

## SUPPLEMENTARY MATERIAL

**Table S1. Non-*Legionella* strains used for validation of specificity of the dPCRgyrA-T831 assay**

Strain	Origin
<i>Acinetobacter baumannii</i>	ATCC 19606
<i>Corynebacterium jeikeium</i>	CIP 8251
<i>Enterobacter cloacae</i>	ATCC 13047
<i>Enterococcus faecium</i>	CIP 5432
<i>Escherichia coli</i>	ATCC 25922
<i>Haemophilus influenzae</i>	ATCC 49766
<i>Klebsiella pneumoniae</i>	ATCC 35657
<i>Moraxella catharalis</i>	Clinical isolate
<i>Neisseria elongata</i>	Clinical isolate
<i>Proteus vulgaris</i>	ATCC 6380
<i>Pseudomonas aeruginosa</i>	CIP 5933
<i>Staphylococcus aureus</i>	ATCC 25923
<i>Staphylococcus epidermidis</i>	CIP 103627
<i>Stenotrophomonas maltophilia</i>	ATCC 17666
<i>Streptococcus agalactiae</i>	ATCC 12400
<i>Streptococcus mitis</i>	CIP 103335
<i>Streptococcus oralis</i>	Clinical isolate
<i>Streptococcus pneumoniae</i>	ATCC 6303
<i>Streptococcus pyogenes</i>	CIP 104226
<i>Streptococcus salivarius</i>	Clinical isolate

**Table S2. *Legionella non-pneumophila* strains used for validation of specificity of the dPCRgyrA-T83I assay**

<b>Strain</b>	<b>Origin</b>	<b>Provided by</b>
<i>L. anisa</i>	ATCC 35290	NRC for <i>Legionella</i> (Lyon, France)
<i>L. birminghamensis</i>	ATCC 73702	NRC for <i>Legionella</i> (Lyon, France)
<i>L. bozemanii</i>	ATCC 33217	NRC for <i>Legionella</i> (Lyon, France)
<i>L. cincinnatiensis</i>	ATCC 43753	NRC for <i>Legionella</i> (Lyon, France)
<i>L. dumoffii</i>	ATCC 35280	NRC for <i>Legionella</i> (Lyon, France)
<i>L. erythra</i>	ATCC 35303	NRC for <i>Legionella</i> (Lyon, France)
<i>L. feeleii</i>	ATCC 35072	NRC for <i>Legionella</i> (Lyon, France)
<i>L. londiniensis</i>	ATCC 49505	NRC for <i>Legionella</i> (Lyon, France)
<i>L. longbeachae</i>	ATCC 33462	NRC for <i>Legionella</i> (Lyon, France)
<i>L. maceachernii</i>	ATCC 35300	NRC for <i>Legionella</i> (Lyon, France)
<i>L. micdadei</i>	ATCC 33218	NRC for <i>Legionella</i> (Lyon, France)
<i>L. oakridgensis</i>	ATCC 33761	NRC for <i>Legionella</i> (Lyon, France)
<i>L. parisiensis</i>	ATCC 35299	NRC for <i>Legionella</i> (Lyon, France)
<i>L. quinlivanii</i>	ATCC 43830	NRC for <i>Legionella</i> (Lyon, France)
<i>L. rubrilucens</i>	ATCC 35304	NRC for <i>Legionella</i> (Lyon, France)
<i>L. sainthelensi</i>	ATCC 35248	NRC for <i>Legionella</i> (Lyon, France)
<i>L. tucsonensis</i>	ATCC 49180	NRC for <i>Legionella</i> (Lyon, France)
<i>L. wadsworthii</i>	ATCC 33877	NRC for <i>Legionella</i> (Lyon, France)
<i>L. worслиensis</i>	ATCC 49508	NRC for <i>Legionella</i> (Lyon, France)

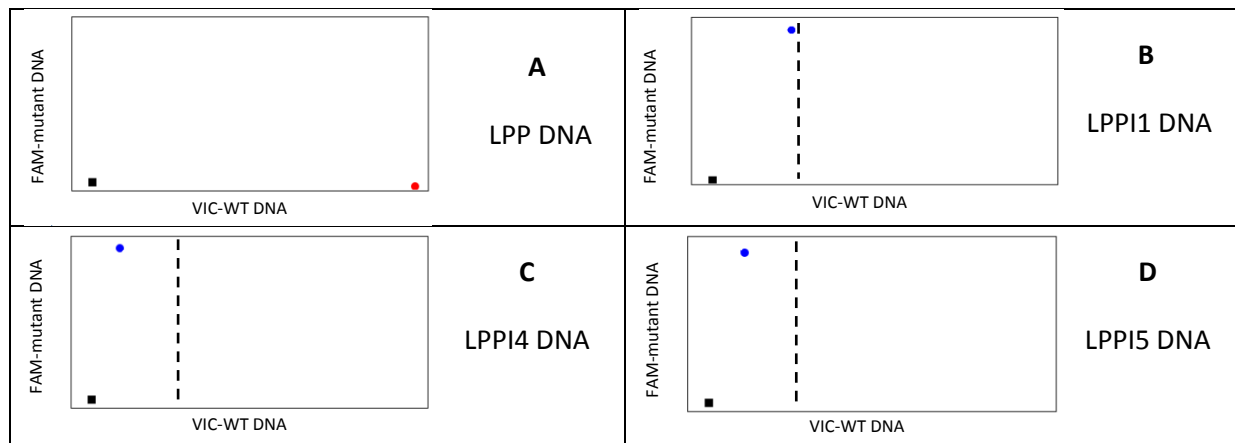
**Table S3. Validation of the dPCRgyrA assays**

Results are represented as number of mutant and wild copies/ $\mu\text{L}$  for each reaction. Numbers in italics represent cases where background noise was observed.

<b>dPCRgyrA assay</b>	<b>DNA sample</b>	<b>Wild copies/<math>\mu\text{L}</math> (VIC)</b>	<b>Mutant copies/<math>\mu\text{L}</math> (FAM)</b>
dPCRgyrA-T83I assay	LPP	1129.90	<i>0.43</i>
	LPPI1	0	1223.00
	LPPI4	0	756.74
	LPPI5	0	734.44
	NTC	0	0
dPCRgyrA-D87N assay	LPP	969.36	0
	LPPI1	993.55	<i>0.69</i>
	LPPI4	<i>0.07</i>	731.17
	LPPI5	<i>0.20</i>	<i>0.07</i>
	NTC	0	0
dPCRgyrA-D87H assay	LPP	1052.20	<i>0.43</i>
	LPPI1	1135.90	<i>0.68</i>
	LPPI4	<i>1.27</i>	0
	LPPI5	<i>0.29</i>	775.44
	NTC	0	0

**Table S4. Linearity and limit of detection of the dPCRgyrA-T83I assay compared to *Legionella* sp. qPCR16S and *L. pneumophila* qPCRmip**

<b>Concentration (genome copies/<math>\mu</math>L) of <i>L. pneumophila</i> DNA as determined by NanoDrop® spectrophotometer</b>	<b><i>Legionella</i> sp. qPCR16S Cq</b>	<b><i>L. pneumophila</i> qPCRmip Cq</b>	<b>Concentration (genome copies/<math>\mu</math>L) of <i>L. pneumophila</i> DNA as determined by dPCRgyrA-T83I assay</b>
19000	20.76	21.88	3790.2
1900	24.04	25.83	305.24
190	28.13	29.89	33.79
19	31.11	33.10	4.07
1.9	35.88	36.76	0.49

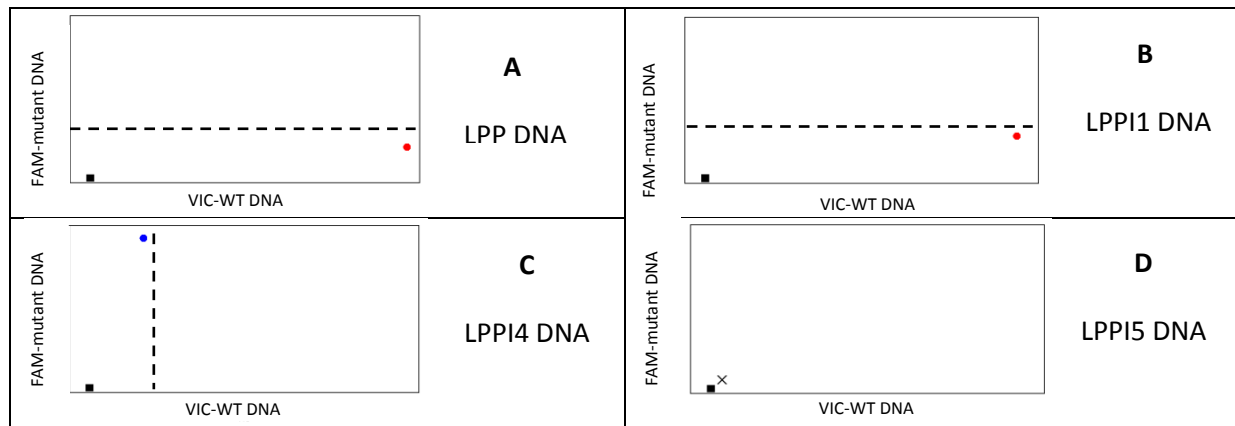


**Figure S1. Validation of the qPCRgyrA-T83I assay**

Graphical representation of StepOne. The StepOne software plots the normalized intensities of the reporters in each sample well on an allelic discrimination graph. Blue points correspond to the detection of mutant DNA. Red point corresponds to wild-type DNA. Black squares are the NTC. Dotted line represents adjusted thresholds.

A) LPP DNA detection with the qPCRgyrA-T83I assay. B) LPP11 (*gyrA*<sup>83</sup> (T83I)) DNA detection with the qPCRgyrA-T83I assay. C) LPP14 (*gyrA*<sup>83</sup> (T83I) + *gyrA*<sup>87</sup> (D87N)) DNA detection with the qPCRgyrA-T83I assay. D) LPP15 (*gyrA*<sup>83</sup> (T83I) + *gyrA*<sup>87\*</sup> (D87H)) DNA detection with the qPCRgyrA-T83I assay.

Mutant probe was highly specific; no FAM fluorescence was detected for the LPP strain. Wild-type probe exhibited a slight cross-reactivity; a slight VIC fluorescence was detected with LPP11, LPP14, and LPP15 DNA. This was circumvented by adjustment of the thresholds.

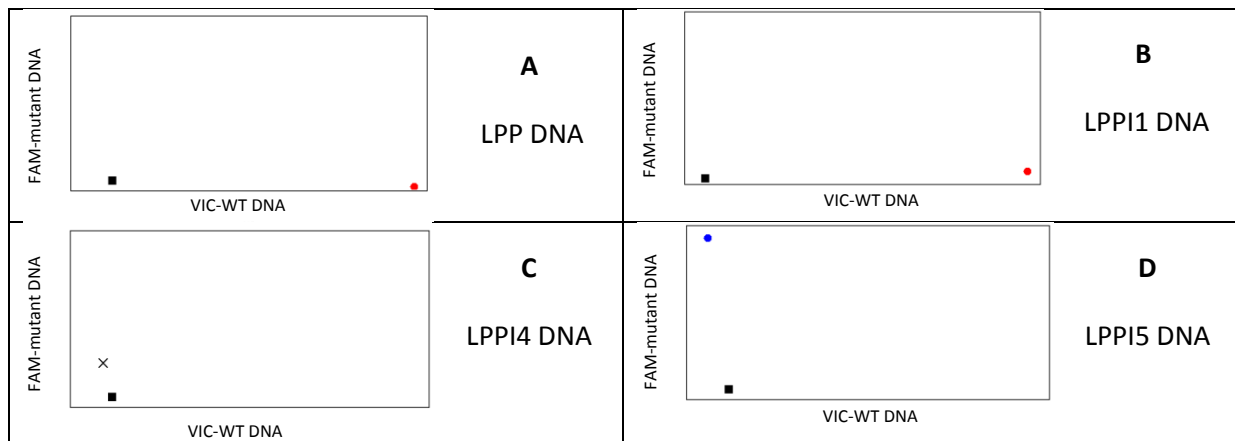


**Figure S2. Validation of the qPCRgyrA-D87N assay**

Blue point corresponds to mutant DNA. Red points correspond to wild-type DNA. Black squares are the NTC. Cross corresponds to undetermined genotype. Dotted line represents adjusted thresholds.

A) LPP DNA detection with the qPCRgyrA-D87N assay. B) LPP11 ( $gyrA^{83}$  (T83I)) DNA detection with the qPCRgyrA-D87N assay. C) LPP14 ( $gyrA^{83}$  (T83I) +  $gyrA^{87}$  (D87N)) DNA detection with the qPCRgyrA-D87N assay. D) LPP15 ( $gyrA^{83}$  (T83I) +  $gyrA^{87*}$  (D87H)) DNA detection with the qPCRgyrA-D87N assay.

Wild-type probe and mutant probe of the qPCRgyrA-D87N assay exhibited slight cross-reactivity circumvented by adjustment of the thresholds. The qPCRgyrA-D87N assay did not show cross-reactivity with the  $gyrA^{87*}$  (D87H) mutation.



**Figure S3. Validation of the qPCR $_{gyrA-D87H}$  assay**

Blue point corresponds to mutant DNA. Red points correspond to wild-type DNA. Black squares are the NTC. Cross corresponds to undetermined genotype.

A) LPP DNA detection with the qPCR $_{gyrA-D87H}$  assay. B) LPP11 ( $gyrA^{83}$  (T83I)) DNA detection with the qPCR $_{gyrA-D87H}$  assay. C) LPP14 ( $gyrA^{83}$  (T83I) +  $gyrA^{87}$  (D87N)) DNA detection with the qPCR $_{gyrA-D87H}$  assay. D) LPP15 ( $gyrA^{83}$  (T83I) +  $gyrA^{87*}$  (D87H)) DNA detection with the qPCR $_{gyrA-D87H}$  assay. The qPCR $_{gyrA-D87H}$  assay was highly specific.