as it limits the service interval, and makes waste management difficult, particularly in remote field or industrial applications. Large energy consumption makes remote or long term operation more difficult and expensive. There can be poor long term performance due to instability of the chemical reagents, and also degradation of any onboard standards / blanks. There is difficulty leaving the lab as numerous microfluidic systems are more chip in a lab than lab on a chip, i.e. they will only operate reliably if extensive and expensive support equipment is arrayed around them.
AQUAWARN directly addresses each of these shortcomings via the following approaches:
It will optimise specifically for on-chip use, a wide range of pre-existing reagent based assays for high priority chemical targets.
It will in parallel optimise the chip and detector systems (e.g. by appropriate selection of LEDs, detectors, material tint and chip design).
It will lever existing microfluidic designs, and integrated systems (e.g. the DCU phosphate analyser) that have solved miniaturisation and simplification of robust low-cost, easy to use integrated systems in similar applications.
It will use ultra-small volumes by using stopped flow analysis in microfluidic chips to reduce reagent consumption and waste production to a few tens of microlitres per measurement.
The reduction in scale, coupled with latching (usually off) valves and pump actuators will deliver low power platforms capable of extended deployment in remote (off grid) applications. Investment in improved reagent and reference material stability (e.g. by excluding microbiology, oxygen and light in reagent cassettes) coupled with frequent self calibration to onboard standards and blanks will enable improved long term performance.
It will further optimise proven complete and autonomous integrated systems that have a track record of high performance operation in small packages, in the field with a complete absence of support infrastructure.
The main results of the project since it began in December 2013 includes a field deployable prototype to detect water pollution within freshwater and wastewater using microfluidic technology. Once developed, the device was tested in situ at a site located near T.E. Laboratories. Testing was carried out over a short time due to device issues and further work is required for long term deployment of the device and sensor as deployment was limited to one week due to time constraints, issues with chips, sensors and firmware and software. The activities of the Aquawarn project were completed within time and within budget.
The research activities and deliverables from the Aquawarn Project have been disseminated both regionally, nationally and international thought oral and poster presentations at conferences and workshops, posts on the project website and social media. Information relating to the project has always been made available to the public and scientific academia where possible. The protection of IP is of great importance for this project.

Description of main S & T results/foregrounds
The main studies and results are summarised below:

Deliverable 3.1 - Optimisation of analytical transducer and manifolds for mass manufacture
This report was submitted in February 2015. Task 3.1 was divided into two blocks of work: a) Primary analytes: optimise assays and explore reagent shelf life and test methods to extend this b) Secondary analytes: develop suitable assays for deployment on lab-on-chip.

Primary analytes: The primary analytes listed agreed by all partners during the first consortium meeting included:

- Phosphate: 0.01-20 mg L-1 (0.1-20 µM)
- Nitrite: 0.0015 -10 mg L-1 (0.1-60 µM)
- Nitrate: 0.01-75 mg L-1 (0.1-1200 µM)
- pH: 4.0-10.0 ± 0.1 pH units

This summary focuses on the work completed to meet the following objectives for these analytes:

- Assay optimisation: Ensure that optimal recipes were used for each analyte;
- Shelf life: Determination of the shelf life of the assays used and use of various techniques to increase the shelf life.

<u>Summary and recommendations for storage of reagents and standards</u>

- Storage bag type had no impact on the results from the current data set; the Flexboy bags are currently employed in the NOC wet chemical system and have been shown to be robust.
- Temperature has a significant impact on the Griess reagent blank measurement. This can be corrected for with frequent blank measurements.
- Mercuric chloride, chloroform or no treatment (only if complete sterility can be assured) are recommended poisons for the nutrient standards; formaldehyde or chloroform should not be used to store phosphate standards.
- All solutions except the nitrite appear to be stable over the 3 month period required in the DOW.

<u>Secondary analytes</u>

The secondary analytes (with desired concentration range in brackets) detailed in the DOW are:

- Arsenic (0.02-0.17 mg L-1 (0.4-3 µM))
- Chemical Oxygen Demand (COD) 1-100 mg L-1 (63-6250 µM))
- Total Organic Carbons (TOC) 1 mg L-1 (1-150 mg L-1 ((83-12450 µM))

The primary goal was to develop assays that were compatible with Lab-on-Chip systems. As they were not included in the first Aquawarn systems, the development goal was to carry these assays forward to future versions of the system.

<u>Arsenic</u>

The main results of the arsenic assay characterisation are as follows:

- At room temperature, 3 hours are required for the colour to fully develop, while increased temperature can accelerate the colour (sensitivity) development.
- Detection limit of 40 nM and 20 nM for As(III) and As(V) respectively under current conditions are within the concentration range of natural unpolluted water

Further work identified includes:

- Determination of the interferences phosphate and silicate.
- Preservation of reagents
- Optimisation of reagent recipe.

<u>Chemical Oxygen Demand (COD)</u>

There are two commonly used methods to assess COD: oxidation with acidified potassium permanganate or potassium dichromate. DCU trialled the potassium dichromate using pre-reacted reagent and a bench-top fluidic system employing a 430 nm LED. This method was found to have good linearity but poor resolution.

Further work would be required to optimise this assay for in situ devices.

<u>Total organic carbon (TOC)</u>

TOC is a measure of water quality (similar to COD) and determines the total quantity of carbon organically bound. It is a useful parameter for assessing water contamination by synthetic organic compounds. The technique is based on the oxidation of organically bound carbon to CO2, and measuring a resulting change in conductivity or pH. Further work would be required to optimise this assay for in situ devices.

Deliverable 3.2 – Optimisation of optofluidic cells

The purpose of Task 3.2 was to improve the performance of the optical cells in order to maximise sensitivity. Axially illuminated cells developed by NERC allow optical cells of at least 100 mm to be manufactured in a single polymer.

Development of microfluidics

NERC designed all microfluidics in collaboration with DCU for all chips produced. All fluidic designs were simple to enable ease of manufacture and ensure production costs are kept as low as is reasonably practicable.

Optical cell optimisation

The tint is important for the operation of the microfluidic devices. Additional LED monitoring was shown to improve LED output and reduce noise resulting from temperature fluctuations. This is likely to be unnecessary for the Aquawarn device as the temperature changes that will be seen in riverine deployments are going to be much smaller - typically a few degrees over multiple hours.

Deliverable 3.3 – Optimisation of analytical transducer and manifolds for mass manufacture

Task 3.3 focused on economising mass manufacturing costs of the Aquawarn fluidic chips by undertaking a cost analysis of all current production processes: layer fabrication, thermoplastic bonding, and finishing/quality control. Several suitable techniques exist for manufacturing the tinted polymer device layers, each with their own advantages and disadvantages.

A concept for an automated electromechanical optical alignment setup has been developed that could accelerate the process further and ensure greater consistency. All of the components and control electronics necessary for such a setup now exist at NOC due to development on other projects. The implementation is planned to be tested.

WP4 – Second Generation Integrated Analytical Detection Strategies

WP4 focused on the development and assessment of second generation analytical platforms for prioritised targets. The main results from this WP are highlighted in 3 key areas of design approach, signal conditioning and fluidic networking where improvements in all areas from the first to the second generation system were completed.

This involved the integration of improved microfluidic chips, detectors and chemistries developed at DCU and NERC (WP2 & 3) with the required hardware, electronics and software components to develop a complete analytical platform with improved performance. This task takes advantage of the significant expertise and experience in the development and integration of analytical platforms developed at DCU during previous projects including the development of the phosphate analyser platform.

Task 4.1 – Design, optimisation and integration on platform components

Task 4.1 focused on the development and assessment of second generation analytical platforms for prioritised targets. This task is complete and was reported in D4.1 in Month 15 (February 2015)

A large amount of work in this task focused on bench testing and optimising the microfluidic chip operation, allowing for the optimisation of mechanical design to give the best result. Benchtop testing consisted of measuring the premixed solutions on the chip and on the verification equipment (UV-Vis spectrometer) to assess the effectiveness of the chemistries. Solutions were then mixed

on-chip and the results verified against the premixed results and UV-Vis spectrometer. This was successfully completed for both phosphate and nitrite.
The nitrate chip was not fully completed at this point as there was an issue with the purity of some of the chemicals used. This prevented the task being completed at this point. The issue was identified and rectified and the nitrate benchtop testing was completed in a later deliverable (D5.2).

Task 4.2 – Design and fabrication of deployable platform
This task focused on and involved integrating the developed and off-the-shelf components into a compact, portable, field deployable unit which will be able to operate independently for long periods of time (target: maintenance-free deployable lifetime of three months).

The individual components, elements and sub-system are manufactured and integrated into the final Gen#2 deployable analytical platform as part of task 4.2. Throughout the design and manufacture focus was kept on two key goals: the timely achievement of all scientific and technical milestones and deliverables in the project. Both the firmware and electronic control system were developed alongside the pumping system. The prototype board was designed in-house.
This WP was completed in July 2015.

WP5 – Field testing of analytical platforms
In this WP, a redesign printed circuit board (PCB) was developed to address design issues. A real time clock (RTC) and SD card were added to the system for real time logging and data capture for customers. The system was deployed near T.E. Laboratories for two weeks. Some issues occurred during deployment including lack of meaningful data collected on the nitrite sensor due to incorrect firmware.

Task 5.1 Laboratory-based assessment of first generation integrated system
This task focused on the evaluation of the detector when evaluating solutions of different pH concentrations. In addition, blind samples were sourced from an SME partner and their pH was estimated.
Samples differing in pH concentrations were examined. The system responded well and in a reproducible manner (R2=0.9971, n=3). The system also performed well during the blind testing with a result of $\leq$ 0.32 pH unit.

Task 5.2 Laboratory assessment of deployable analytical system
This task tested the performance of the integrated field-deployable analytical system under laboratory conditions, including testing of relevant environmental samples, and analytical specifications obtained. This task follows on closely from and brings together the outputs from deliverables D2.1, D2.2, D5.1, D4.1 and D4.2.
A redesigned printed circuit board (PCB) was developed to address issues with the design. A real time clock (RTC) and SD card were added to the system to allow for real time logging and data capture.
The pH system was calibrated using a range of pH buffers (4, 5, 7, 8 and 9) supplied by SME partners. A high degree of linearity in the range between pH 4 and 9 (R2 = 0.9716) was reported.
The phosphate system was calibrated using a range of spiked phosphate solutions (1µM, 6µM, 10µM, 18µM, 22µM). Linearity between 6µM and 22µM (R2 = 0.9184) was achieved.
The deployable nitrite sub-system was calibrated using a range of spiked nitrite dilutions (0.1µM, 1µM, 5µM, 10µM, 20µM). For the range between 0.1 µM and 20µM linearity was reported (R2 = 0.9796).
During benchtop testing the nitrate chip proved extremely sensitive e.g. to flow rate. These issues can be addressed within a deployable system and once addressed, the flow rate of the Gen2 system can be optimised for the nitrate chip.

Task 5.4 Life Cycle Analysis (LCA) and detailed cost analysis

This task included a comprehensive cost analysis of the elements and the totality of the analytical system. Both the mechanical and electronic elements of the system performed well during testing. A number of design changes to address outstanding issues and to reduce both operating and manufacturing costs were also detailed. Reducing the overall power consumption of the system was identified as one of the key areas for future development. This would increase deployment time and reduce the number of batteries required to run the system. This could be readily achieved by optimising the command set and power management of the firmware, without the need to redesign any mechanical elements of the system. This WP was completed in November 2015 (M24).

Potential impact and main dissemination activities and exploitation results

The AQUAWARN project will have a significant impact and potential market growth for European environmental legislators and enforcement bodies. Using a deployable system that incorporates early detection of pollution will help reduce pollution incidents which will in turn generate a positive impact to citizens. This project will also improve and strengthen commercial competitiveness of the SMES by increasing their portfolio of products and expertise, thereby positively impact each of the SMEs by enabling them to increase sales and profitability, open new markets for future product sales, increase staff levels, outsource the research to the RTDs, extend professional networks with other SMEs and raise their international profile, exploit research results, acquire technological know-how, access to knowledge and IP, minimise the gap between research, innovation and ultimately commercialisation of a product with an identified and quantified market gap.

The expected results associated with the AQUAWARN project includes a functional AQUAWARN device, sensor technology and microfluidic platform device design, software control and monitoring systems, reagent chemistry and reagent delivery systems and manufacturing IP.

The final expected results include a fully functional deployable device to detect water pollution within freshwater and wastewater using microfluidic technology using primary analytes of phosphate, nitrite, nitrate and pH.

The main dissemination activities include attending exhibition/trade fairs, networking events and presenting at conferences. Other dissemination activities include: posters, press releases, inclusion on the Environmental Protection Agency's (EPA, Ireland) Droplet website which is a database of Research Outputs: Projects, Literature and Environmental Technologies.

Updating of the project website, LinkedIn and Twitter pages were done regularly throughout the project so that members of the public and scientific community are aware of Aquawarn activities.

<u>Dissemination activities from December 2013-November 2015</u>

- Pollutec Horizons, Paris, December 2013. TelLab visited this event to understand the requirements and changes in the market. - Arablab, Dubai, March 2014. TelLab exhibited at this event with Aquamonitrix/Aquawarn on the stand. This was a technology showcasing event and networking day.
- Analytica 2014, Munich, March 2014. TelLab exhibited at this event and included a summary of Aquawarn on the stand. Analytica is an International Trade Fair for Laboratory Technology Analysis, Biotechnology and the Analytica conference. This tradeshow showcases products from the scientific instruments industry and is held every two years.
- Water Research Council Innovation Day, UK, April 2014. TelLab exhibited at this event and had some discussions regarding the Aquawarn project.
- Water, Energy, Technology and Environmental Exhibition (WETEX) Exhibition, Dubai, April 2014. WIS attended this event.
- 4th Instrumental Analysis Conference, Barcelona, October 2014: Dublin City University attended this event and submitted a poster presentation in relation to the Aquawarn Project.
- ArabLab 2015, Dubai, United Arab Emirates, March 2015. This yearly analytical industry trade show reaches markets in the Middle East and Africa, China, Asia and the Indian Sub-Continent. TelLab has attended this event for the last 3 years and in 2015, TelLab's exhibition included an Aquamonitrix/Aquawarn section on the stand. http://www.arablab.com

- Pittcon 2015, Chicago, USA in March 2015. This is the world's largest annual conference and expo for laboratory science that offers innovation from leading exhibitors and a diverse technical program. TelLab attended this event.
- Achema 2015, Frankfurt, June 2015. This trade show highlights industrial water technology. TelLab attended this event and its exhibition included an Aquamonitrix/Aquawarn section on the stand. http://www.achema.de/
- Remtech 2015, Ferrea, Italy, September 2015. The 9th Remediation Technologies and Requalification of Territory Exhibition is a large event that showcases products and services from the Industrial Products Industry related to measuring and monitoring remote sensing techniques, coastal dynamics, river mouths, lagoon and coastal passages. Over 4,000 visitors and 180 exhibitors attended this event in 2014. This was attended by RT Environmental. http://www.remtechexpo.com/en/.

Presentations
- Arizona State University Strategic Research Alliance Symposium, Dublin City University, Ireland - April 2014. Information on Aquawarn project included as part of presentation on Nutrient Sensing Research at DCU.
- 8th Irish Earth Observation Symposium (IEOS 2014), Maynooth, Co. Kildare, Ireland. Dublin City University attended this event and gave an oral presentation on the Aquawarn project.
• H2020 Environmental Networking Event, Dublin, Ireland in June 2015. TelLab had a stand at this networking event held in Enterprise Ireland offices. They presented an Aquawarn poster on the stand and discussed the project with companies and researchers. In addition, DCU attended this networking event and gave a short presentation on Aquawarn.
• 67th Irish Universities Chemistry Research Colloquium, Maynooth, Ireland. 25-26 June 2015. Duffy, Gillian and Murphy, Kevin and Diamond, Dermot and Regan, Fiona (2015) An autonomous phosphate sensor for remote continuous monitoring. DCU attended this event with a poster presentation and had discussions regarding the Aquawarn project. Please follow the link for further details and to view the poster presentation:
https://www.maynoothuniversity.ie/chemistry/chemistry-colloquium-2015
• 9th European Waste Water Management Conference and Exhibition, Manchester, UK in October 2015. The European Waste Water Management Conference deals specifically with problems and solutions for the management and treatment of wastewater. DCU attended and gave a presentation regarding Aquawarn to a wide audience.
• EPA National Information Day on H2020 Societal Challenge 5, Dublin, Ireland in October 2015. TelLab attended this event and gave a short presentation on Aquawarn.
• Alliance to Coastal Technologies, Washington D.C. USA on 12th August 2015. TelLab attended the programme event, submitted a poster and had discussions relating to the Aquawarn project. http://www.act-us.info/

Publications
• "Autonomous Reagent-Based Microfluidic pH Sensor Platform" Perez de Vargas Sansalvador et al., (2015)
• "An assessment of reagent and standard shelf life for deployable in situ devices" (in progress) - NERC are working on this publication and currently, do not have a set date for completion.

Press releases
• Press release on the DCU website on the 16th of January 2014. A short summary of the project was announced detailing the purpose, use and target agencies associated with Aquawarn. https://www.dcu.ie/news/2014/jan/s0114e.shtml. This is available to the public to view on the project website.

• A general press release was created in October 2015 highlighting details and outcomes of the project. These details have been distributed on the project website, LinkedIn and Twitter project pages and have been made available to all partners on the project drop box folder.

Future Dissemination Work

Dissemination activities will continue and all SMEs will display the technology on their stands at forthcoming trade shows.

TelLab will attend Analytica in Munich, Germany in May 2016. This is the 25th International Trade Fair for Laboratory Technology Analysis, Biotechnology and Analytica Conference. This tradeshow held every 2 years showcases products from the scientific instruments industry. TelLab attended this event in 2014 and obtained customer feedback regarding the Aquawarn device.

It is important to note that each company may use their own trade name for the technology. In the case of TelLab (coordinators), this will be Aquamonitrix. Much dissemination has already taken place using this name rather than the Aquawarn project name. The ACT Nutrient Challenge will also provide very significant global coverage with a full profile of the technology launching on the ACT site in February 2016. The Aquawarn website will continue to stay live to highlight further tradeshows, exhibitions etc. that the consortium partners attend such as Analtyica in Munich. Details of this event is located on the project webpage. In addition, the LinkedIn and Twitter project pages will continue to stay live. In the future, it is important to note that the Aquawarn device will not be sold on www.aquawarn.com. Further discussions are required when the commercialisation process has begun.

Exploitation Results

Both RTE and WIS have strong links with instrumentation companies and these links could be used to provide a route to market. Other possibilities include exploiting the market directly or via each SME's distribution chain.

At the time of this report, TelLab is currently building 3 prototypes which will be used in the ACT Nutrient challenge. A 4th prototype will be built in conjunction with RTE who will take one and work on the design of the electronic components and advise on customer interfaces.

Future Exploitation

A patent check will be performed on the existing technology and the use of strategic commercial partners for specific components / production will be investigated.

Address of project public website and relevant contact details

The actual website is available at www.aquawarn.com. The content of the website has been managed by TEL and will continue to be live to inform potential customers of our presence at important trade shows and exhibitions such as Analytica in Munich, Germany in May 2016.

- Web designer: Carlos Cantón.
- Web coordinator: Lorna Cooper: researchoffice@tellab.ie; Sandra Lacey: slacey@tellab.ie