

Troubleshooting

Checklist for Quality:

- 1. Methods**
Are you using standard or validated, clearly documented methods for isolation, identification and enumeration of Legionella spp.?
- 2. Culture media**
Is your culture media allowing optimal isolation of Legionella spp.? Do you have sufficiently challenging quality control procedures for your culture media?
- 3. Control strains**
Do you use control strains, at appropriate levels, for all the processing procedures?
- 4. Processing**
*Is the percentage recovery of legionellae as expected following heat and acid treatments?
Do you undertake trend analyses for these processes? Are you aware of the limitations of the method used?*
- 5. Equipment**
Is all the equipment used for the procedures (incubators, refrigerators, measuring instruments, waterbaths etc.) calibrated and monitored regularly?
- 6. Staff Training**
Are the staff who perform the examinations fully trained and familiar with all the procedural steps?
- 7. Internal Quality Control (IQC)**
Do you have adequate IQC procedures in place with documented guidelines for dealing with IQC failures?
- 8. Good Laboratory Practice (GLP)**
*Do staff adhere to GLP at all times?
If cross-contamination has occurred with EQA samples it can also occur with routine water samples.*
- 9. Clerical Procedures**
*Are your laboratory numbering and clerical procedures adequate?
If you have reported EQA results incorrectly this may also happen with routine water samples*

Identification of *Legionella* spp.

There are at least 50 species of *Legionella* of which at least 20 have been associated with human disease. The species that is most commonly isolated both from the environment and from infections is *L.pneumophila*; this species can be divided into at least 16 serogroups on the basis of surface antigens. *L.pneumophila* serogroup 1 is the most common cause of outbreaks of Legionnaires' disease and, within serogroup 1, the strains most commonly associated with disease share a common epitope, as revealed by monoclonal subtyping. It is important, because of the risks to human health, to differentiate routinely between *L.pneumophila* and the other *Legionella* species and to be able to distinguish serogroup 1 from the other serogroups of *L.pneumophila*. However, monoclonal subtyping is normally performed only by specialist reference laboratories. Participants who do not identify *Legionella* spp. other than *L.pneumophila* should, where appropriate, report their results as '*Legionella* sp. not *L.pneumophila*' rather than '*Legionella* sp.'.

Incomplete or misleading identification of *Legionella* spp. from water samples may have serious public health implications. Correct reporting of the serogroup is essential for outbreak investigations.

False Positive Results

False positive results may arise due to cross-contamination, either from other test samples or control strains, mis-identification of background micro-organisms that are present in the sample, or sample-handling and clerical errors. False positive results may result in unnecessary treatment of the water-source with serious financial implications.

False Negative Results

There are many reasons for false negative results including failures with processing, culture media or equipment, insufficiently sensitive methods, inadequately trained staff or sample-handling or clerical errors. False negative results may have serious public health implications.

Outlying Results for Enumerations

Outlying results may be due to errors such as preparation of dilutions, selectivity of culture media, errors in counting, mis-calculation, mis-identification, mis-reporting or sample-handling or clerical errors. Laboratories are advised to investigate the reasons for any anomalous results, particularly if they are occurring repeatedly. Participants who report consistently low levels should audit all stages of their procedure that might allow organisms to be lost, e.g. filtration, centrifugation and pre-treatment. Participants reporting consistently high counts should assess their identification processes but should consider also whether they use methods that differ from those used by the majority of other participants*. Incorrect results for enumerations can give a misleading impression of the severity of the risk associated with the sample.

Repeat Samples

Participants should, where possible, determine the reason(s) for mis-identification, false positive and false negative results and outlying counts for enumerations. Repeat samples should be requested to ensure that causes of error have been eradicated. Repeat samples are free of charge, provided they are dispatched with the next distribution of samples. A handling fee will be charged for immediate dispatch.

Help and Advice

The Scheme Organisers will help participants to resolve issues relating to the testing of environmental water samples for *Legionella* spp. Participants should also contact FEPTU to discuss any issues relating to the schemes in general, data analysis or performance assessments.