ScanVIT™: The new technology for the fast and accurate identification and quantification of *Legionella* and *L. pneumophila* in water samples

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Bacteria belonging to the genus *Legionella* are waterborne pathogen bacteria. We know today that the genus *Legionella* includes 40 species, which all have been implicated in human disease (legionellosis). *Legionella pneumophila* is responsible for approximately 90% of all reported infections and is thus the causative agent of a life-threatening lung infection which is known as the Legionnaire`s disease.
Legionella are found ubiquitously in natural and man-made aqueous systems and are able to tolerate a wide range of growth conditions, including temperatures of 0 to 63 °C, pH values of 5.0 to 8.5, and dissolved oxygen concentrations of 0.2 to 15 ppm in water.
Legionella are frequently detected in hot-water plants, swimming pools (particularly whirlpools) as well as cooling towers, air-conditioning systems, humidifiers and evaporative condensers. As intracellular parasites of protozoa (e.g. amoebae, ciliates) or socialized in biofilms or sediments they can also survive unfavourable life conditions, for example, extreme temperatures and water chlorination. Thus, they are able to coexist in our drinking and hot water supplies.
During the last years an increasing number of outbreaks resulting in fatal casualties from drinking water contaminated with *Legionella* were reported world-wide.

How can this be prevented?
Public health can only be protected by the routinely maintenance of the pipe system, monitoring of the microbial status and suitable disinfection actions (like heat-and-flush procedure, chlorination, UV radiation etc.). Analysing and testing for *Legionella* is the only key to assessing risks and in determining whether or not preventive and corrective measures are working.
Only if these bacteria can be detected in an appropriate time effective preventive action can be carried out to minims contaminations and to stop the spread of the infection.

Today's standard detection of *Legionella* is based on a long cultivation procedure that lasts between 7 to 14 days. In many cases this detection time is too late for checking the microbial status of a given sample in order to avoid dangerous contaminations.

For this reason over the past few years an increased development of microbiological methods has taken place in order to shorten the long and unacceptable detection times for *Legionella*. But these modern methods are often hampered by different facts: First, they can be too specific by detecting

only *Legionella pneumophila*. Second, the robustness of the tests is too low and third, they require often a molecular-biological knowledge which demands a special education of the staff in the labs of the water-suppliers.

Therefore, a new method was developed which identifies *Legionella* in water samples by using dye-labeled gene probes. This method is based on the so-called VIT™ (vermicon identification technology) and is called ScanVIT™

What is the basis of VIT? Over the billions of years of evolution, every bacterium has developed special signatures on their genetic material. Some of these signatures are typical for a certain species, some are typical for different species of the same genus. Based on huge databases these signatures can be compared and identified to use them as target positions for dye-labeled gene probes.
Gene probes are tiny pieces of DNA, which are labeled with a fluorescent dye. They can be programmed so that they find the special signatures within the bacteria cells and bind to them. So, they illuminate the bacteria from inside. The final results under a fluorescent microscope are red or green shining bacteria cells which are easily identified by their colour. The advantages of the technique are the speed and the specificity which are outstanding among modern technologies. Moreover, the handling of the test is very easy and can be performed even with no molecular-biological skill of the personnel.
During the last 3 years a variety of VIT-testkits for different applications and for the rapid and specific detection of different bacteria have been developed. They are used nowadays in many laboratories around the world where fast and specific microbiological results are needed.

The state-of-the-art detection system ScanVIT offers the applicant many benefits in one single test. All viable bacteria of the genus *Legionella* which are present in the sample are detected, as well as all *Legionella pneumophila* are specifically identified.. In addition the result of the ScanVIT analysis is not only a qualitative conclusion regarding Legionella is present or absent in the sample, it also retrieves the actual counts and thus enables a quantitative finding. ScanVIT-Legionella relies on specific dye-labeled gene probes which identify the corresponding *Legionella* species. The practical performance and evaluation needs no special molecular-biological skills.
In this test micro-colonies of *Legionella* grown on a filter membrane are treated with the labeled gene probes in the ScanVIT-reactor, both provided with the kit. After an incubation step for 90 min the gene probes are washed away and the filter membrane with the specifically labeled cells is evaluated. The evaluation step can take place by using a fluorescence microscope or can be carried out by a scanner. The scanner device detects and quantifies the labeled micro-colonies of *Legionella* and *Legionella pneumophila* species within 10 min. The whole test procedure of ScanVIT takes 3 hours with a handling time of only 30 min.

All in all ScanVIT results are available after 48 hours, which means a time saving of between 5 and 12 days for the *Legionella* detection and quantification in comparison to the conventional method.

Thus, ScanVIT reduces significantly the detection time and appropriate counteractive measures can be taken against *Legionella* contaminations in time.

By nearly enabling an online microbial status of water systems this detection system will effectively help to avoid outbreaks of Legionnaire`s disease.