



Hydro-X Legionella Testing Method

- All laboratories used by Hydro-X are UKAS approved and use methods in line with British Standards.
- The Legionella testing method is performed in 3 key stages -

Stage One – Selective Plating

The sample is filtered to retain any micro-organisms. This filter is then treated in 3 ways. Firstly it remains as it is (untreated). This allows for any potentially damaged Legionella to be detected. Secondly it is added to an acid buffer. Legionella are acid tolerant compared to a lot of other water borne micro-organisms. Thirdly it is heated to 50°C and held at this temperature for 30 minutes, again Legionella are fairly heat tolerant compared to some other microbes. These 3 processes are designed to promote the detection of Legionella and hold back other non Legionella bacteria. Following these processes the sample is transferred to a Legionella selective agar culture media which is supplemented with reagents designed to promote the growth of Legionella but inhibit the growth of other bacteria. These plates are then incubated at 36°C for 10 days.

Stage Two – Confirmation

The agar plates are checked periodically during the incubation. Any colonies that are observed and are deemed to be possible Legionella are then transferred to 2 derivatives of the initial Legionella selective agar. The first plate contains a reagent called L-cysteine, the second plate does not contain L-cysteine. L-cysteine is an essential growth requirement for Legionella. Therefore if the suspect organism grows on both plates (i.e. with and without L-cysteine) it cannot be a Legionella organism. If the suspect organism grows only on the plate containing L-cysteine then it is most likely to be Legionella.

Stage Three – Agglutination Test

These organisms that only grow in the presence of L-cysteine are mixed with a latex solution coated with antibodies that are specific to the antigens found on the surface of individual Legionella bacterium. We use 3 such latex solutions. The first contains antibodies to *Legionella pneumophila* serogroup 1, the second contains antibodies to *Legionella pneumophila* serogroups 2 to 14 and the third contains antibodies to a 7 different common Legionella species (*L. longbeachae*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei* and *L. anisa*). If the mixture of organisms and latex forms a clump with one of the 3 solutions then the sample is reported as being positive for Legionella.

Using the three stages listed above and appropriate laboratory techniques such as correct labelling of samples, aseptic technique and single use/sterile equipment, presence and count of Legionella bacteria can be accurately reported, and the chances of reporting false positive Legionella results are almost entirely eliminated.