XP-Design Assay *L. pneumophila* SG1 - Technical Report

May 2025

This Technical Report, specific to the XP-Design Assay *L. pneumophila* SG1, is intended to be used as a complement to the general XP-Design Assay User Guide (document #10000241371).

Assay Information

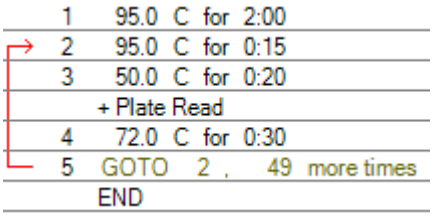
Assay Name	XP-Design Assay *L. pneumophila* SG1
Assay Type	TaqMan
Catalog Number	qFSD74624492
Assay Description	The XP-Design Assay *L. pneumophila* SG1 is a ready-to-use primer and probe solution for the detection of a specific DNA sequence unique to *Legionella pneumophila* SG1, in isolated colonies or in water samples.
Results Interpretation	Refer to the iQ-Check *Legionella* User Guide (document #10000134675): • Isolated bacterial colonies: see Section 9 • Water samples: see Section 7.D

Scope of Use

Products used to generate technical data:

Real-Time PCR Primer-Probe Mix	XP-Design Assay *L. pneumophila* SG1
Real-Time PCR Supermix	iQ Multiplex Powermix (catalog #17007081)
Real-Time PCR Instrument	CFX Opus Deepwell Real-Time PCR System
Experimental Samples	• Isolated *Legionella* colonies • Water samples: potable water, non-potable water (swimming pool), cooling tower water

Protocol used for the technical study:
(refer to the XP-Design Assay User Guide for detailed information on the protocol)

Sample Preparation	• Isolated bacterial colonies: see Section 9 of the iQ-Check *Legionella* User Guide • Water samples: prepare DNA extract with the Aquadien DNA Extraction and Purification Kit (catalog #3578121)
Fluorophore	FAM
Thermal Cycler Protocol	 See Section 7.B of the iQ-Check *Legionella* User Guide for detailed instructions on setting up the thermal cycler protocol.

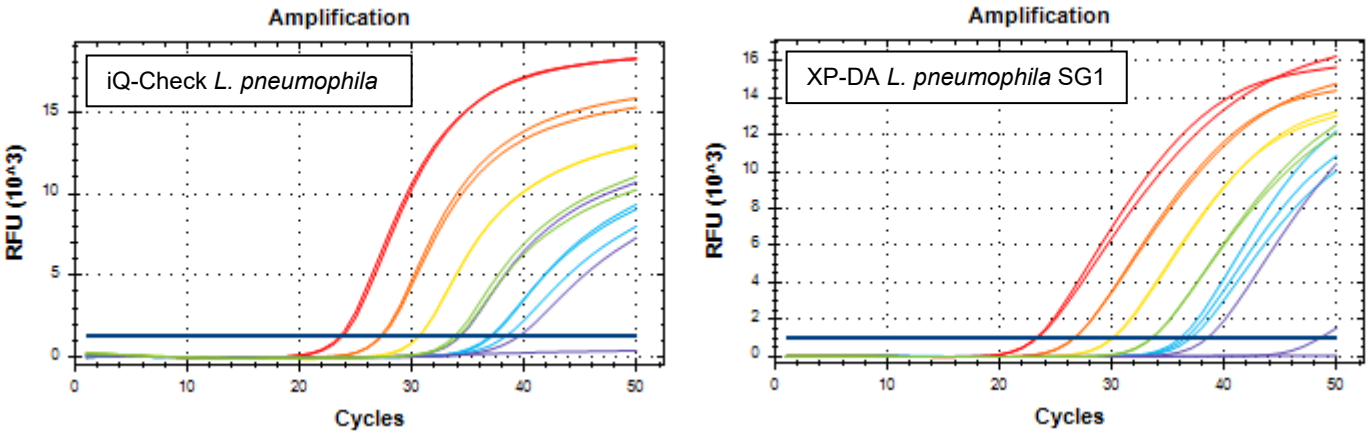
Experimental Testing of the XP-Design Assay *L. pneumophila* SG1

Specificity	The specificity of the XP-Design Assay *L. pneumophila* SG1 was verified on a collection of 57 strains, consisting of: - 25 *L. pneumophila* SG1 strains - 14 *L. pneumophila* non-SG1 strains, representing all serogroups 2–15 - 5 *Legionella* non-*pneumophila* strains (*L. anisa*, *L. bozemanii*, *L. dumoffii*, *L. feeleii*, and *L. micdadei*) - 13 non-*Legionella* strains from various bacterial species (*Aeromonas hydrophila*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Burkholderia cepacia*, *Clostridium perfringens*, *Escherichia coli*, *Flavobacterium johnsoniae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus vulgaris*, *Serratia marcescens*, and *Stenotrophomonas maltophilia*)
Limit of Detection	Similar to iQ-Check *Legionella pneumophila*
Efficiency	99% Assay efficiency was determined using a five-point standard curve generated from 10-fold serial dilutions of a target DNA. An efficiency of 100% represents a perfect doubling of template at every cycle. Acceptance criterion was 90-110%, the typical range of good assay efficiency
R^2	0.9977 The R^2 represents the linearity of the standard curve and how well the standard curve data points fit the linear regression line. Acceptable values were > 0.99

Robustness to Inhibition	Artificially spiked water samples were used to verify that target detection was not impeded by matrix inhibition. Matrices used: potable water, non-potable water (swimming pool), cooling tower water. Certain matrices, for example some cooling tower water samples, may contain PCR inhibitors. Matrix performance verification (see Section 6 of the iQ-Check *Legionella* User Guide) or addition of an internal amplification control (IC) as described below is recommended prior to analysis.

Experimental results obtained with the XP-Design Assay *L. pneumophila* SG1:

Amplification curves of a DNA extract from *L. pneumophila* strain ATCC 33152, serially diluted in H_2O, tested in parallel with iQ-Check *Legionella pneumophila* and with XP-Design Assay *L. pneumophila* SG1.



The following table shows the Cq values obtained for each concentration level of target:
(N/A: no amplification detected)

		Cq Values Obtained					
		iQ-Check *L. pneumophila*			XP-DA *L. pneumophila* SG1		
	Dilution Factor	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
	1	23.81	23.54	Not tested	23.33	23.21	Not tested
	10	27.30	27.19	Not tested	26.57	26.64	Not tested
	100	30.60	30.55	Not tested	30.01	30.12	Not tested
	1E+03	34.14	33.76	Not tested	33.56	33.48	Not tested
	1E+04	36.97	38.34	37.12	36.50	36.09	37.03
	1E+05	N/A	39.50	34.29	48.51	38.46	N/A

Addition of the Universal Exogenous qPCR Positive Control (IC)

The Universal Exogenous qPCR Positive Control can be added to the XP-Design Assay *L. pneumophila* SG1 test to serve as a PCR internal control.

Assay Name	Universal Exogenous qPCR Positive Control for TaqMan Assays (Cy5-QXL670 Probe) Kit
Assay Type	TaqMan
Fluorophore	Cy5
Catalog Number	RT-IPCC-Q02 (Eurogentec)
Assay Description	The Universal Exogenous qPCR Positive Control for TaqMan Assays (Cy5-QXL670 Probe) Kit contains: - a DNA template (IPC DNA) - a ready-to-use primer and probe solution for the amplification and detection of this DNA template (IPC mix) It is used to monitor PCR inhibition.

To use the Universal Exogenous qPCR Positive Control as a PCR internal control for the XP- Design Assay *L. pneumophila* SG1 assay, supplement the XP-Design Assay *L. pneumophila* SG1 PCR mix with 0.75 µl IPC mix + 0.25 µl IPC DNA per test. Refer to the following PCR Mix Calculation Guide to prepare the required volume of PCR master mix containing the XP-Design Assay *L. pneumophila* SG1 reagent, iQ Multiplex Powermix, IPC mix and IPC DNA. Use **21 µl** of this master mix per test (instead of 20 µl when no IC is added to the reaction).

PCR Mix Calculation Guide for tests carried out with IC

Total number of samples and controls	XP-Design Assay (µl)	iQ Multiplex Powermix (µl)	IPC mix (µl)	IPC DNA (µl)
1	7.5	12.5	0.75	0.25
2	18	30	1.8	0.6
3	24	40	2.4	0.8
4	33	55	3.3	1.1
5	39	65	3.9	1.3
6	48	80	4.8	1.6
7	57	95	5.7	1.9
8	63	105	6.3	2.1
9	72	120	7.2	2.4
10	78	130	7.8	2.6
11	87	145	8.7	2.9
12	96	160	9.6	3.2
13	102	170	10.2	3.4
14	111	185	11.1	3.7
15	117	195	11.7	3.9
16	126	210	12.6	4.2
17	135	225	13.5	4.5
18	141	235	14.1	4.7
19	150	250	15.0	5.0
20	159	265	15.9	5.3
21	165	275	16.5	5.5
22	174	290	17.4	5.8
23	180	300	18.0	6.0
24	189	315	18.9	6.3
25	198	330	19.8	6.6
26	204	340	20.4	6.8
27	213	355	21.3	7.1
28	222	370	22.2	7.4
29	228	380	22.8	7.6
30	237	395	23.7	7.9
31	246	410	24.6	8.2
32	252	420	25.2	8.4
33	261	435	26.1	8.7
34	270	450	27.0	9.0
35	276	460	27.6	9.2
36	285	475	28.5	9.5
37	294	490	29.4	9.8
38	300	500	30.0	10.0
39	309	515	30.9	10.3
40	318	530	31.8	10.6
41	324	540	32.4	10.8
42	333	555	33.3	11.1
43	339	565	33.9	11.3
44	348	580	34.8	11.6
45	357	595	35.7	11.9
46	363	605	36.3	12.1
47	372	620	37.2	12.4
48	381	635	38.1	12.7
49	387	645	38.7	12.9

Total number of samples and controls	XP-Design Assay (µl)	iQ Multiplex Powermix (µl)	IPC mix (µl)	IPC DNA (µl)
50	396	660	39.6	13.2
51	405	675	40.5	13.5
52	411	685	41.1	13.7
53	420	700	42.0	14.0
54	429	715	42.9	14.3
55	435	725	43.5	14.5
56	444	740	44.4	14.8
57	453	755	45.3	15.1
58	459	765	45.9	15.3
59	468	780	46.8	15.6
60	477	795	47.7	15.9
61	483	805	48.3	16.1
62	492	820	49.2	16.4
63	498	830	49.8	16.6
64	507	845	50.7	16.9
65	516	860	51.6	17.2
66	522	870	52.2	17.4
67	531	885	53.1	17.7
68	540	900	54.0	18.0
69	546	910	54.6	18.2
70	555	925	55.5	18.5
71	564	940	56.4	18.8
72	570	950	57.0	19.0
73	579	965	57.9	19.3
74	588	980	58.8	19.6
75	594	990	59.4	19.8
76	603	1005	60.3	20.1
77	612	1020	61.2	20.4
78	618	1030	61.8	20.6
79	627	1045	62.7	20.9
80	636	1060	63.6	21.2
81	642	1070	64.2	21.4
82	651	1085	65.1	21.7
83	657	1095	65.7	21.9
84	666	1110	66.6	22.2
85	675	1125	67.5	22.5
86	681	1135	68.1	22.7
87	690	1150	69.0	23.0
88	699	1165	69.9	23.3
89	705	1175	70.5	23.5
90	714	1190	71.4	23.8
91	723	1205	72.3	24.1
92	729	1215	72.9	24.3
93	738	1230	73.8	24.6
94	747	1245	74.7	24.9
95	753	1255	75.3	25.1
96	762	1270	76.2	25.4

XP-Design Assays are not experimentally wet-lab validated.

They should be validated prior to use by the end-user.

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