AQUAVALENS

Powerful water - purity and safety tested

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 11.5

Report on the experience of users of the technology and their recommendations for implementation

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Authors: A. López-Avilés and S. Pedley, University of Surrey, UK

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AQUAVALENS

Executive Summary

This report describes the content and outcomes of the analysis of data collected among users of the AQUAVALENS technologies. These include laboratory staff from the Instituto Superior Técnico in Portugal, the James Hutton Institute in Scotland, Teagasc in Ireland, CETaqua in Spain, and the University of Belgrade in Serbia, who have been piloting the AQUAVALENS technologies.

For small water systems which are the subject of investigation under Work Package 11, a questionnaire was developed to evaluate the experience of users of the AQUAVALENS technologies in three case study countries: Portugal, UK and Serbia in order to gather their experiences and their recommendations for the routine use of rapid analytical methods for monitoring small water systems. In addition, the views and experiences of other users of the AQUAVALENS technologies were sought among a large water supplier providing treated water in Spain: CETaqua, and among users of the technologies in Ireland whose experience involved testing water used in food preparation.

The questionnaire was developed by researchers at the University of Surrey specifically for users of the AQUAVALENS technologies. The University of Surrey has not been involved in the development, validation or use of the AQUAVALENS methods, and was therefore unbiased when formulating the questions, analysing the data collated and presenting the results.

The objectives of this deliverable are:

- To obtain feedback about the experience of laboratory staff using AQUAVALENS analytical methods
- To discuss findings from experienced staff in various countries using these technologies
- To draw conclusions and recommendations for future work, including practical recommendations for the implementation of these analytical methods, and
- To highlight areas where improvements may be needed

From data analysed and comments included by respondents in the questionnaires it can be discerned that the experience of users of the technologies is varied depending largely on whether all the elements of a method or process were available. Some methods (e.g. qPCRs) have been widely reported as easy to use and having potential in the water industry. However, all respondents noted that the methods require further development, that they require laboratories to have the necessary equipment, which can be expensive; and that some of the steps in the methods can be time-consuming and are dependent on skilled technicians. Also, some respondents reported that the recovery rates of the organisms tested were low, and that this is still a cause for concern about the methods.

Despite of these setbacks, respondents said that the AQUAVALENS analytical methods save time and money because the bacterial, protozoal and viral pathogens can be concentrated from water using a single filter, and that PCR techniques are quicker than the traditional methods for isolating pathogens, which can take several days. Therefore, PCR techniques and the proposed AQUAVALENS method can offer a major advantage in critical situations such as outbreaks of disease.

Specifically for small water supplies, all users of the AQUAVALENS technologies agreed that water samples cannot be concentrated in the field, and large volumes of water are required that need to be transported into laboratories. Thus the AQUAVALENS analytical methods cannot be used on site, which would be desirable for small water supplies. On the other hand, all respondents answered that rapid on-line testing systems detecting increased burdens of microbiological contamination would be beneficial for small water supplies, suggesting that this might be the future for testing for pathogens in small systems.

Finally, regarding barriers, all users highlighted that these are not yet accredited methods accepted under EU directives, and thus the techniques are not currently favoured by laboratories.

Based on the data analysed the following conclusions can be drawn:

- There is great potential for the AQUAVALENS technologies to be implemented by the water industry.
- Questions on the use of these technologies in the water sector, and more specifically, in small water supplies, could not be fully answered by the participants piloting these technologies.
- It is recommended that further research with widespread pilots in a variety of environments and scales should take place in order to ascertain the viability of the AQUAVALENS analytical methods in both large and small water supplies, and from the technical, economic and environmental perspectives.
- In parallel it is recommended that research takes place with a strong element of stakeholder participation to outline the policy and regulation aspects that would be needed in order for technologies such as the ones developed under the AQUAVALENS project to be widely implemented throughout the water sector in Europe.

Contents

1. Introduction

The AQUAVALENS consortium has brought together small and medium-sized Enterprises (SMEs), industries, universities and research institutes with the mission of protecting the health of European citizens from contaminated drinking water and water used in food processing. This is being achieved by the development of sustainable technologies to enable large and small water systems' managers, and food growers and manufacturers to better ensure the safety of their water supplies.

The Work Packages in Clusters 1 and 2 of the AQUAVALENS project have developed and validated analytical methods for the detection of several priority pathogens in water. Work package 11 (WP11) in Cluster 3 of the project, aims to implement and test the potential use of these analytical methods for monitoring small water supplies across three European countries: Scotland, Serbia and Portugal. WP12 in Cluster 3 of the project, aims to implement and test the potential use of these analytical methods for monitoring water in food preparation across four European countries: the UK, Serbia, Ireland and Portugal.

In order to provide greater safety assurance, solutions were specifically developed for improving pathogen detection within Small Water Systems. These platforms, which have been validated for many water types and paired with AQUAVALENS optimised concentration methods, are presented below.

1.1. The AQUAVALENS analytical methods

A major challenge in food and water microbiology is the concentration of microorganisms (pathogens) that are present in low numbers. This is particularly true for enteric viruses and protozoa. Therefore, the concentration of microorganisms from large volumes of a sample of water is required. Until recently, all three kingdoms (bacteria, viruses and protozoa) were concentrated using different methodologies and in separate filters or cartridges. This is mainly as a consequence of these microorganisms being very different in their sizes, morphology and constitution. AQUAVALENS partners set to develop a method that would allow for the concentration of all three kingdoms using a single cartridge and elution method. The method developed relies on the use of hollow fibre filters, commonly used in dialysis, followed by a secondary concentration using polyethylene glycol (PEG). This method enables the concentration of large volumes of water to a small volume that is then analysed by molecular biology.

After the concentration of microorganisms, the conventional way to detect bacteria is by growth on artificial media, protozoa by immune-magnetic fluorescence, and viruses by cell culture or molecular biology techniques. However, most of these techniques are quite laborious, and time consuming and results can take several days. Polymerase Chain Reaction (PCR) is a fast and sensitive method that allows the detection of specific organisms. AQUAVALENS partners Ceeram and GPS™ have in recent years developed kits for an easier and repeatable approach to PCR. These kits are robust, specific, sensitive, ready and easy-to-use, and reliable as they include internal controls to avoid the possibility of false negative results (positive and negative controls are included).

Based on the technologies describe above, and following optimization with a ring trial performed amongst AQUAVALENS partners, the techniques and processes were validated and shown to be effective in concentrating microorganisms in low numbers. Thus, the method developed under AQUAVALENS can lead to a faster and more sensitive way of combined detection of microorganisms/ pathogens from multiple kingdoms using one single cartridge.

Research is on-going but the growing knowledge about the applicability of AQUAVALANS methods to small water supplies in Europe, and potentially elsewhere, is promising and could lead to the improved protection of the health of European citizens and beyond.

1.2. Objectives of this deliverable

Users of the AQUAVALENS analytical methods in WP11 and WP12 were questioned on their experience using these methods via a questionnaire that is included in Appendix A. These include laboratory staff from the Instituto Superior Técnico (IST) in Portugal, the James Hutton Institute (JHI) in Scotland, Teagasc in Ireland, CETaqua in Spain, and the University of Belgrade in Serbia, who have been piloting the AQUAVALENS technologies.

This questionnaire was developed specifically for users of the AQUAVALENS technologies by researchers at the University of Surrey, who have not been involved in the development, validation or use of the AQUAVALENS methods, and were therefore unbiased when formulating the questions, analysing the data and presenting the results.

The objectives of this deliverable are:

- To obtain feedback about the experience of laboratory staff using AQUAVALENS analytical methods
- To discuss findings from experienced staff in various countries using these technologies
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- To highlight areas where improvements may be needed

2. Approach to the analysis of the experience of users of the AQUAVALENS analytical methods

In total, eight questionnaires were received from users of AQUAVALENS analytical methods. The responses have a wide geographical spread, including users from Spain, Portugal, Scotland, Ireland and Serbia, with multiple questionnaires received from staff in the IST and JHI whose feedback was combined to present one set of answers per country. Data from these questionnaires were therefore compiled and segregated by country, analysed and results are presented below.

It should be noted that not all respondents were able to answer all the questions in the questionnaire, and the following caveats should be considered:

Teagasc and representatives from the IST, University of Belgrade and CETaqua stated that they could not answer all the questions fully as they are still implementing the methods.

CETAqua representatives answered the questions as a large water supply (large water system) and thus could not answer questions 21, 25 and 26 related to small water supplies. They answered questions 3, 4, 5, 6 and 7 based on routine analyses (not AQUAVALENS methods), and provided a caveat around their understanding of "water supply" as "treated water".

Users of the analytical methods in Serbia have provided some comments alongside some of the survey questions specifying their answers in relation to specific parts of the AQUAVALENS methods (e.g. only related to PCR kits, or the use of on-line adenosine triphosphate (ATP)-based monitoring methods) or for the complete procedure (including filtration, concentration, Polymerase Chain Reaction (PCR) etc.).

Data analysed and results from the analysis are described in more detail in the next section, which is followed by Conclusions and Recommendations at the end of this report.

3. Data analysis

Feedback from users of the AQUAVALENS technologies for the detection of pathogens in small water supplies and water used in food preparation was collected. More specifically, feedback on the technologies and processes developed under the project was sought in order to ascertain if and how the techniques can be applied to help protect the health of Europeans and people beyond the borders of the European Union. The following headings have been created to facilitate the presentation of answers provided by all respondents under a single or various questions on the same topic.

Source and appearance of water sampled

Water samples were collected from rivers and springs (surface water), wells, boreholes (groundwater) and from communal drinking fountains. Most of the sampled water was reported to have satisfactory colour and smell with the exception of one location in Portugal were water appeared to have high levels of turbidity. Also some water sampled in Ireland was post irrigation so was turbid as a result, and in Scotland the tap water's appearance was satisfactory, but water from peat-land areas can be highly coloured.

Samples location and previous tests for pathogens

In Portugal water was sampled from communal fountains and one borehole. In Ireland water for food preparation was collected at the end of the water supply network i.e. from a tap before irrigation. Post irrigation water was also collected. In Scotland water was collected from wells and holding tank or collection chamber pre-treatment. Treated water was also sampled from tap in house (post-treatment). In Spain the respondent is a large water supply and they relate their questions to treated water from tanks and pumping stations. In Serbia water samples are being taken at the well itself and at the borehole (end-user place).

Most locations where water was sampled have been reported as having been tested for pathogens in the past using conventional methods[1], except for Serbia where the answer to this question was negative.

Volume of water collected

Most samples were of 20 litres (l) except for Scotland where 50 l –samples were collected, Serbia where 10 to 20 l-samples were collected, and Spain where the large water supply specified that they collected 1000 l –samples for viruses, 100-800 l-samples for protozoa, 1 l-samples for Legionella bacteria, and direct samples for culture methods.

Sample stabilisation at the point of treatment or before

None of the samples were stabilised (meaning neutralising any possible residual disinfectant such as chlorine by adding sodium thiosulphate). The large water supplier in Spain was an exception and they reported that they used sterilized bottles containing sodium thiosulphate for the microbiological samples.

[1] Portugal: Culture methods: Membrane lauril sulphate; Slanetz and Bartley and Vinde-fois culture media. Ireland: standard methods outlined by the Drinking Water Directive (DWD). Scotland: tests done by Scottish Water. Spain large water supply: Culture, traditional methods: Aeromonas, Pseudomonas, Enterococci, Salmonella, HPC 22ºC & 37ºC, total coliforms, E. coli, C. perfringens; PCR: Legionella spp, Legionella pneumophila; PCR: Mycobacterium, Acanthamoeba, Naegleria; Filtration, IMS, Epifluorescence: Giardia & Crypto.

Was the AQUAVALENS pre-defined treatment /concentration method followed?

The complete AQUAVALENS procedures are being followed in Portugal and Ireland, but are reportedly not followed in Scotland, Spain and Serbia.

The alternatives to the AQUAVALENS methodology are:

Scotland is using the REXEED – 25A filters. For the secondary concentration stage they are using the PEG precipitation method instead of the Centricon method. For the DNA extraction stage an alternative method is being used as the users of the technology in Scotland do not have access to Minimag.

Spain did not follow the AQUAVALENS method in routine analyses.

In Serbia due to the unavailability of REXEED -25A filters in the country, all samples are tested using the filtration method developed by ITP based on charge change, filtration and subsequent testing of the collected filtrate. Afterwards, DNA/RNA is extracted and CEERAM and GPS PCR kits are being used.

Advantages and problems of the AQUAVALENS method

The feedback from Portugal is that water samples cannot be concentrated in the field. Samples need to be transported to the laboratory, and thus the technique cannot be used on site.

Ireland reported that there is no Irish distributor of the filters. Scotland reported that they encountered problems with the Centricon filters leaking, which isn't acceptable in a mixed laboratory, where equipment is also used for "clean" samples, due to the risk of cross contamination.

Spain emphasised the advantages of the method in that Virus, Bacteria and Protozoa can be concentrated with the same filter saving time and money. They, however, pointed out that some partners had difficulties getting the Centricon filters, Nuclisens: nucleic acid for extraction, and laboratory instruments such as the Centricon: specific centrifuges for large sample volumes were not available in all laboratories. Some users also had problems when running the PCR kits, and there was a comment about the need for a specific microscope and software that are also not available in all laboratories.

In Serbia the REXEED -25A filters recommended by the AQUAVALENS method are not available in the country and direct purchase from the Japanese company was not possible due to Customs rules that require Customs Services to approve each package delivered to the Serbian market. As the Faculty of Agriculture is not a medical institution and filters are made for dialysis and medical purposes, they need permission to legally import the filters.

Sample preparation in the field and methods reliability

All respondents emphasised that they could not do any of the sample preparation in the field with the water samples needing to be transported to the laboratory, which means the techniques cannot be used on site.

One respondent (equivalent to 12.5% of the total surveyed) concludes that the methods are reliable although the majority answered that they could not comment on the methods' reliability as tests are still on-going as the demonstration process is still under development.

How easy to use are new PCR kits compared to other PCR techniques?

60% of the respondents said that the PCR kits are very easy to use giving them a score of 5 (in a scale of 1 to 5), while 40% of the respondents stated that the PCR kits were similar to other PCR methods on the market and equally "ready to use", giving them a score of 3 (in the scale of 1 to 5).

Limitations with the AQUAVALENS techniques

Portuguese users of the technologies reported their concerns with low recovery rates of the tested Viruses, Protozoa and Bacteria (target organisms). Users in Ireland reported the loss of bacteria during the PEG precipitation step and stated that the method is time consuming and requires training. Users in Scotland reported that there are no standards available for Ceeram kits to enable them to quantify the target organism, and that they don't know whether the qPCR positives are from live or dead cells. Users in Spain highlighted the problems of some equipment not being available to all partners (see details above under problems of the AQUEAVALENS method) and reported that the Nuclisens extraction procedure is very manual.

Most time consuming steps in the new water testing methods

The following processes were reported as the most time consuming by all respondents: secondary concentration (Centricon): concentration of sample and usage of the filter for the concentration. Elution of pathogens from ultrafiltration cartridges and extraction of pathogens. Filtration of the samples using DEUF. However, overall respondents confirmed that PCR is quicker than traditional methods such as incubation.

Are new methods valuable for commercial laboratories serving water supplies?

Respondents included comments such as '*PCR methods will be the future of water testing'* with PCR ready and easy to use kits already available.

They also reported PCR kits are highly sensitive and have commercial value as a tool for detection of microorganisms but some fine-tuning is required because the method of concentration of pathogens allows for detection of 3 different 'kingdoms', but there are low levels of recovery for targeted microorganisms.

Most users (except of Serbia, thus 80% of those users surveyed) also said that new methods would allow water suppliers to provide a greater selection of tests to enhance assurance of the quality of their water. The respondents in Serbia reported that considering the complete procedure starting from water filtration using REXEED filters, concentration etc. there is some concern because commercial laboratories always go for a standard method of examination that is approved (see under barriers below).

The Spanish large water supply found the new concentration methods with REXEED, PCR targets, new online control etc. very valuable although they are still testing.

Barriers for laboratories to use the AQUAVALENS kits?

Some users pointed to barriers such as that some laboratories do not have the qPCR equipment, the expensive price of the necessary equipment and the need to have trained technicians. In addition to equipment not always being available in regulatory laboratories, possible slow turnarounds, large volumes of water for each sample needing to be transported, processed and stored, and some labour intensive steps were also seen as barriers. Users reported that some pieces of equipment could be improved also.

However, the biggest barrier mentioned by all users is that the methods are not a standard method for pathogen detection in drinking water, i.e. PCR is not yet an accredited method. The large water supplier in Spain saw regulation as the main barrier and commented that the qPCR techniques and results are not yet accepted in European Directive legislation. Another user reported that the whole procedure would require for laboratories seeking accreditation according to ISO 17025 to perform a complete validation procedure.

Rapid on-line testing systems suitability and small water supplies

All respondents answered that rapid on-line testing systems detecting increased burdens of microbiological contamination would be beneficial for small water supplies. In a scale of 1 to 5 (1, not very beneficial and 5, very beneficial), 40% of respondents gave a score of 4 and the remaining 60% of respondents answered that it would be very beneficial to have these on-line testing systems (score 5).

Regarding where to install this on-line systems, most respondents said that right after the treatment/ disinfection process, i.e. after the filters and UV, or before the water becomes available to the consumers. After the treatment system it would demonstrate when pathogens are likely to be breaking through, but used before treatment it may give an earlier indication of water quality changes which could alert the user that their treatment system may become compromised – especially if for example particulates or organics accompany the pathogens which is common.

The large water supply in Spain recommended to monitor all critical points of the plant and also critical infrastructures such as big tanks, hospitals supply, etc. whereas the Serbian partner advised that the on-line system should be ideally introduced in a place where action can be taken if the on-line system showed unsatisfactory results. This would point to installation near the disinfection system if exists or where it is planned to be introduced.

Methods to develop for the benefit of water supplies

The following were reported as potential useful methods to be developed for the water industry: a fast, reliable method that can be used on site, preferably on-line detection systems. Bioassay to test for live cells or a method with a faster turnaround than 2 to 3 days. An automated sampler with the REXEED filter that could concentrate virus, bacteria and protozoa at the same time. An ATP online analyser. An automated method with subsequent disinfection system that would disinfect water when it is out of microbiological safety limits.

Feasibility of adopting these techniques for small water supplies

25% of respondents said it is feasible to adopt the techniques for use by small water supplies but not with the current methods developed. 50% of respondents reported they do not know and the remaining 25% answered negatively. Respondents commented that the current methodology would be difficult to apply for the end-users: small systems and might not be useful to search for multiple pathogens at all times, suggesting that more testing is required. On-line monitoring systems may be more suitable for mall water supply systems according to users, depending on the water source and if it is possible to take action after the on-line system detects something wrong.

Examples of systems where reportedly it may be feasible to adopt the techniques include systems such as Kinloch and Kirkmichael water treatment works in Scotland which are run by Scottish water where the filters could be incorporated into the system and then transported to their labs for analysis . However it may not be considered worthwhile for laboratories to invest in the training and equipment needed when they could potentially outsource "investigations" should there be an incident, making it difficult for techniques to be adopted routinely and for investment to be considered worthwhile for occasional use.

It should be noted that the Spanish large water supply did not comment on the questions specifically designed for small water supplies.

Barriers for these techniques and their use in laboratories dealing with small water supplies

The following table represents the barriers that users found as more important for small water supplies to ask their laboratories or implement themselves the AQUAVALENS analytical methods. The main barriers are perceived to be related to regulation not demanding testing for pathogens.

Table 1 Barriers for implementation of AQUAVALENS techniques for small water supplies

Barriers for these techniques and their use in laboratories dealing with small water supplies?	Number of responses as provided by 8 respondents
No barriers/no perceived barriers	-
Users not demanding pathogen-testing	2
Regulation not demanding testing for pathogens	5
Regulation demanding standard methods (*AQUAVALENS techniques not standard & require validation)*	5
Reluctance of laboratories serving small water supplier to implement because of: -cost of equipment -running costs (e.g. filters, increase in electricity/energy bill) -maintenance -legal responsibility/liability -not wanting to increase bills / pass on costs to customers	 6 4 3 3 4
I don't know	-

Advantages /opportunities for small water suppliers and regulators to ask laboratories to adopt these techniques

The following table represents the advantages that users found as more important for small water supplies and regulators to ask their laboratories to adopt the AQUAVALENS analytical methods. The main opportunities are perceived to be related to limiting risk and expenses related to microbiological contamination of water, and to improving public health.

Table 2 Advantages /opportunities of AQUAVALENS techniques for small water supplies

Advantages /opportunities for small water suppliers /regulators to ask their labs to adopt these techniques	Number of responses as provided by 8 respondents
Limit the risk and expenses derived from microbiological contamination by predicting and identifying the source of faecal pollution	6
Keeping check on potential polluters to encourage them to comply with regulations	1
Improving public health by limiting the risk of disease	4
Limiting costs of treating and /or compensating users in a contamination incident	4
Other, see comments below	2

Could the AQUAVALENS methods/kits be evolved to be used on site, e.g. by the owner of a water supply?

The majority of respondents answered negatively to the above question rating their answers 1 to 3, in a scale where 1 is very unlikely and 5 is highly likely. This suggests that other techniques may be more suitable for on-site testing.

4. Discussion

All users of the AQUAVALENS technologies agreed that water samples cannot be concentrated in the field, and have to be transported to the laboratory, thus concluding that the techniques and the AQUAVALENS methods are not yet suitable for use on site, which would be desirable for small water supplies. On the other hand, all respondents answered that rapid on-line testing systems detecting increased burdens of microbiological contamination would be beneficial for small water supplies, suggesting that this might be the future for testing for pathogens in small systems.

Users in Portugal and Ireland also commented that concentrating pathogens allows for detection of 3 different 'kingdoms': Virus, Bacteria and Protozoa, but there are low levels of recovery for targeted microorganisms.

Also, the complete AQUAVALENS procedures are only being fully followed in Portugal and Ireland, but are reportedly not fully followed in Scotland, Spain and Serbia. There are problems of availability of the recommended REXEED -25A filters in Ireland and Serbia where there are no distributors of the filters. In Serbia, these filters could not be imported due to Customs rules on imports of medical equipment (these filters are used for dialysis). In Scotland users of the techniques encountered problems with the Centricon filters leaking which presents a risk of contamination in the laboratories.

The majority of users of the AQUAVALENS analytical methods answered that they could not comment on the reliability of the methods because tests are still on-going as the technology demonstration process is still under development. However, despite these setbacks, all respondents said that the PCR kits are very easy to use or similar and equally "*ready to use*" than other PCR kits available in the market. Moreover, one of the respondents in Portugal concludes that the methods are reliable, and the large water supplier in Spain emphasised the advantages of the AQUAVALENS methods in that Virus, Bacteria and Protozoa can be concentrated using the same filter, saving time and money in the concentration and elution step.

Overall, respondents confirmed that PCR kits are highly sensitive and faster than traditional methods such as incubation and this seems to be one of the most innovative aspects of the AQUAVALENS project in that existing techniques such as PCR, are being adapted for use in the water sector where rapid testing of pathogens in water during disease outbreaks is critically important.

Moreover, PCRs are recognised as having commercial value as a tool for detection of microorganisms although some fine-tuning is required to, for example, improve low levels of recovery for targeted microorganisms. Also, the lack of accreditation of these new methods in the water sector is seen as a major barrier that needs to be addressed to make them standard methods.

In summary, from the comments included in the questionnaires it can be discerned that the experience of users of the technologies is varied depending on which technology they have used, but some methods (e.g. qPCRs) have been reported as easy to use and having potential in the water industry. However, these methods are said to be expensive, time consuming and depend on skilled technicians. In terms of processing the samples, large volumes of water are required that need to be taken into laboratories, and the technologies are not easy to use in the field. Moreover, a common remark made by users is that these are not yet accredited technologies accepted under EU directives, and thus they are not favoured by laboratories yet.

5. Conclusions and Recommendations

Users have stated the specific advantages of rapidly testing for a range of pathogens versus the current practice of testing for indicator bacteria that takes longer and involves several steps: first test for indicators (especially coliforms) that would point to specific pathogens to be tested. Then find and use specific pathogen detecting techniques. Hence, AQUAVALENS novel techniques offer a faster way of testing for pathogens when compared with conventional methods.

AQUAVALENS technologies can help compared to traditional methods and standard PCR kits. These advantages are critical during disease outbreaks. The advantages of the new methods are improved effectiveness and speed of testing/detection of pathogens. Respondents confirmed that PCR is faster than traditional methods such as incubation and this can be one of the most innovative aspects of the AQUAVALENS project in that existing techniques such as PCR, are being adapted for use in the water sector, where rapid testing of pathogens in water during disease outbreaks is critically important.

However, on-line systems for the detection of pathogens are considered to have more potential for small water supplies and the laboratories that serve them.

A number of questions could not be answered by the participants especially around the use of these technologies in the water sector, and more specifically, in small water supplies. Thus it is recommended that further research with widespread pilots in a variety of environments and scales should take place in order to ascertain the viability of the AQUAVALENS analytical methods in the water industry, both in large and small water supplies, and from the technical, economic and environmental perspectives.

In parallel it is recommended that research takes place with a strong element of stakeholder participation to outline the policy and regulation aspects that would be needed in order for technologies such as the ones developed under the AQUAVALENS project to be widely implemented in the water sector in Europe.

It is also recommended that the AQUAVALENS project findings and the technologies are disseminated among commercial laboratories involved in small water supplies and food preparation.

Appendix A

Semi-structured questionnaire for Users of the AQUAVALENS technologies

AQUAVALENS Powerful water - purity and safety tested

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

Work Package 11 Small Systems – Pathogen detection in small water supplies across Europe. Guiding questions for Deliverable D11.5 -Month 48: 'Report on the experience of users of the AQUAVALENS technology and their recommendations for implementation'.

Feedback on this questionnaire will be followed by telephone interviews with *Nada/Andreja, Ricardo/Silvia, Lisa/Richard/Claire/Eulyn, Claudia and Kaye*

New analytical techniques developed under the AQUAVALENS project can detect pathogens in water supplies and track down the origin of microbiological contamination, e.g. faeces of cattle, pigs etc. The implementation of these techniques as part of water safety risk assessments for small water supplies can limit the risk and expenses derived from microbiological contamination by predicting and identifying the source of faecal pollution. Please answer the appropriate option or options when multiple answers are provided, and give as much detail as possible on other questions.

1. Where does the water for the water supply/ies you have monitored when using the AQUAVALENS methods come from?
   - Surface Water (e.g. from a river, spring or lake)
   - Groundwater: wells
   - Groundwater: boreholes
   - Communal fountains
   - Water stores
   - I don't know
   - Other, please state (e.g. rainwater)

2. Was the appearance (colour, smell, taste) of the water sampled from the water supply/ies satisfactory? If you are not sure, please circle the third option: I don't know
    - o Yes
    - o No If no, please specify what is unsatisfactory
    - o I don't know

3. As far as you know, has the water supply/ies you have sampled ever been tested for pathogens in the past?
    - o Yes
    - o No
    - o I don't know

4. If the answer to 3 is yes, what methods were used to identify pathogens? Please provide details if you know

5. Where in the water supply network did you collect your samples? Please provide details

6. What volumes of water sample did you take on average?

7. Did you stabilise the sample at the point of treatment or before?
    - o Yes , if so, please explain what process/es
    - o No

8. Did you follow the AQUAVALENS pre-defined treatment/concentration methods?
    - Yes
    - No   If no, please explain what process/es you followed (e.g. filtering, elution, re-concentration, other)

9. If yes to the above question, are there any advantages or problems that you foresee in relation to the AQUAVALENS methods (e.g. availability of filters)? Please provide details

10. Was any part of the sample preparation done in the field?
    - Yes , if so, please explain what process/es
    - No

11. Are the new methods reliable?
    - Yes , if so, please explain what process/es
    - No

12. In scale of 1 to 5, how easy is it to use the new kits compared to other PCR techniques? (1 being not very easy and 5 being very easy)

    1 2 3 4 5

13. Are there any limitations with the AQUAVALENS techniques? Please provide details

14. What were the most time consuming steps in the new water testing methods? Please provide details

15. Do you think the new methods are valuable for commercial laboratories serving water supplies?
    - Yes If so, please provide details
    - No If no, please explain why not in the next question

16. In your opinion, are there any barriers for laboratories to use the AQUAVALENS kits? If yes, please provide details (e.g. not standard methods for pathogens detection in drinking water)
    - Yes If yes, please specify
    - No

17. In your experience, would an on-line testing system that could rapidly detect an increased burden of microbiological contamination be beneficial to the owners/operators of small water supplies?
    - Yes
    - No
    - I don't know

18. If yes to the above question, can you please indicate how beneficial the on-line system would be in a scale of 1 to 5? (1 being not very beneficial and 5 being very beneficial)

    1 2 3 4 5

19. Where in the system would you introduce such a method? Please provide details

20. Do you have any suggestions as to what kind of method you would like to see developed that you think would benefit water supplies? Please explain/ provide details

21. Given that there are improved techniques available such as those of AQUAVALENS that can detect, separate groups of pathogens (be it bacteria, viruses or protozoa), and track down the origin of these pathogens in water supplies, do you think it is feasible for small water supplies to adopt these techniques?

   - o Yes
   - o No
   - o I don't know

22. If yes, where would you apply them? Please provide details

23. What do you perceive as the main barriers for these techniques and their use in laboratories dealing with small water supplies? (tick or circle as many as you want)

   - o No barriers/no perceived barriers
   - o Users not demanding pathogen-testing
   - o Regulation not demanding testing for pathogens
   - o Regulation demanding standard methods which require no validation (*AQUAVALENS techniques are not standard methods)*
   - o Reluctance of laboratories serving small water supplier to implement because of:
     - cost of equipment
     - running costs (e.g. filters, increase in electricity/energy bill)
     - Maintenance
     - Legal responsibility/liability
     - Not wanting to increase bills / pass –on costs to customers
   - o I don't know

24. What are your main challenges in microbial /pathogen testing at the present time and how would you propose to overcome them? Please detail

25. What to you perceive to be the advantages /opportunities for the owners /regulators of small water supplies to encourage them to ask their labs to adopt these techniques? (tick or circle as many as you want)

    - Limit the risk and expenses derived from microbiological contamination by predicting and identifying the source of faecal pollution
    - Keeping check on potential polluters to encourage them to comply with regulations
    - Improving public health by limiting the risk of disease
    - Limiting costs of treating and /or compensating users in a contamination incident
    - Other, please detail

26. Could the AQUAVALENS method/kit be evolved to be used on site, e.g. by the owner of a water supply? Please grade your answer 1 to 5 (1 being very unlikely, 5 being highly likely)

    1 2 3 4 5

Other comments or suggestions, please detail here