



Understanding vPCR results for risk assessment evaluation

Water microbiology : *Legionella*

Doc Code 450000132-00

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Understanding vPCR results

Although the aim of any vPCR procedure is to be near of 100% of efficiency in DNA neutralization from dead cells, the natures of samples or the cell status not always make it possible.

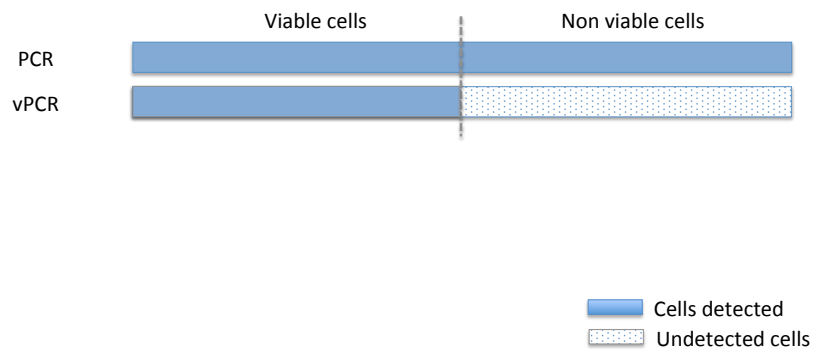
- Some DNA-intercalating dyes will have access to the DNA in viable cells, showing false negative results
- Dye or light may not be able to penetrate in all dead microorganisms, overestimating the number of viable cells (false positive)

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Understanding vPCR results

Theoretical approach: vPCR only will detect live cells

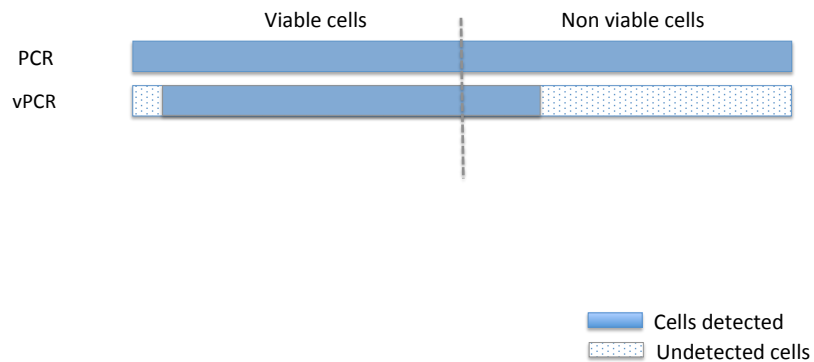


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Understanding vPCR results

Reality: often vPCR not is completely specific

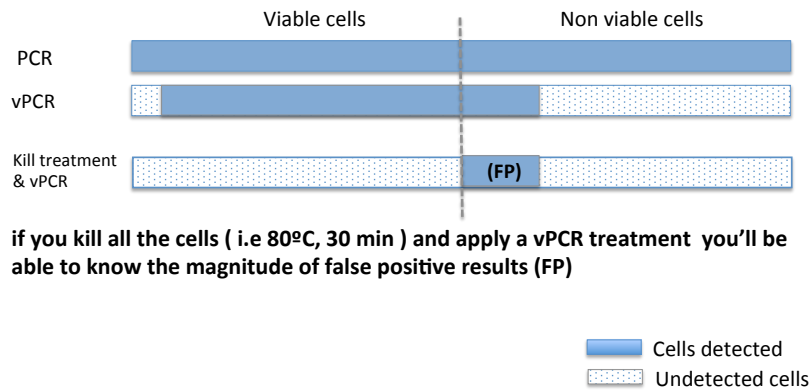


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Understanding vPCR results

Practical approach:



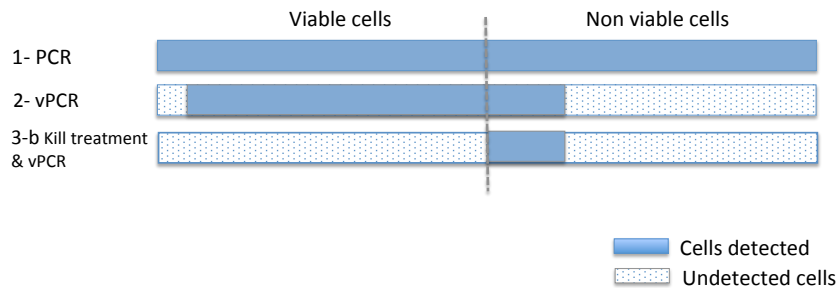
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Understanding vPCR results

Practical approach:

Through the sample analysis with three different points of view is possible to obtain a clear idea about the levels of live cells, and the % of live cells regarding to the total level.



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Understanding vPCR results

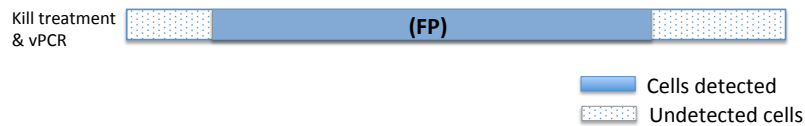
The estimation of the minimum percentage of live cells, is possible with this triple approach.

Aliquot	Workflow	Result
①	vPCR	Theoretical live cells
②	80°C, 30 min & vPCR	False positive live cells
③	PCR	Total

Total cell level = ③

% live cells level = $[(① - ②) / ③] \times 100$

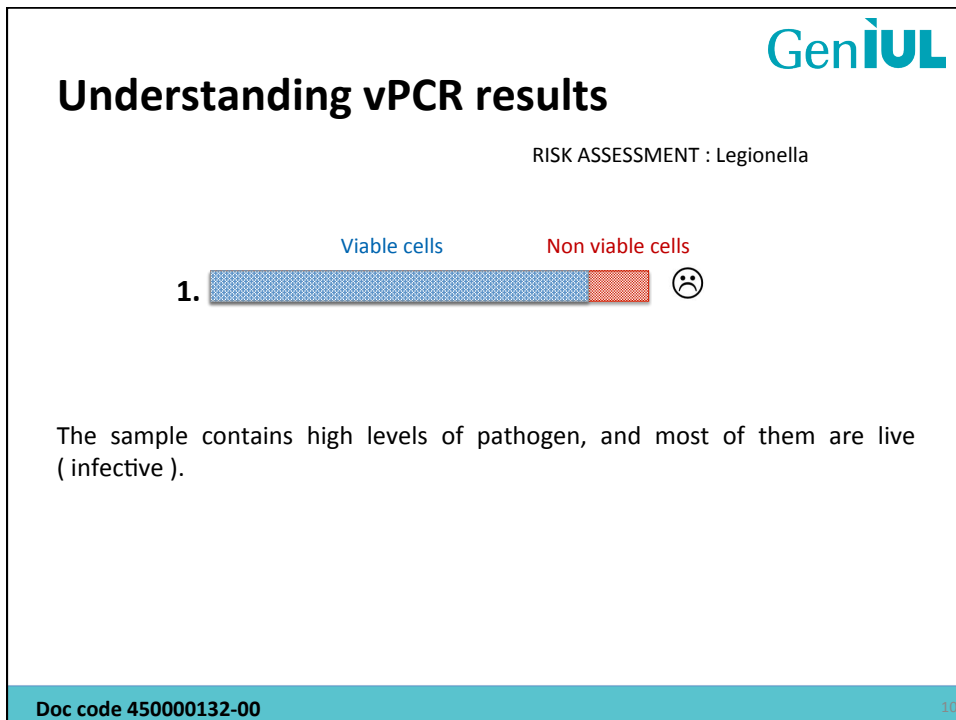
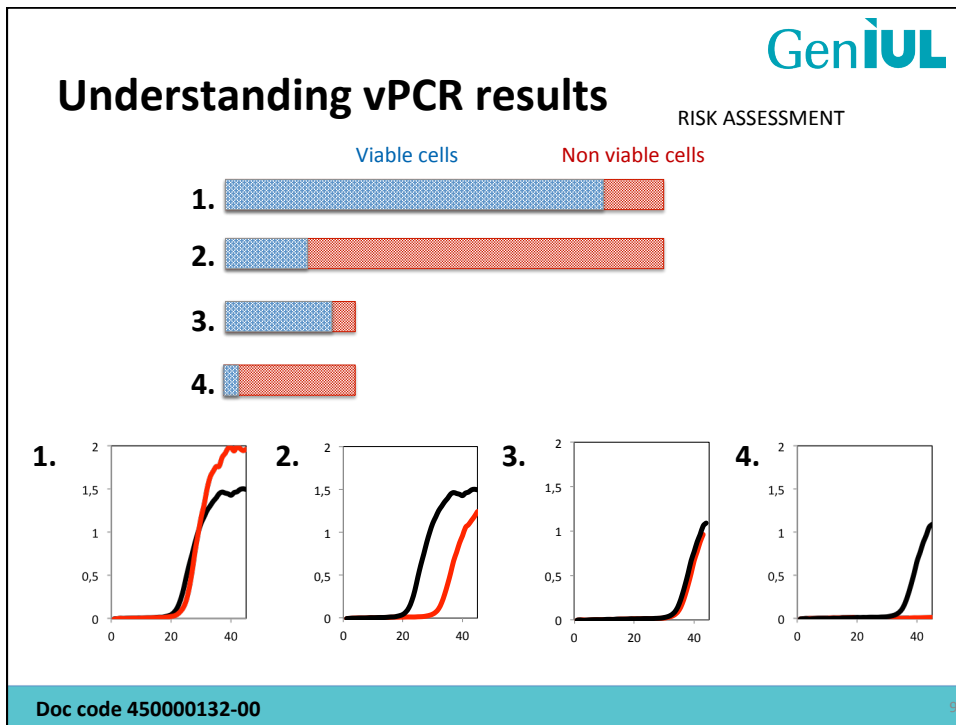
Too many bias?



The sample has been treated in order to kill all the cells, and still there are detecting a lot of live cells?

Probably the sample contains a lot of background (DNA, organic matter..) that reduces the treatment efficiency

If you are obtaining signal reductions lower than 9 Ct, repeat the procedure with 1/10 dilution



Understanding vPCR results

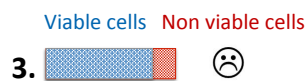
RISK ASSESSMENT : Legionella



The sample contains high levels of pathogen, however most of them are dead (non infective). If we are monitoring a disinfection procedure the results are promising. However if it's a prospective analysis, the results also are showing low levels of infective microorganisms, maybe it's result of a pathogen regrowth.

Understanding vPCR results

RISK ASSESSMENT : Legionella



The sample contains low levels of pathogen, however most of them are live (infective). The results are showing an scenario with pathogen regrowth potential, however the health risk is low.

Understanding vPCR results

RISK ASSESSMENT : Legionella

Viable cells Non viable cells



The sample contains low levels of pathogen, and most of them are dead (non-infective). The results are showing an scenario with low pathogen regrowth potential and the health risk is low.

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Understanding vPCR results

RISK ASSESSMENT

Viable cells

Non viable cells



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