eurofins | Water Hygiene Testing

Legionella Counts and Serology Counts

Legionella count are calculated as per BS EN ISO 11731:2017 Water quality – Enumeration of *Legionella*

ISO 11732 says 'To estimate the number of colony forming units of *Legionella* in the original water sample, select the plate or set of plates (from the same culture medium) showing the maximum number of confirmed colonies per water volume. Take the dilution into account. Do not average the counts from different methods, treatments or culture media, as these are not replicates'.

There are 3 treatments used in the testing of *legionella*, untreated, heat-treated and acid-treated, the latter two are used to lower the levels of competing microorganisms such as *Pseudomonas* which could make the untreated plate unreadable if present in very high levels.

On the agar, *Legionella* can appear as many different looking colonies (morphologies), as described in ISO 11731 which says: "Colonies of *Legionella* are white-grey in general but can also appear in other colours…." Blue, purple, green, red with yellow edge, yellow…. Some species fluoresce under ultraviolet light, some do not; are all mentioned here. This list is not definitive, as new species can be found.

For each morphology found, we take a number for confirmation.

According to ISO 11731, for the total count, we take the highest count that confirm that is seen on one treatment and multiply up by the dilution factor. This is a total of all types of *legionella* that are present on that treatment and confirm.

Note: The calculation is based on:

- Untreated or heat-treated colony - from 25 colony forming units (cfu)
- Acid-treated colony – from 250cfu

The total is the highest count seen of colonies that all confirm from one plate.

As part of the confirmation, we test for *L.pneumophila* serogroups, splitting these into SG1, SG2-14 and also for *Legionella* species, not *pneumophila*.

However, the other plates may have lower numbers of *Legionella* that confirm and are of different serology. For the estimation of the serology groups, we report the highest of each serology.

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Example

Plates

Untreated - **13** colonies, **12** confirm as species and 1 as SG2-14

Heat-treated - **2** colonies, both confirm as SG1

Acid-treated - **1** colonies, both confirm as SG2-14

Calculation

- Total count as **13** colonies (x dilution) · Highest count seen on any treatment (12 species + 1 SG1 from same treatment.

  Total count is **13** x 25 = 325. Species count is **12** x 25 = 300

- SG1 count as **2** colonies (x dilution) based on highest count on any treatment.

  2 x 25 = 50

- SG2-14 count as **1** colony (x dilution) based on highest count on any treatment.

  1 x 250 = 250

Reported as

Legionella	325 cfu/volume
Legionella pneumophila SG1	50 cfu/volume
Legionella pneumophila SG2-14	250 cfu/volume
Legionella species not *pneumophila*	300 cfu/volume

The total count does not have to match the sum of the individual species or serogroups as these are estimates and not replicates.

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