Aquavalens Project

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation."

Grant agreement number: 311846

Deliverable D15.4

Report on the specific leadership role of that Europe can take on the health water related risk.

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Contents List

This report will introduce and comment on the attached brochure from the point of view of aiding European role in dealing with health water related risk. The interpretation is here in this report and the main stories are in the attached brochure produced by the Aquavalens Consortium.

New stories from the Aquavalens team

Implications of the results of Deliverable Report 15.4

Implications of the results for the Work Package (WP 15.)

The brochure and the introductions provided here for it, do describe some of the subjects where Europe has an opportunity to create a leadership role for itself. Using the carbon footprint assessment technology to assess the alternative water tests, was done for the first time by this consortium we believe. In Deliverable 15.2 on derived cost of food and water borne infections, we noted such calculations are complex. Microbiologists agree that prevention is quicker, cheaper and simpler than cure when it comes to water and food borne infections. Finally this report links to Deliverable 15.3 on the feasibility of the application of the Aquavalens platforms in developing or Low and Middle Income countries. Some of us expected our colleagues from Africa to say that many of the technologies and tests developed and improved would be too expensive. In practice the answer appeared to be more nuanced and some of the technologies we have improved were of interest to partners in Africa. [See Deliverable 15.3]

Implications of the results for this Cluster

The dissemination of the consortium breaks into five areas: the > 60 peer-reviewed books and articles published, the > 250 dissemination activities largely carried out in conferences and industry meetings, the five main themed presentations at key conferences selected by the consortium and finally the brochure of stories that forms part of this deliverable. The brochure gives us a handy easily-accessible summary of some of the consortium achievements. It can work as a calling card for the consortium and a way to reach the stakeholders we have been unable to reach so far. It has shown us that some of the approaches explored are very likely to continue to develop quickly. Examples would be the carbon footprint assessment techniques, the use of Water Safety Planning for small water systems, and the simplification of the qPCR technology by developing products that are easier to use. Online warning systems for small and large water supply systems are also popular machines which offer a number of attractive benefits to system operators – to begin with the cost per tests is low and speed of result is good. There are too many developments to list here.

Implications of the results for the whole project

The brochure and the introduction to it provided here has summarised a number of areas in which Europe is playing a leading role now in the water health risk. We aim to consider one or two follow-on projects such as a COST Action bids which could further cement a lead role for Europe.

Indicate key external stakeholders interested in the results of Deliverable Report 15-4

EU FP7 programme. External Scientific Advisers. Small and large water companies. Water researchers. Umbrella groups like MSF or WaterAid which are active in potable water provision.

Which internal partners should your deliverable be sent to? *All*

All partners. This is especially relevant for SME partners and the Executive Group.

Item 1

Robust molecular systems for detection and typing of health significant enteric viruses.

By Dr Silvia Monteiro Instituto Superior Tecnico, Lisbon and Prof Albert Bosch, University of Barcelona.

This story was produced by two of our scientists working in different countries. Sylvia worked mainly on the small water system testing and Professor Bosch is a very experienced virologist. It starts on page 4 in the attached "Key Findings" Brochure produced by Aquavalens and attached to this deliverable.

They present a clear and compelling argument in favour of adaptation of our regulations on potable water supply to also include tests for well-known viruses or protozoa / parasites which we know cause disease in humans. This is a subject which has been debated at length by our research team.

We have currently very little influence over or access to water regulation staff. It would appear to be an excellent challenge for a follow-on COST Action study to organise at national or European level some presentations to water regulators by key staff from Aquavalens. In this respect Professor Jenni Colbourne, a science adviser to Aquavalens and ex UK Water Regulator can probably be very helpful. We believe this would be an excellent investment as there are already a number of tests available for water-borne viruses and protozoa which are reliable, quick and accurate.

Item 2

Advancement of quantitative and sensitive detection of pathogenic bacterial species and genotypes in drinking water using molecular techniques.

By Prof Manfred Höfle, Helmholtz-Zentrum fuer Infektionsforschung, Braunschweig, Germany.

The second story on page 6 in the Aquavalens “Key Findings” brochure outlines two challenges frequently facing staff assessing water quality. Many waterborne pathogens are difficult to detect because they do not grow in a laboratory even though they are still infective. The team outlined how Next Generation Sequencing can be applied to help overcome this difficulty, and the importance of having access to genome standards of reference strains of the targeted individual species.

Then the team tackled the second challenge frequently facing water testing staff, the fact that bacteria are often present in very low concentrations as well as being difficult to grow in the laboratory. They applied whole genome enrichment [WGE] to low abundance bacteria. The developed approaches provide new molecular surveillance tools to monitor major water-borne pathogens such as *Vibrio cholerae, Escherichia coli, Salmonella enterica, Legionella pneumophila* and *Pseudomonas aeruginosa* in quantitative and qualitative terms. Thankfully from a clinician point of view they may also offer more insight into virulence factors linked to pathogens by identifying the presence of specific genes linked to virulence directly from DNA of aquatic environments.

Item 3

Molecular markers for the detection and genotyping of waterborne protozoan parasites.

By Professor Rachel Chalmers, Public Health Wales, UK.

This story appears on page 9 in the Aquavalens brochure titled "Key Findings".

The team studying waterborne protozoan parasites starts by explaining how and why they wanted to develop a test for *Toxoplasma gondii* contamination in water. As the burden of disease caused by toxoplasmosis is high and the clinical picture can be very worrying, gaining more insight into transmission in the environment was felt to be a significant step forward. A sensitive and specific test for the detection of *T gondii* in water was developed for the first time and then tested on 1427 water samples. It is pleasing to see that further support and funding was obtained to investigate more about the nature, origin and thus implications of the results.

Professor Chalmers goes on to discuss Cryptosporidium. She summarises that there was a lack of genetic markers for assessing the relationship between Cryptosporidium samples during epidemics. Her team sequenced the entire genome so that they could find new markers for a genotyping scheme. This will help find out whether patients have been infected from the same source; this could be livestock, wildlife or other people.

The final part of the story describes a rural area where spread of Cryptosporidium from animals to people had been a problem and how it was quite successfully addressed.

Item 4

What is polluting my water? Microbial Source Tracking to determine the source of faecal pollution in water.

By Professor Anicet Blanch, University of Barcelona.

This story appears on page 12 in the Aquavalens brochure titled "Key Findings".

Determining the origin of faecal pollution in water helps produce a better health risk assessment of water resources. Aquavalens has selected combinations of indicators that allow the identification of the faecal pollution sources based on predictive models. The application of this technological approach in the water sector can facilitate not only an improvement in the use and re-use of water resources in different European regions, but also the development of a cartography for the load and the origin of faecal pollution of waters at continental level.

Professor Blanch's team pointed out that there are three reasons at least to want to determine the faecal source of pollution in water: Assessment of the microbial risk, mapping of catchments and search for solutions to conflicts between land users. His team noted that there is already a lot of interest in this technology in the USA where there seems to be more interest in charging polluters or at least taking them to court, to help protect source water for drinking plants. Another lesson we noticed over the course of the five years or research is that most water companies are paying more attention to the composition of their source water for safety's sake and also to develop better ways of treating water successfully with a minimum of chemicals added. For all the reasons noted here there has been a lot of interest in this research into Microbial Source Tracking.

Item 5

Advanced qPCR innovations for microbial testing in water: Knowledge transfer through a small-medium enterprise (SME)

By Dr Antonio Martinez-Murcia, Genetic PCR Solutions™ / University Miguel Hernandez, Alicante.

The author, a professor of Microbiology at the University Miguel Hernandez, Alicante, presents a brief overview on the experience of exploiting R&D results by transferring knowledge to end-users thorough entrepreneurial activities. On page 14 in the attached “Key Findings” Brochure produced by Aquavalens, he describes how the laboratories of GPS™ have innovated with some existing technologies based on genetic methods to adapt sophisticated procedures to market needs, following simple schemes and considering relevant validation requirements. The skills of GPS™ to develop pathogen detection methods in a period of few weeks have provided a very powerful capacity of response to the emergence of outbreaks in the health system. The opportunity to demonstrate the above activity has been shown on several occasions with success during the last few years: for instance, in 2016, the World Health Organisation (WHO) declared the Zika Virus outbreak to be a Public Health Emergency of International Concern and GPS™ developed and validated a qPCR kit for its detection. Right now in 2018, the Hospital La Fe (Valencia) is testing a new kit for the greatest outbreak in Europe of *Candida auris*.

Item 6

Sample processing to maximise recovery rates of pathogens in large volume water samples.

Dr Anna Charlotte Schultz, Technical University of Denmark, (DTU), National Food Institute (FOOD), Diagnostic Engineering.

Micro-organisms, such as viruses, bacteria and parasites, that may cause illness, can be present in water in such low numbers that they are difficult to detect and yet still represent a major threat to human health. To maximise the chance of detection, a large volume [10-1000 litres] of water needs to be sampled and concentrated to less than a few millilitres or microliters that can then be analysed.

Overall, the results obtained showed that all three concentration methods could recover bacteria viruses and parasites from 60-100 litres of spiked water samples.

We are not yet able to offer an “online machine” which can filter and analyse large samples automatically online or straight from the pipe, but much progress has been made in this direction. What’s more one of the large water suppliers active in the consortium described these improvements in methods as one of the most interesting developments to come out of our work from their point of view.

Item 7

Detection of pathogens in water with DNA techniques – a pragmatic approach.

By Dr Antonio Martinez-Murcia, Genetic PCR Solutions™, Alicante, and Dr Jakob Ottoson, Swedish University of Agricultural Sciences, Uppsala.

AQUAVALENS argues in favour of adaptation of our regulations on potable water supply to also include molecular tests for detection of pathogens we know cause disease in humans. However, a prerequisite for regulated molecular testing of water in terms of cost and test accuracy is the development of validated ready-to-use kits. The authors are Antonio Martinez, a biochemist and entrepreneur developing rapid diagnostic tests for detection of microorganisms and Jakob Ottoson, who uses the results in microbial risk assessment. They describe how molecular testing has been simplified to the extent that it can be used in any laboratory setting through the development of GPS MONODOSE. The story starts on page 21 in the attached "Key Findings" Brochure produced by Aquavalens and attached to this deliverable.

MONODOSE has being commercialized worldwide (not only in Europe, but mostly in middle eastern countries such as The Emirates, Kingdom of Saudi Arabia, Jordan, Egypt etc.) and during last 3-4 years GPS™ have developed, produced and validated qPCR kits that contains all reagents to perform detection and quantification for >370 specific microbial targets, for example, Toxoplasma gondii, Campylobacter jejuni, Zika virus, Candida auris, etc.

Item 8

Automated sampling, filtration and detection systems for water

Professor Marc Desmulliez, Heriot Watt University, UK

This story can be found on pages 24-26 in the attached “Key Findings” brochure.

The development of automated filtration, sampling and / or detection platforms is highly desirable to improve water testing, reduce cost of analysis, human handling time and potential for contamination.

The detection of low amounts of microbial pathogens in water requires the analysis of large volumes [60-1000 litres] of water using various filtration techniques to reduce water volume while retaining the microorganisms. The potential to automate the filtration process exists. Aquavalens succeeded in demonstrating that it was indeed possible to build low cost automated filtration and sampling systems for the three kingdoms of viruses, bacteria and protozoa. This result is of great importance for water supplies for the developing countries and small water systems, where costs prevent the use of existing commercial filtration systems. Aquavalens also demonstrated that, using a novel type of agitation using megasonic energy, membranes and filters, the sampling and filtration method could be simplified to make centrifugation superfluous, and be carried out with less manpower, making it an attractive commercial proposition for water companies.

Though this work is not quite complete an automated platform was designed, manufactured and tested for the rapid detection of living bacteria by measuring Adenosine Tri-Phosphate (ADP). The team used a microfluidic system to measure ATP levels with the high sensitivity which is necessary for drinking water testing.

In a parallel piece of research the team also enabled Vermicon to improve the performance of their optical imaging systems, designed to count E Coli and coliform bacteria in water samples.

These results ought to be more widely disseminated and commercialised to the wider scientific and industrial communities as well as the general public. The automated filtration and sampling systems are to be open source for all to take up. In that respect the creation of a COST action proposal will be very welcome to guarantee that results of Aquavalens are used for the benefits of all.

Item 9

Demonstrating the reliability of fast molecular pathogen detection: standardisation and validation

Claudia Stange and Professor Dr. Andreas Tiehm, Technologiezentrum Wasser, Karlsruhe, Germany

The story on page 28-30 of the Aquavalens brochure show the active efforts being made to standardize and validate the new analytical methods and platforms for the detection of waterborne pathogens, that have been developed within the project. Validation of the techniques is essential for their acceptance and forms the basis for practical applications including routine testing.

The results of the molecular kit assays were consistent with current standard microbiological analysis, highlighting the potential of PCR (Polymerase Chain Reaction) methods for rapid and specific microbiological analysis of potable water. These findings were consistently also confirmed by inter-laboratory ring tests. In additions, validation of an automated ultrafiltration system for the concentration of pathogens from water samples was performed. The positive results for the validation of the concentration system underlined the usability and practicability of this method for field applications.

Item 10

Evaluating the newly developed technologies to detect pathogens in large water systems across Europe

By Clàudia Puigdomènech, Project Manager, Cetaqua, Spain

This story was produced by WP10, a multidisciplinary team formed by large water companies, research centres, SMEs and universities from different countries. It starts on page 32 in the attached "Key Findings" Brochure produced by Aquavalens and attached to this deliverable.

They present a record of how we tested innovative and advanced techniques for microorganism monitoring in large water systems. They focused on concentration and recovery techniques, consisting on large volume filtration with Rexeed^{TM} that allows simultaneous concentration of the three kingdoms; molecular detection methods, consisting mainly on qPCR and FISH and online detection systems (BACTcontrol), as an early warning system.

Results obtained so far show a great potential for the improvement of microbiological sampling and pathogen detection in terms of time, money and human resources. In addition, these techniques can enable water operators to improve Water Safety Plans and assess health risk of the exposed population.

Item 11

Small Water Systems – Pathogen detection in small water supplies across Europe

By Dr Alma López-Avilés and Prof Stephen Pedley, University of Surrey and Dr Ricardo Santos, Instituto Superior Técnico, Lisbon

This story was produced by Aquavalens scientists working in the UK and Portugal specialising in water resource management, micro-biology and small water systems testing. The story starts on page 36 in the attached "Key Findings" Brochure produced by Aquavalens and attached to this deliverable.

The work undertaken under the Aquavalens project to test Small Water Systems (SWS) in Portugal, Serbia and Scotland has improved our understanding of the health-risks associated with these systems. Microbiological results show the presence of pathogens in SWS in all three study countries, which reinforces the need for improved regulation on potable small water supplies across Europe to include tests for well-known bacteria, viruses and protozoa that cause disease in humans. This is of great strategic importance given the large number of treated and untreated SWS across Europe which often constitute the only available water supply for many remote rural communities.

Additionally, the work carried out on SWS has provided us with an insight into the views of these communities of water users. Their response to surveys shows that they generally welcome new tests that can provide them with rapidly available information about the quality of their water supplies, especially during disease outbreaks. However, owners of SWS expressed some concerns about the cost of testing and about further regulation, which indicates the need for more work raising awareness and liaising with local and regional authorities about the need for basic treatment of all water supplies. The teams in Surrey, Belgrade, and Lisbon can provide skills in stakeholder engagement and awareness raising to disseminate the Aquavalens methods, as well as to engage with policy makers and regulators at regional, national and EU leadership level.

Item 12

Not just drinking water; water safety for food production

Dr Kaye Burgess, Teagasc, Ireland.

This story focused on the key role of water in food production, both as an ingredient, but also during production and processing and was fittingly included within the *Aquavalens* framework. Water contamination t poses a major risk to the food industry, particularly for foods which are not cooked prior to consumption, such as salads.

The story described the evidence driven identification of foods and microorganisms most commonly associated with produce related disease outbreaks. This informed the selection of sampling sites in four countries where water sampling was undertaken using *Aquavalens* methodologies throughout 2017.

Water quality was in general found to be very good, with no confirmed pathogen detection. The project partners noted that the growers that were involved with the project were extremely interested, engaged and cooperative throughout the sampling campaigns. Engaging with the project was seen positively from their perspective as it provided greater information on their water quality and also educated them on potential risks. It also exposed them to new testing methodologies which can be potentially added to their risk management protocols.

Item 13

Management of water quality with improved detection techniques: applications for water safety plans

Dr Maria J Gunnarsdottir, University of Iceland.

This story is summarizing the work done to give recommendation on the best practice to implement the improved detection technique developed in Aquavalens into preventive management as a water safety plan. The work started with gathering information on the main challenges and risk factors in the twenty water supplies that participated in testing the Aquavalens platforms. These were 5 large supplies and 15 small supplies. The "Key Findings" for this work starts on page 40 in the brochure produced by Aquavalens. The analyses of impact on safety management is presented in deliverable D13.4.

Data from monitoring trials was compared to results from regular surveillance monitoring according to EU directive on drinking water to assess water quality. Survey was made from the users of the AQV platforms on capability and challenges encountered. These results were compared to the steps in water safety planning using WHO's manual for large supplies.

Many incidents of pathogens were detected in the trial, with norovirus, *Campylobacter*, and *Cryptosporidium* being the most frequent. The results from the supplies show high faecal contamination in raw water, and even in treated water in the small supplies, so risk-based management as water safety plan should be applied in the supplies. Improved knowledge of water quality and of the presence of pathogens will have an impact on water safety management in many ways. Improved monitoring that detects simultaneously and quickly the most common waterborne pathogens and indicators has a great early warning potential in preventive management.

The main recommendation given as a result of this work is to implement requirement in the drinking water directive of risk-based approach in all water delivery. It should also include improving infrastructure, leakage control and renewal planning, as well as regular external audit of the water safety management. Water supplier and regulators are advised to improve monitoring and include concentration and recovery protocols for improvement of microbiological detection limits.

Item 14

Carbon Footprint of Aquavalens novel technologies

By Dr Carmen Torres and Prof Maria J. Figueras, Universitat Rovira i Virgili

This story was produced by two of our scientists working in different areas of research. Carmen Torres works in environmental analysis and management of processes using Life Cycle Analysis methodologies, and Professor Maria J. Figueras is an expert microbiologist with an extensive experience in water microbiological methods. It starts on page 42 in the attached "Key Findings" Brochure produced by Aquavalens and attached to this deliverable.

The story shows how the implementation of the innovative technologies developed in the project have positive implications in the fight against climate change. The greenhouses gases produced along the life cycle of the novel platforms were computed through the calculation of the Carbon Footprint. This indicator was used in the comparison with conventional methods, achieving a significant reduction when all the potential of the AQUAVALENS technologies is exploited in exhaustive water analyses entailing bacteria, protozoa and viruses. These results contribute, even in its limited scope, to the objectives of EU 2020 energy strategy actions for the reduction of greenhouse gases emissions, the increase in the use of renewable energies and the improvement in the energy efficiency.

Derived from the interdisciplinary collaboration, the research has also an important academic interest due to the novelty of the research. Although there are few works using Life Cycle approach for the environmental evaluation of the operation of analytical facilities, there is no literature reporting the application of these methodologies to water analysis methods, nor even to analytical procedures in general. Besides, the study creates added value in terms of a link to economic benefits. The Carbon Footprint of the Aquavalens technologies correlates with the consumption of fossil energy, mainly in activities of transport and use of equipment. This direct relation implies that a reduction of the Carbon Footprint also entails economic savings due to lower energy expenses.

Item 15

WP 13 Case story – Water safety plan and Aquavalens

Dr Maria J Gunnarsdottir, University of Iceland.

This case story was produced as a part of deliverable D13.4 to assess evident base of safe water with systematic preventive management as water safety plan and possible impact from improved monitoring. Water quality information and other relevant information on lessons learned were gathered from three water supplies in Iceland, two small and one large.

Water safety plans have mostly been implemented in large water supplies (>5000 people), and drinking water is generally considered safe when delivered from large water supplies. However, many studies have shown that non-compliance is much higher in the small water supplies than the large ones. One of the United Nations Sustainable Development Goals, to be achieved by 2030, is the universal and equitable access to safe and affordable drinking water for all, with indicators as improved drinking water facilities and compliance to standard. Both are very relevant in connection with the results of the AQV project. Aging infrastructure has been recognized as one of the main risks to water safety, and improved monitoring, of e.g. pathogens, has the potential to assisting preventive management.

In 1995 drinking water was defined as food in Icelandic legislation and water supplies were required to use preventive management to secure the safety of all food. In 2013 over 80% of the population had drinking water from a water supply with WSP. In Iceland it has been shown that non-compliance with water quality standards are 3.7 times more likely to occur in supplies that are without WSP compared to supplies with WSP, when comparing before and after implementation. In 2012 there were 796 regulated water supplies in Iceland of which 748 serve fewer than 500 inhabitants, 39 served 500 to 5000 and 9 served more than 5000. Most of those are without any systematic preventive management and these are the ones that have been shown to be most at risk of non-compliance with standards. The small supplies often serve many more people than the local inhabitants, when tourists and summerhouse dwellers are included.

In the beginning there were great challenges in implementing WSP as no guidelines were available and especially guidance for the small supplies was lacking. In 1998 three neighboring communities initiated cooperation to implement WSP. Two are small villages (i.e. one is a greenhouse village with 1600 inhabitants and the other is a fishing village with 1200 inhabitants), and one is a larger commercial town with 5000 inhabitants. Two of them succeeded in implementing WSP shortly afterwards, whereas one of them did not succeed until 2007 because of internal structure changes in the community. Information on monitoring water quality are now available for 20 years with eight to sixteen years of WSP. The water quality has improved in the three communities following the implementation of WSP, and at two the improvements are statistically significant, at the fishing village and in the commercial town.

In the greenhouse village non-compliance in heterotrophic plate counts (HPC) at 22°C, total coliform and *E. coli* were high in the five-year period before WSP but have since decreased. The fishing village had no non-compliance in total coliform and *E. coli* before WSP. Nevertheless, with compliance there was improvement with a significant HPC 22°C decrease, indicating a more hygienic system. The commercial town finished the WSP implementing process in 2007 and since then there have been no non-compliance incidents (<0 in 100 ml) in total coliform or *E. coli* and there has also been great reduction in fluctuation in HPC 22°C which has rarely come close to the Icelandic regulation limit of 100 in 1 ml after WSP implementation as shown on Figure 1.

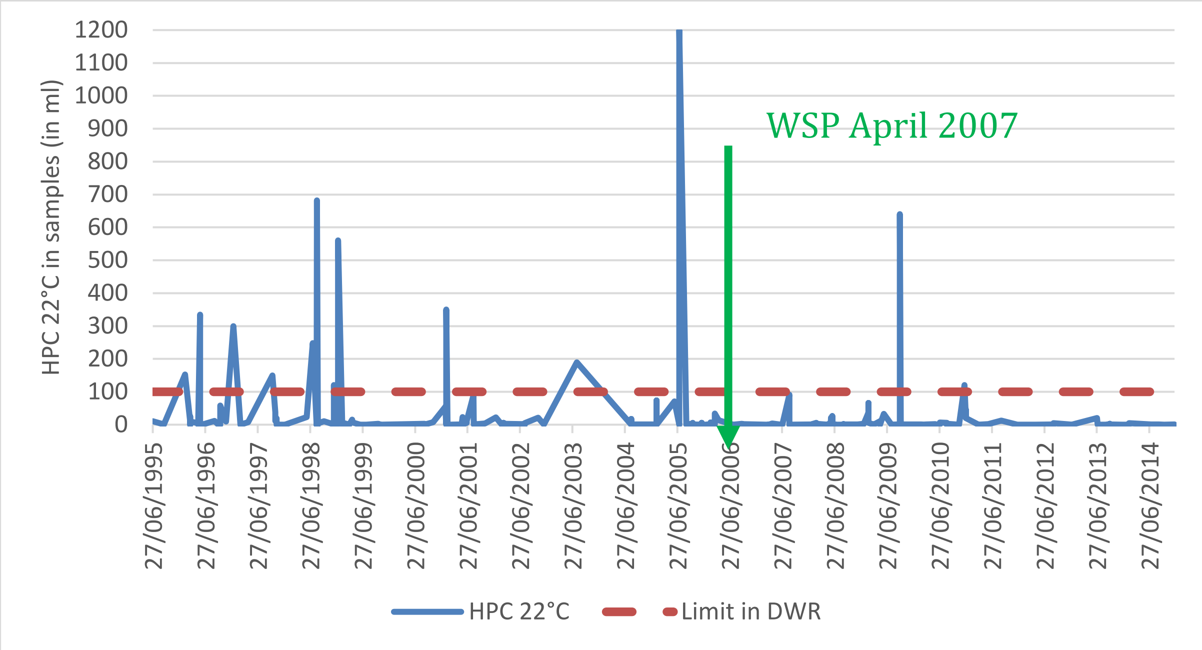

Figure 1. Water quality in HPC 22°C in commercial town before and after WSP.

The main conclusion of these case studies is that implementing WSP has been beneficial for the communities and provided safer drinking water. In an interview with the personnel responsible for the implementation process in two of these supplies some additional important points came forth. For example, it was not considered expensive to implement and they recognised many advantages of cooperating with the other two communities and also within the water sector. The lesson learned from the process was in their own words *"It is important to have involvement of staff that are to carry out the WSP task in all the process and to maintain continuous training."* Support from the authorities was also considered important for succeeding, especially for the smaller supplies. The authorities in the greenhouse village were supportive and considered the WSP endeavour to be beneficial to attract new businesses to the community.

The initiative for this endeavour of risk-based management was that the supplies were to fulfil new regulations. However, this was greatly helped by several factors such as the desire to use this opportunity to improve the systems and acquire knowledge about infrastructure and the system as a whole. There was also an underlying fear of not being in control if something went wrong which systematically tackling the problems alleviated. Working together with neighbouring communities made the job easier and cheaper and it also helped to have a strong national platform where the staff of the suppliers participated actively.

The challenges still to be addressed, which can explain some of the problems that still exist, are that a large part of the pipes in the towns are old and worn out and need to be renewed. These towns are built on porous ground and water leakage disappears easily down into the soil without appearing on the surface, and is therefore difficult to detect. The problem is also that sewage pipes lie near to the water pipes. Regular external audit of WSP, which is the responsibility of the Local Competent Authority, is also lacking.

The platforms and the developed techniques in the Aquavalens project can have a clear impact on the improvements of the WSP, both for large and small supplies, not the least those described in this case study. This includes operational monitoring with molecular methods that detect quickly and simultaneously the most common waterborne pathogens as well as assisting in validating whether the control measures that have been implemented as a part of WSP are working as they are intended. The AQV platform, with online monitoring, has the potential to give early warning of biomass, coliform or *E. coli* and allow faster closing down of sources where needed and thus prevent contaminating drinking water. In addition, the AQV platforms can be important in detecting early and timely impact from natural hazards on water quality such as extreme weather events, earthquakes and volcanic eruptions. Such events have triggered water contamination in Iceland and online monitoring, as presented in the AQV platform, could increase safety.

Item 16

“Simplicity and pragmatism, keys to success in the implementation of sophisticated technologies”

Dr. Antonio Martinez-Murcia, Genetic PCR Solutions™ – University Miguel Hernandez, Alicante, and Dr. Jakob Ottoson, Swedish University of Agricultural Sciences, Uppsala.

The genetic technique known as “quantitative polymerase chain reaction (qPCR)” is a highly sensitive method for detection of specific parts of the DNA that can be used as fingerprints providing information of which pathogenic organisms you may have in your water sample and how many of them. In order to find your fingerprint you make millions of copies of it so it can be visualized. This technique has been available for more than three decades but this is, one of many, an illustrative example of how our R&D resources may not be exploited. One reason for this is because the implementation hasn’t received the adequate attention. A partner from our consortium, **Genetic PCR Solutions™ (GPS™)**, has articulated this sophisticated technology to provide an easy ready-to-use product. The key for a wide implementation with success was, once more, “simplicity”, a consequence of what we learned during the Aquavalens project. Real time PCR is not new, but since the time frame for our Platform development in order to provide (validated) methods for the field studies was limited we could not reinvent the wheel. Instead, we took off directly from the target development in cluster 1 with the purpose of finding suitable “fingerprints” for prioritized pathogens. With some modifications of those findings, we could develop a method suitable for the detection of all pathogens at the same time. However, there were some obstacles to overcome in order for this method to be useful for water monitoring, e.g. robustness of detection, the detection of multiple targets at the same time and potential of automation.

The idea behind our developed technology for detection of pathogens in water is basically the same as the simple **freeze dried soup**. It may not be the tastiest dish, however, it is cheap, easy to distribute and anyone can make it resulting in almost the same taste. Instead of adding hot water to a cup, you add your sample to a test tube. How similar would a dish made from the same recipe by two different chefs taste? Due to small differences, in for example raw products, kitchen models, cooking times and measuring cups, they would most likely taste different. This is also the fact when different laboratories analyse samples taken from the same water source at the same time. Despite following a strict protocol (e.g. recipe) differences in results can be seen from testing of standardized samples across, and even within, laboratories. Therefore we wanted a method to be so simple that anyone could perform the testing without prior learning, giving rise to a similar and reliable result.

There are more than a hundred different diseases that can be transmitted via drinking water. We have within the project prioritized the most important ones. Still these are more than a handful. Having one analysis for all of them would save valuable time and money. As different dishes work better with specific seasoning and cooking times, analyses of different microorganisms have their certain prerequisites. Of course, preparing different dishes in the same cooker is not possible because the different flavours would mix with each-other. For the same reason, the results from several PCR reactions for different pathogens in the same tube, so-called multiplex, is unpredictable. Therefore our approach was to prepare different recipes with the same cooking times and intensity of fire. Just as you can prepare several different kinds of freeze-dried soup adding water from the same kettle in different cups, we have made it possible to run several PCR reactions for many different pathogens simultaneously, but in separated tubes.

Many PCR problems derive from the tedious handling needed to set up the assay for testing a high number of samples, a considerably time-consuming process. Just like freeze-dried soup, in the MONODOSE dtec-qPCR kit, the qPCR reagents for each specific pathogen in this kit is dehydrated in ready-to-use tubes. And just like adding hot water to the freeze-dried soup, it only requires the addition of samples, i.e. the extracted nucleic acids, to perform the qPCR. With many samples to process, a machine will likely do it better and more efficiently. Another advantage with prefabricated ready-to-use kits is that variation in performance is minimized. This will make results more robust and comparable between measurements and laboratories. For our purpose, these were the most important criteria.

Fig. 1. GPS™ MONODOSE qPCR kit for detection of *Listeria monocytogenes*. Each bag contains 24 single PCR ready-to-use tubes with all dehydrated qPCR reagents for *L. monocytogenes* detection in and you just need to add the sample and run the qPCR in the thermocycler.

This simple method, which is a real-time PCR technique, can be performed without prior learning and produces comparable, reliable and fast results, which is key for testing products normally consumed rapidly after treatment, such as water. If genetic detection of pathogens in water will be a future regulatory standard, this will push the market demand for automated detection systems. AQUAVALENS companies are on track to provide the best solutions for them.

Item 17

New findings on the little-known emerging pathogen species belonging to the *Campylobacter* related genus *Arcobacter*

By Dr Maria José Figueras and team from the Faculty of Medicine of the University Rovira i Vigili, Reus, Spain.

This story was produced by Professor Figueras and her team, who since 2006 have been investigating the taxonomy and epidemiology of the genus *Arcobacter.* They discovered 12 of the 27 species currently existing in the genus. Notice that the SME partner Genetic PCR Solutions™ (by GENETIC ANALYSIS STRATEGIES S.L.), has developed quantitative PCR kits for the fast identification of *Arcobacter* and for species of the related genus *Campylobacter*.

Within the framework of the Aquavalens project the team of Prof Figueras formed by Drs. Arturo Levican, Luis Collado and the PhD students Nuria Salas-Massó and Alba Peréz-Catalunya published 11 research articles on *Arcobacter*. In the latter they evaluated five molecular identification methods for the characterization of the 17 species that embraced the genus, indicating important shortcomings for some commonly used m-PCR methods that will hamper the recognition of the true incidence of the different species. In this sense it has been discovered that the importance attributed to *A. butzleri* is the result of a bias introduced by the enrichment step, because when direct culturing was used in parallel *A. cryaerophilus* dominated in wastewater. Interestingly the latter species has been associated with a case of recurrent diarrhea in a young man and all the clinical cases associated with the different *Arcobacter* species were reviewed. It was recognized through a metagenomic analysis of wastewater that *Arcobacter* was one of the dominating genera, and as such, it could be used as signature for sewage contamination. It was demonstrated that during the summer months when the water temperature is high *E. coli* fails to predict the presence of *Arcobacter* spp. present in water and shellfish. The research group contributed with the discovery of more than 10 of the species that include the genus and 7 new additional species were recognized during this investigation and are in the process of description. Furthermore the specific capacity to adhere to and invade the Caco2 human intestinal cells by the species described up to 2013 was demonstrated.

So in conclusion we can indicate that the Aquavalens project contributed extensively to elucidate the role of *Arcobacter* in water and it’s impact on human health. The publications are available at the web site of the Aquavalens project.

Item 18

Small Sites Treatment Stations: Engineering and technology improving populations microbiological water quality

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Small sites are unique population clusters which, due to their small size, their geographic location, the lack of infrastructures (among other factors) have access to microbiologically improper water. Characterizing small sites in terms of their water systems debilities rely most of the time on:
(a) raw/ source water stored in tanks which lack maintenance, so, it is common to find very dirty tanks, with biofilm, and dead animals like mice and birds or mud;
(b) the raw water is then distributed to the populations, with no treatment or relying on a very rudimentary one (with a lot of dependency on the manpower intervention);
(c) the water quality changes a lot with the weather seasonality (both during floods and dry weather);
(d) lack of information about the existing supply water systems (which rely on old people's knowledge);

During the AQUAVALENS project, three Treatment Stations (2 in Portugal and 1 in Serbia) were designed and implemented in each of the chosen small sites. The main challenges faced during the conception phase, were how to overcome all the constraints observed (already listed below) and still provide microbiologically proper water to the population. So, when designing and engineering the solution for these small systems water problems, it was mandatory to **focus on the PEOPLE** rather than on the technological solution. Focusing on people includes facing the lack of resources (infrastructures, workforce, mobility, etc.), and noting the site location as well as the population fluctuation.

With this in mind it was possible to establish the requirements for the Small Water Treatment Stations: **efficient disinfection performance, integrated and stand-alone systems, with low maintenance and with automatic operation**. In order to fulfill all these requirements and to overcome the lack of local physical infrastructures, two of the system were assembled in a 10' sea container (See Figure 1), and the other in a skid mounting configuration (See Figure 2), all of them relying on a plug-in approach.

The inclusion of these configurations, specifications and functionalities were crucial to guarantee a good and permanent functioning, supervision and monitoring of the disinfection treatment (See Figure 3).

The next phase was to decide which water treatments should be used. Attending to the raw water quality (low organic matter content) and several other parameters it was decided that the disinfection steps would include:

a. Oxidation of the residual organic matter, iron, manganese, etc and pre-disinfection of the water source;
b. Water filtration, for oxidized matter and suspended solids removal;
c. Storage of the filtered water; with measurement and adjustment of the residual chlorine;
d. Distribution of the water to the served population;

The systems were designed for a daily consumption of **0.15 m^3/ habitant**.

The AQUAVALENS project covering three small sites to provide the microbiological characterization of the water before and after the disinfection approach and to relate these events with illnesses. The second big challenge was how to address the water sampling, on the assumption that **an external event (like a flood) can drastically change the water quality** and put in 'danger' the population. Given this the Small Water Treatment Stations were engineered to have two sampling modes: sampling by event and sampling by routine. The event sampling, is triggered by turbidity, which according to WHO guidelines (World Health Organization, Guidelines for drinking Water quality, 4th edition, 2011), can be an (indirect) indicator of microbiological contamination.

The treatment stations are also prepared to monitor on-line the following parameters from the water: turbidity, pH, chlorine. These parameters will allow a better understanding of the events that occurs in the source water and to fine tune the levels of chlorine in the treated water. Furthermore, each of the 3 systems is equipped with telemetry systems, which allows the **information to be continuously monitored** (and gives an alarm if any parameter is below or above the defined and expected ranges or when sampling events occur) and registered daily at the webpage: www.diveil.com.

The AQUAVALENS Project, apart from the inherent scientific and technological breakthroughs changed permanently the water quality, and thus the health of the local population, of the two Small Sites in Portugal, since these Treatment Stations will be donated to the Municipalities. Indeed, this is an example of how a project *helps to tackle the problem of poor water quality in Small Water supplies* and **improve the quality of people's lives**.

Figure 1: Treatment water conditioning assemble on a 10’ sea container

Figure 2: skid mounting configuration

Figure 3: view of the control, sampling and monitoring