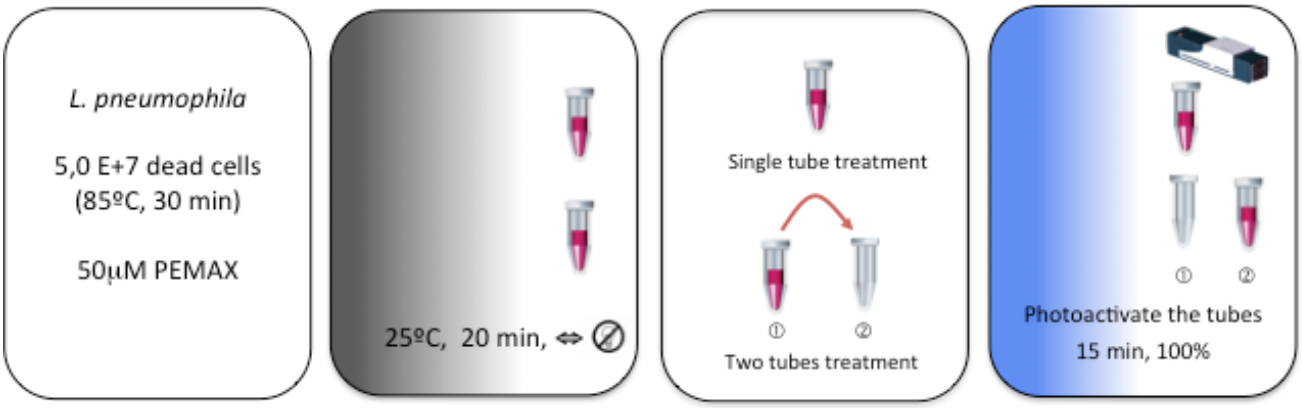
Understanding the tube contribution in vPCR results

Even in VIABILITY PCR procedures well-optimized, most protocols are unable to obtain an absolute signal reduction. A quick calculation about DNA and dyes stoichiometry proves that the current reagent concentration ranges should be enough to full neutralization of nucleic acids. However, practical experience shows that a persistent DNA fraction from dead cells that cannot be neutralized.
On the other hand, it's well known that microtube surface interacts with the sample, retaining nucleic acids and proteins. Different research activities conducted in our R&D laboratory concluded that a fraction of this DNA remains inaccessible to vPCR reagents and this interaction results in a release of non-neutralized DNA during subsequent DNA purification procedures. This fact results in one of the mains contribution to false positive results in vPCR.
In order to overcome this drawback we propose a two-step procedure, which is detailed below.



TUBE CONTRIBUTION TO FALSE POSITIVE RESULTS:

Following the above depicted method, the same experiment has been performed using 3 different brands of tube.

The results have been compared with the conventional treatment of a single tube. The data presented in the graph demonstrates that:

- The 0,005% of DNA remains linked to tube.
- The two-step procedure improves signal reduction, being in some cases near to detection limit.