

Rapid testing in the monitoring of *Legionella* in complex environmental matrices

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Background flora gives inconclusive results by culture based techniques

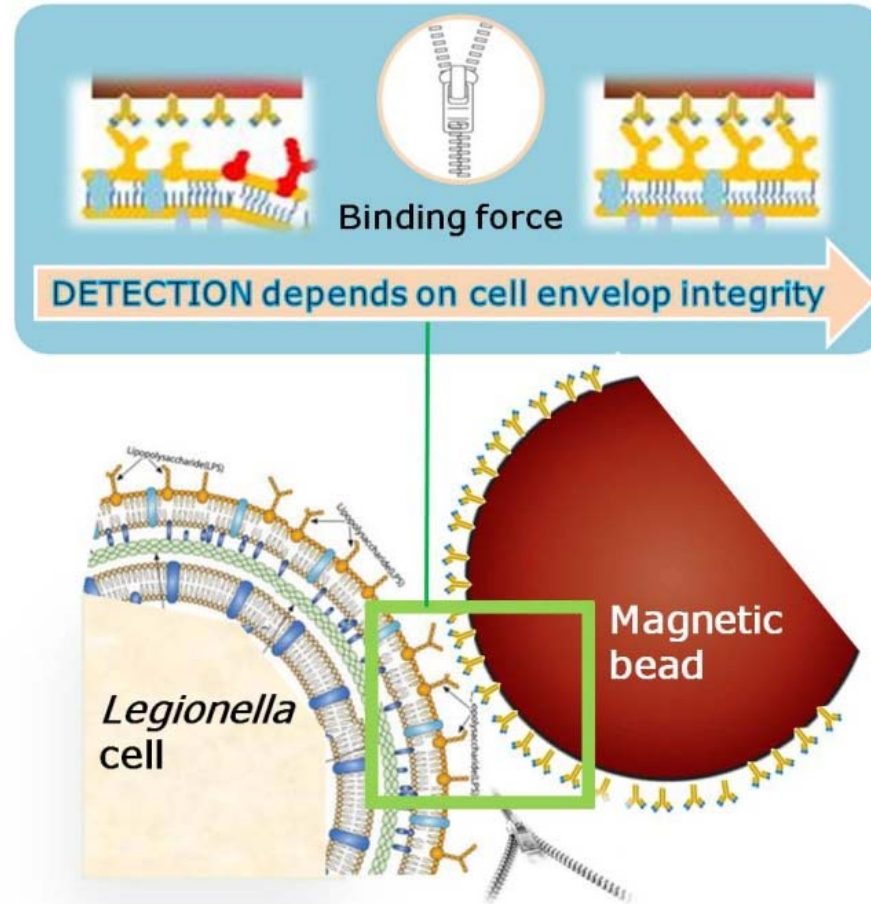
Inhibitors

gives inconclusive results by PCR based techniques

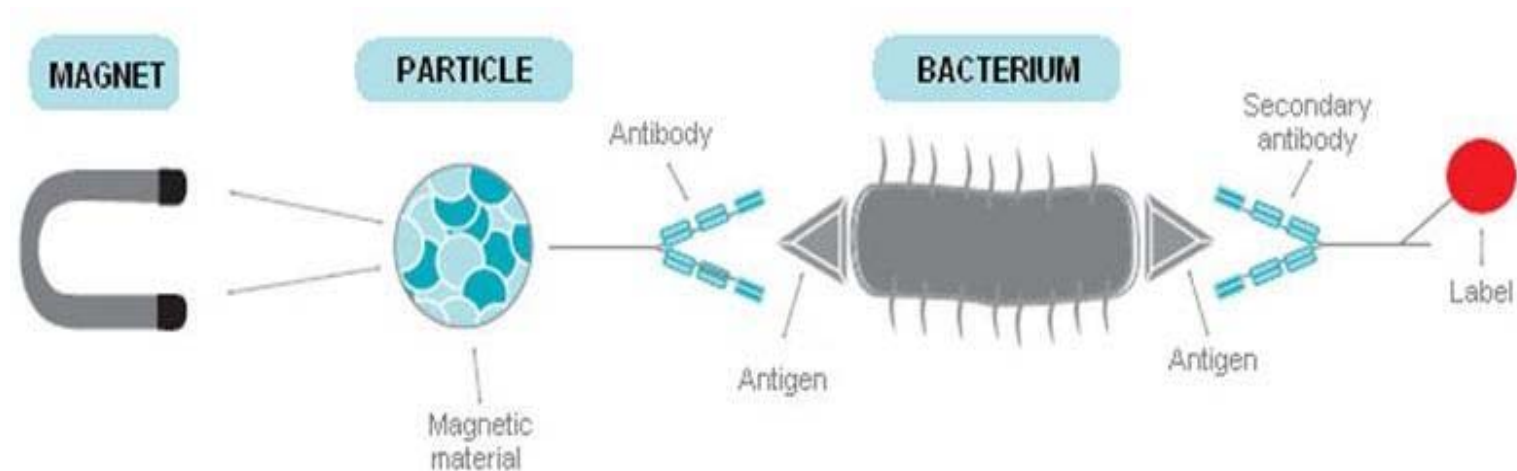
Technical approach:

Immuno-magnetic method (IMM)

- The capture of IMM dependent on integrity of cell envelope



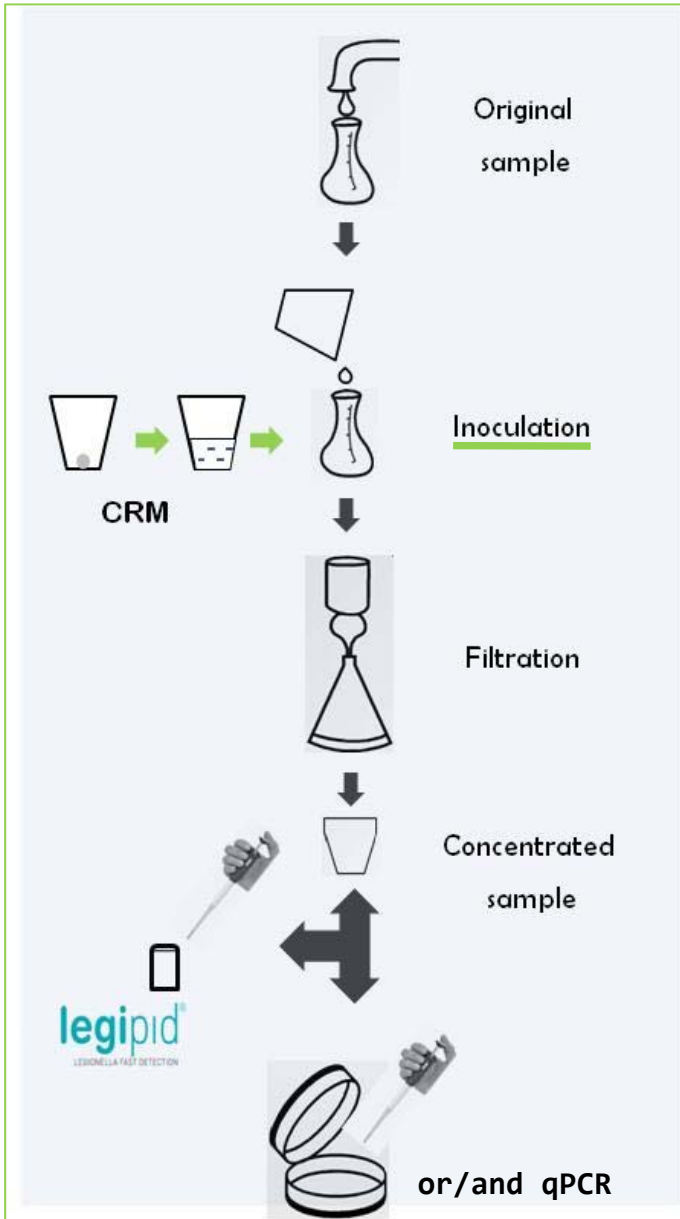
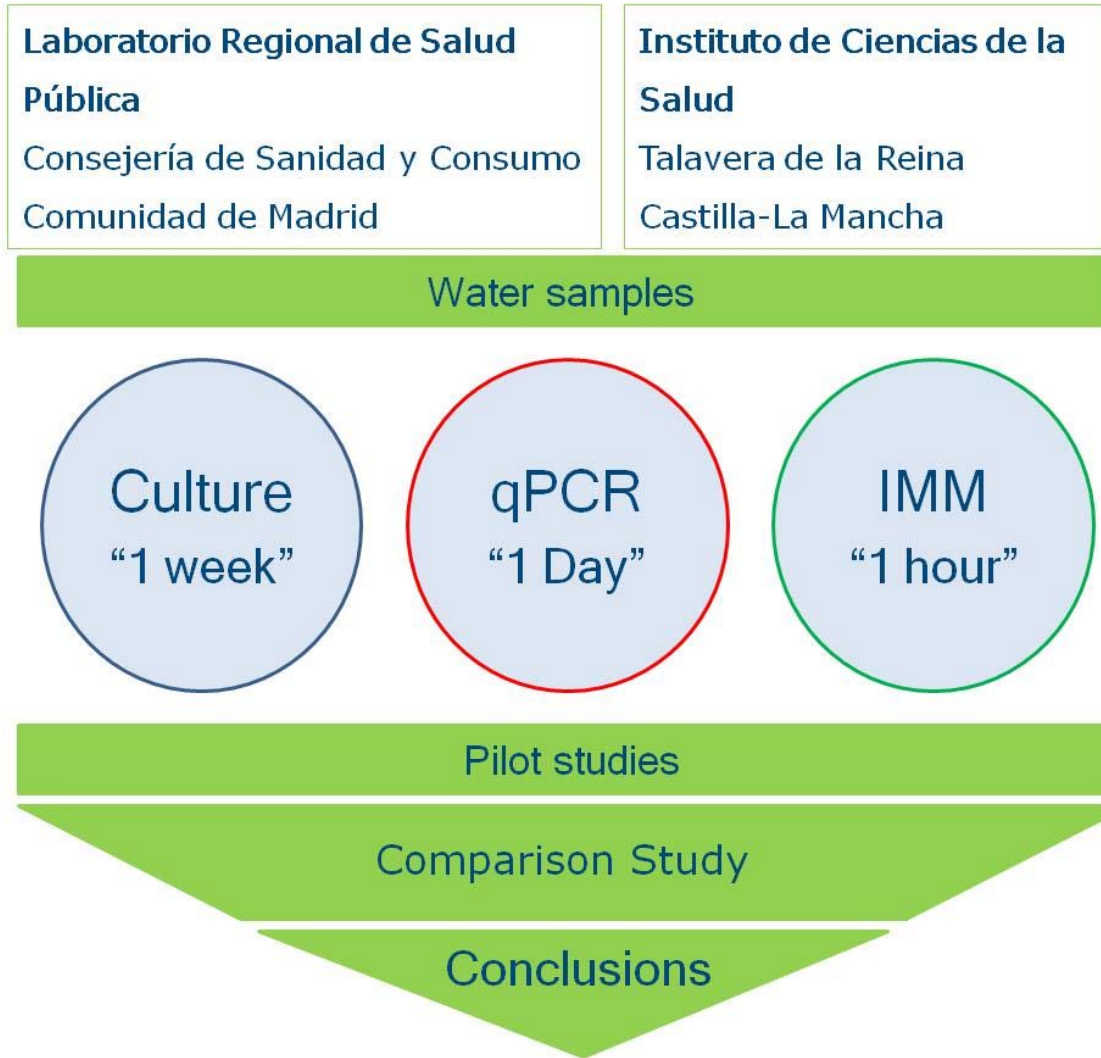
- IMM utilises magnetic immuno-beads to capture *Legionella* achieving purification & concentration at the same time.



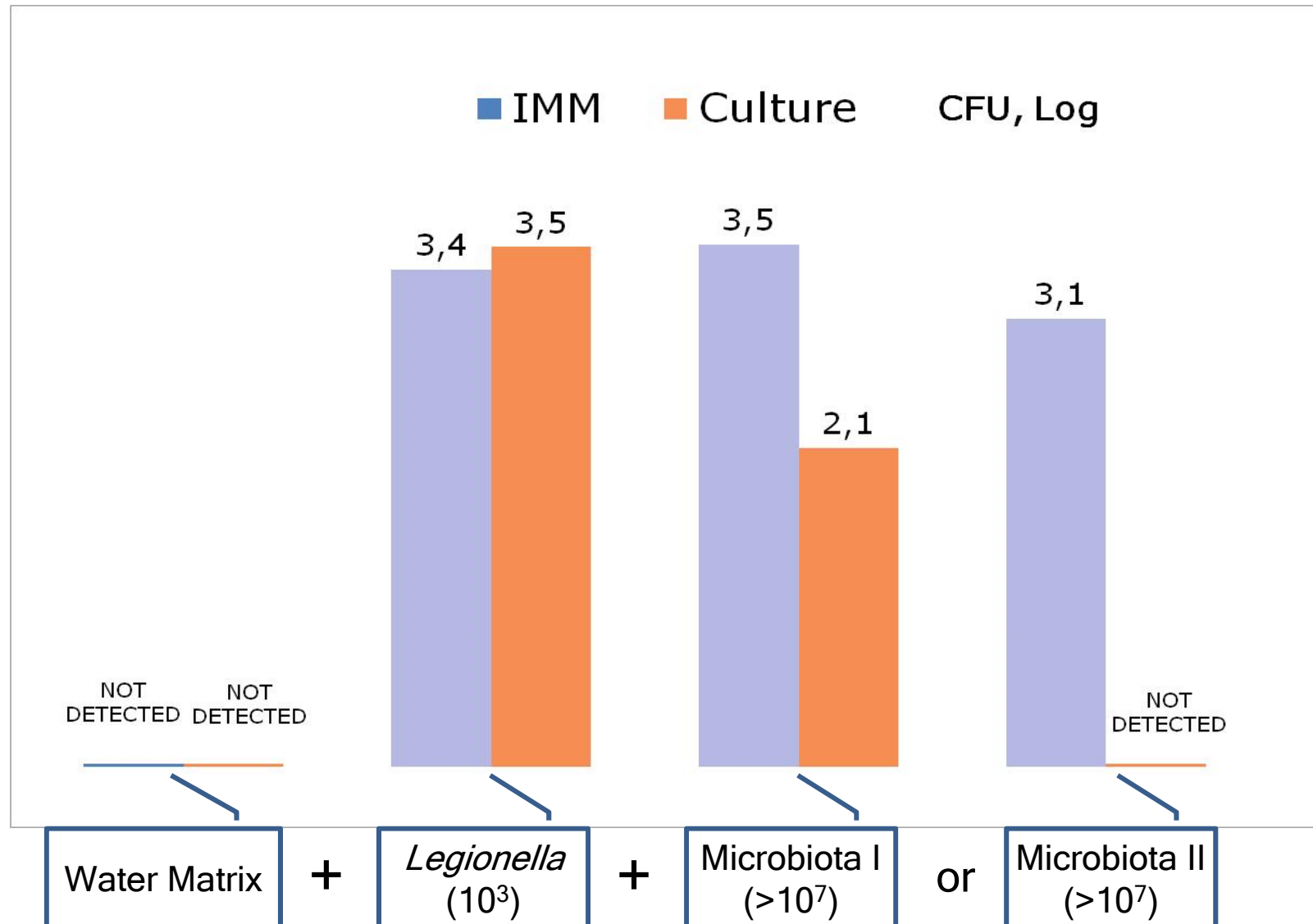
legipid[®]
LEGIONELLA FAST DETECTION

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER **111101**

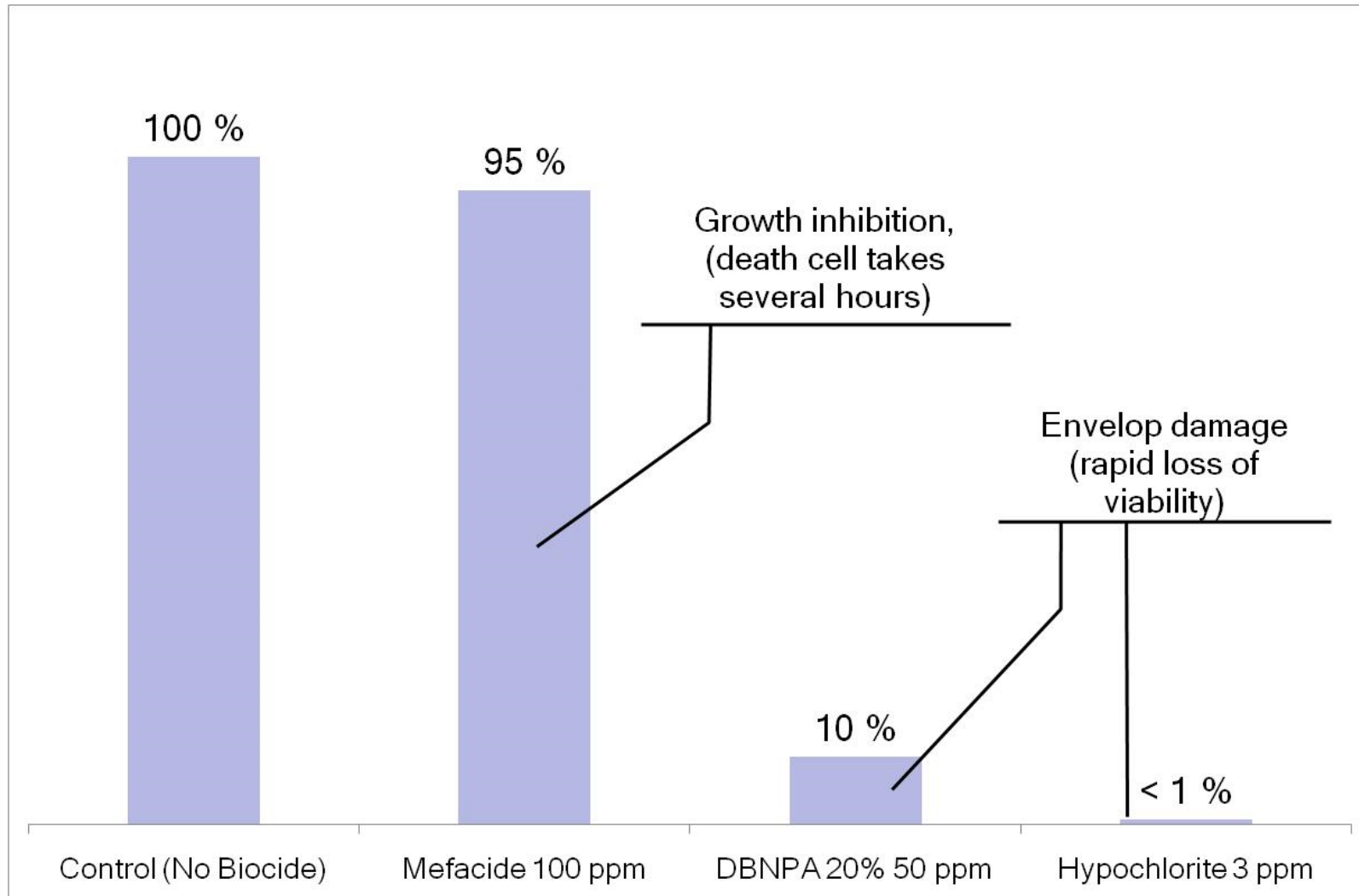
Laboratories evaluated the IMM by comparison with culture and qPCR



💧 Culture but not IMM lost viable *Legionella* when microbiota was added

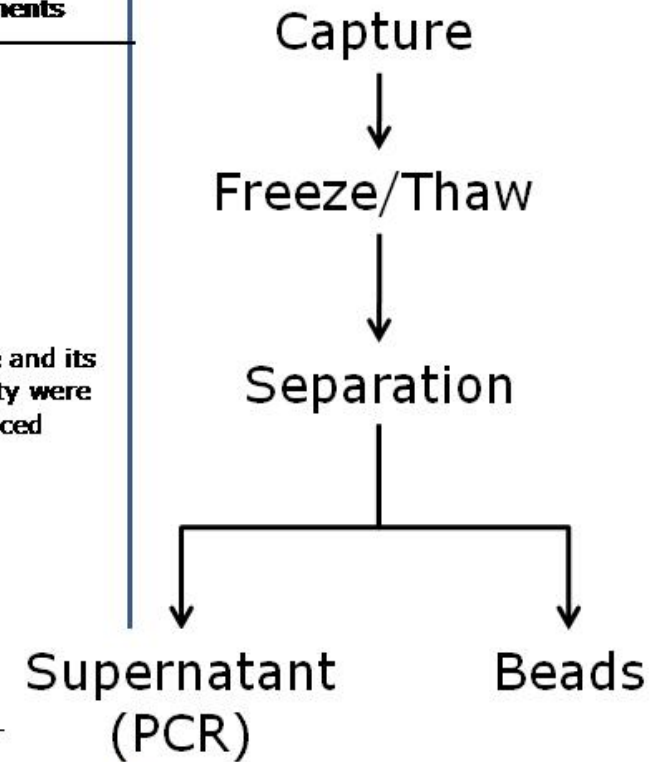


- IMM signal was reduced by the effect of biocides on *Legionella*



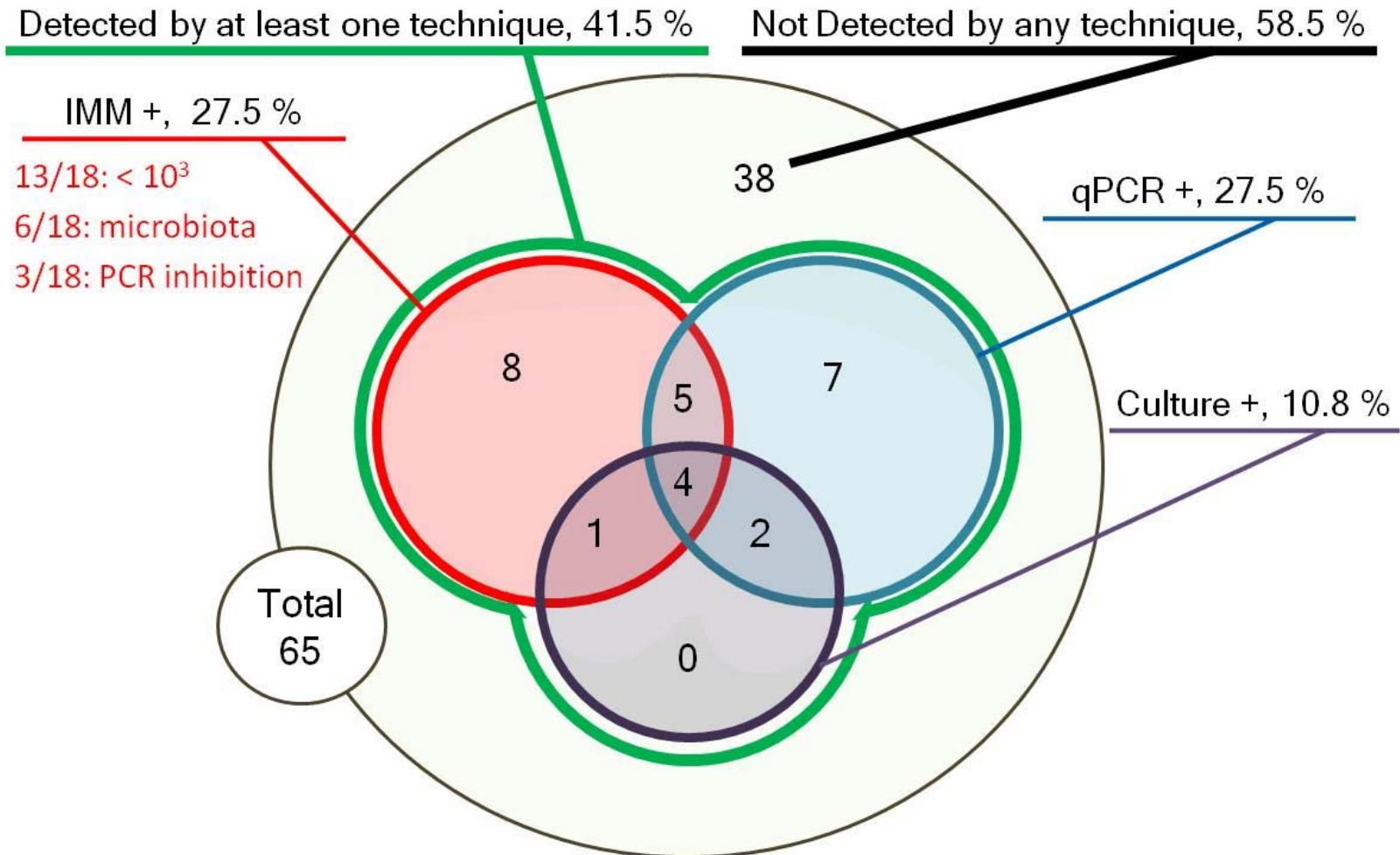
💧 Immunomagnetic separation could be useful to reduce inconclusive results by PCR (ongoing studies)

Sample no.	Replicate no.	PCR	IMS + PCR	Comments
1	1	Positive	Positive	
	2	Positive	Positive	
	3	Positive	Positive	
	4	Positive	Positive	
2	1	Inhibition	Positive	Cq value and its variability were reduced
	2	Negative	Positive	
	3	Positive	Positive	
	4	Inhibition	Positive	
3	1	Positive	Positive	
	2	Inhibition	Positive	
4	1	Inhibition	Negative	
	2	Negative	Negative	



Results from a comparative study.

Non-growth based methods achieved higher detection rate than culture method.



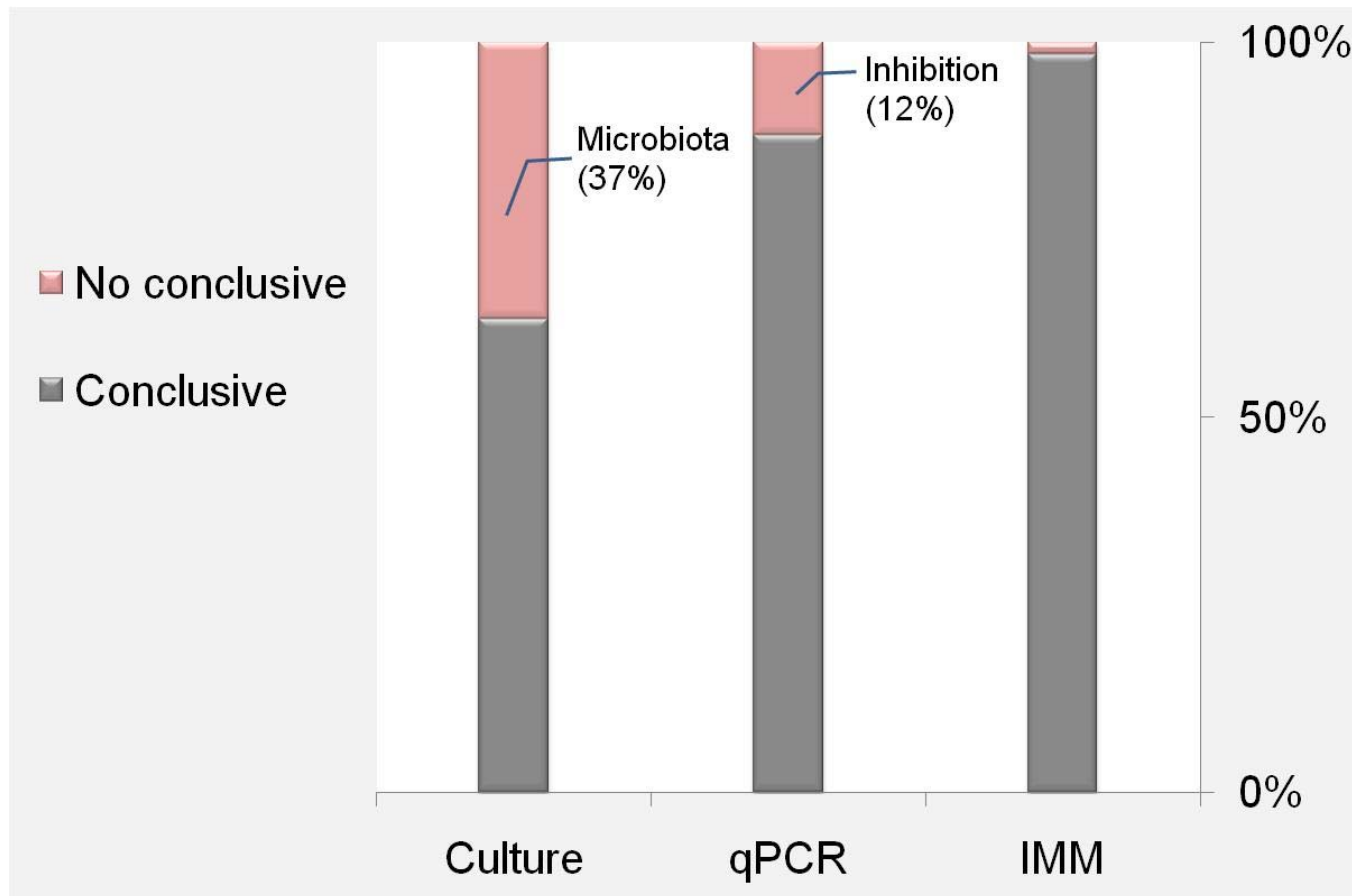
- 💧 Action and alert levels for IMM were comparable to those derived from the culture (coincidence 94%)

		IMM- Legipid no.			Total
		Action $\geq 10^4$ CFU l ⁻¹	Alert $\geq 10^3$ CFU l ⁻¹	Satisfactory $< 10^3$ CFU l ⁻¹	
Legionella spp					
culture no. (*)	Action $\geq 10^4$ CFU l ⁻¹	0	0	0	0
	Alert $\geq 10^3$ CFU l ⁻¹	0	2	1	3
	Satisfactory $< 10^3$ CFU l ⁻¹	1	2	59	62
Total		1	4	60	65

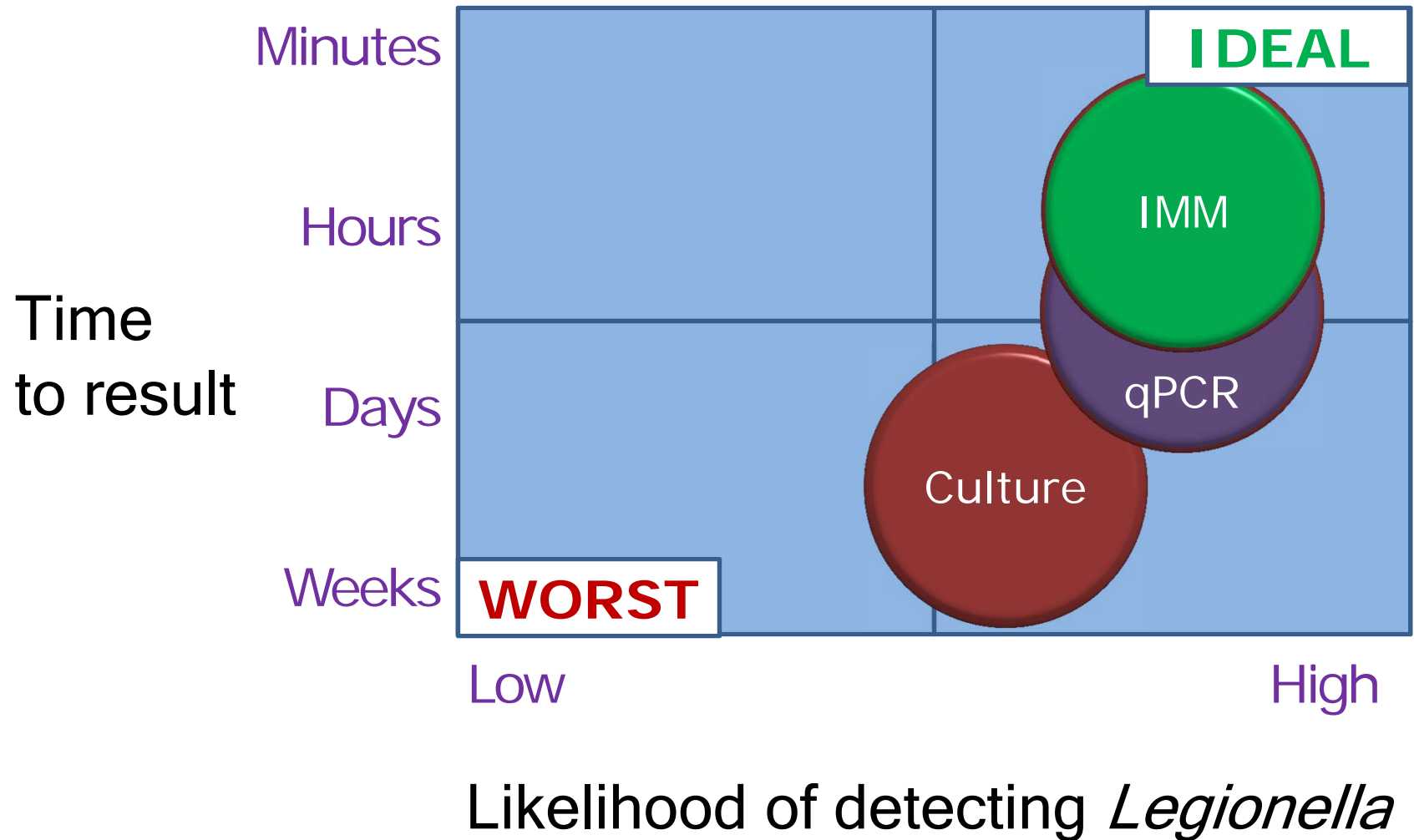
(*) There is not an accepted correspondence between PCR and culture

💧 Purification of *Legionella* allowed to deliver even more profitable information

The proportion of no conclusive results decreased by eliminating or minimizing interfering agents over detection



- IMM could be a complementary tool for the intended use of prevention.



- Summary: *Legionella*-binding magnetic beads were useful for rapid testing in environmental samples
 - Enhances consistency of the results by minimizing the background flora/inhibitors
 - Focus on viability because capture event depends on the integrity of cell envelop
 - Reduces time to result
 - Cost effective and simple to use



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