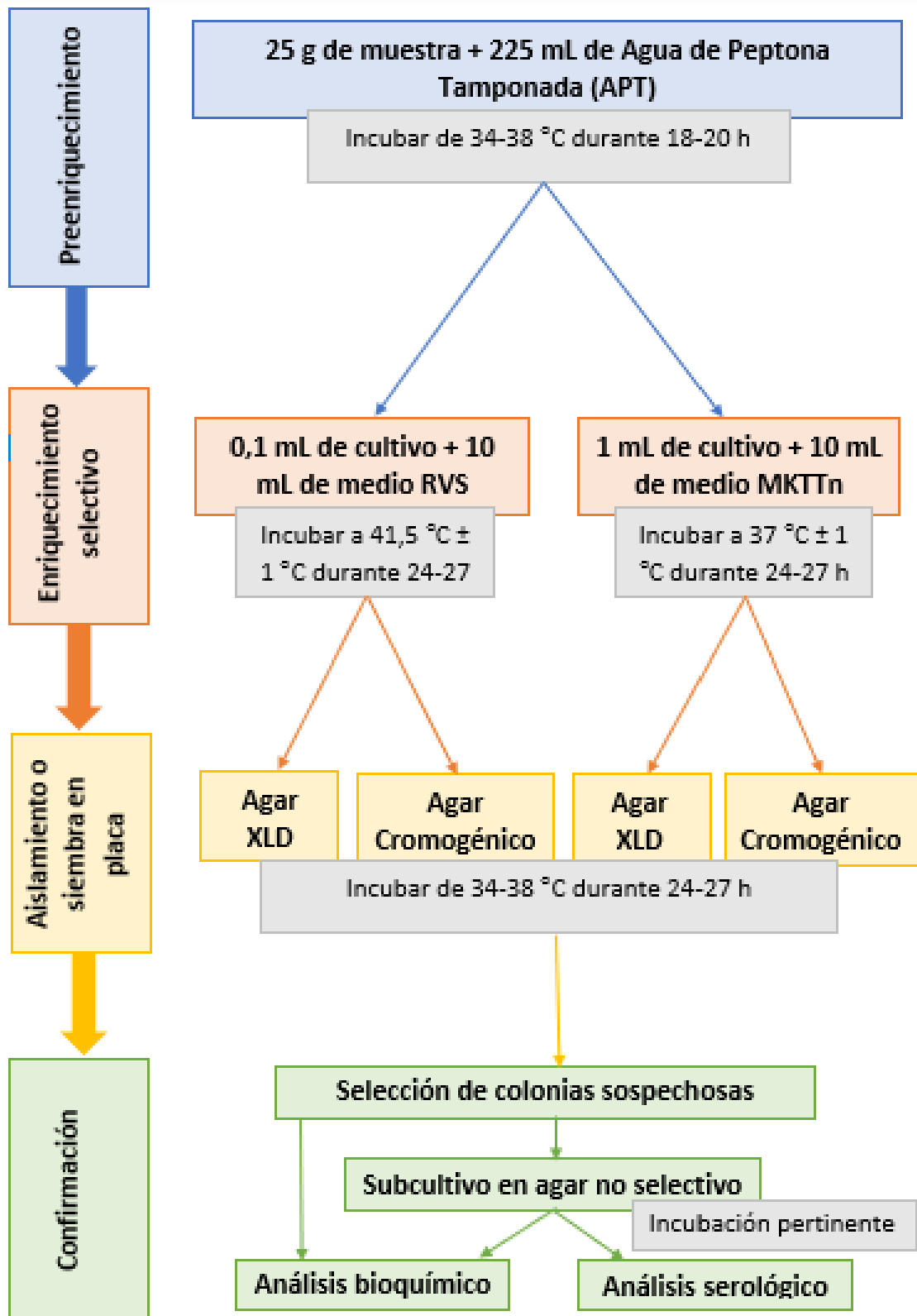
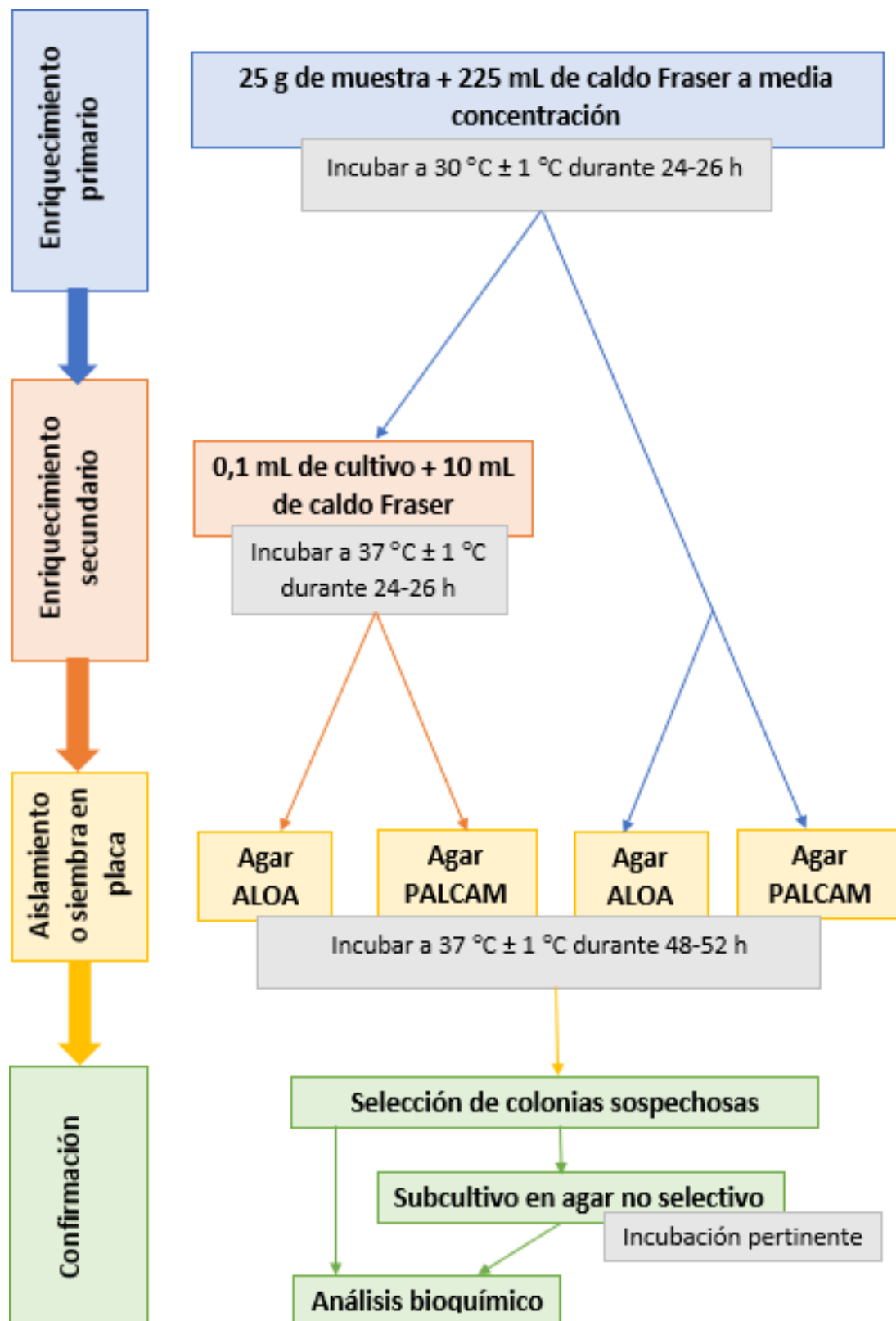


7. ANEXOS



Anexo 1: Diagrama del procedimiento de detección de *Salmonella* spp. en productos alimenticios para el consumo humano siguiendo la Norma UNE-EN ISO 6579-1:2017.



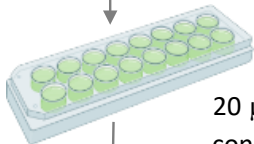
Anexo 2: Diagrama del procedimiento de detección de *Listeria monocytogenes* en productos alimenticios para el consumo humano siguiendo la Norma UNE-EN ISO 6579-1:2017.

Salmonella spp.

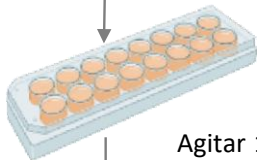
25 g de muestra + 225 mL de Agua de Peptona Tamponada (APT)

Incubar a 37 °C ± 1 °C durante 18-24 h

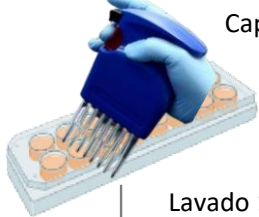
1 mL muestra/pocillo



20 µL reactivo concentración

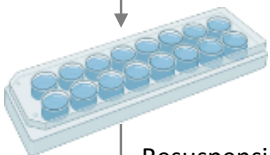


Agitar 10-20 min a 900 rpm

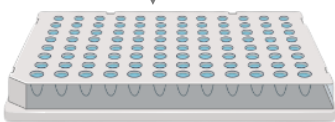


Captura 30 seg

Lavado 10 seg en 1 mL tampón de lavado



Resuspensión en 45 µL tampón resuspensión



Incorporar 30 µL a tubos de amplificación

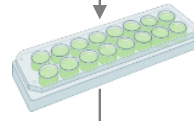


Listeria monocytogenes y *Listeria* spp.

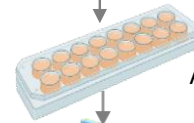
25 g de muestra + 225 mL de caldo Fraser a media concentración

Incubar a 30 °C durante 22-26 h

1 mL muestra/pocillo



20 µL reactivo concentración

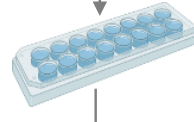


Agitar 10-20 min a 900 rpm

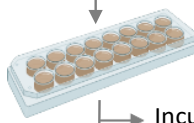


Captura 30 seg

Lavado 10 seg en 1 mL tampón de lavado



Resuspensión en 0,5 mL de caldo Fraser a media concentración



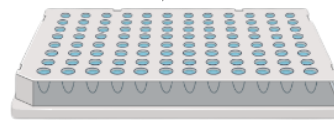
Incubar a 30 °C durante 4-6 h

Agitar 10-20 min a 900 rpm

Captura 30

Lavado 10 seg en 1 mL tampón de lavado

Resuspensión en 45 µL tampón resuspensión



Incubar a 60 °C durante 15-60 min

Incorporar 30 µL a tubos de amplificación



Anexo 3: Diagrama del procedimiento de detección de *Listeria monocytogenes*, *Listeria* spp. y *Salmonella* spp. en productos alimenticios para el consumo humano mediante el sistema comercial Assurance® GDS de la empresa Merck.

BIOCONTROL

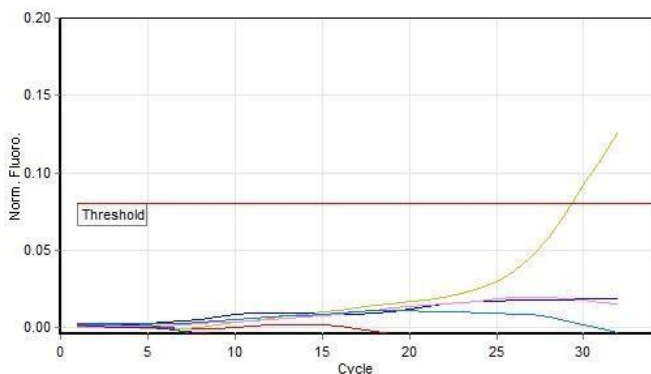
Assurance GDS Results Report

Run Information

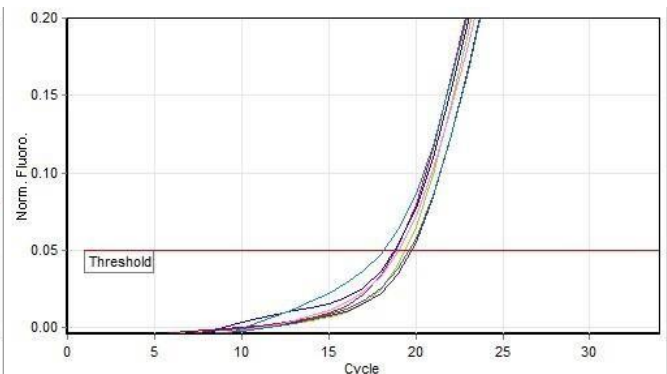
Run Name	BioControl Assay 36 Wells 2022-04-04 (1)
Run Start	04/04/2022 11:01:23 am
Run Finish	04/04/2022 12:26:39 pm
Operator	Assurance GDS User
Notes	SEGUNDA PRUEBA
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Salmonella*

Salmonella



Internal Control



No.	C	Name	<i>Salmonella</i>	<i>Salmonella</i> result	Ct	Assay	Kit
1	■	EF100	Negative	-		<i>Salmonella</i>	011321-02
2	■	EF101	Positive	+	29.35	<i>Salmonella</i>	011321-02
3	■	EF102	Negative	-		<i>Salmonella</i>	011321-02
4	■	EF92	Negative	-		<i>Salmonella</i>	011321-02
5	■	EF93	Negative	-		<i>Salmonella</i>	011321-02
6	■	EF94	Negative	-		<i>Salmonella</i>	011321-02
7	■	CONTROL NEGATIVO	Negative	-		<i>Salmonella</i>	011321-02

BIOCONTROL

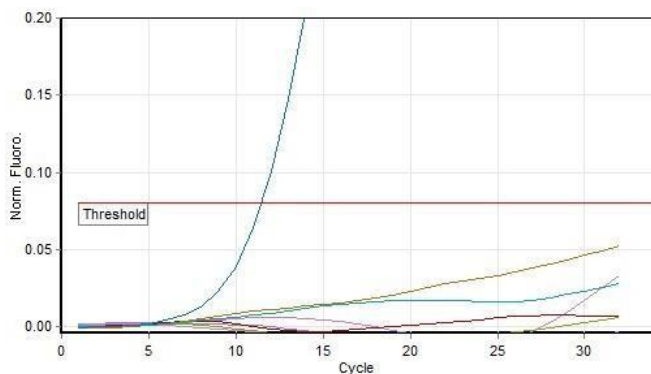
Assurance GDS Results Report

Run Information

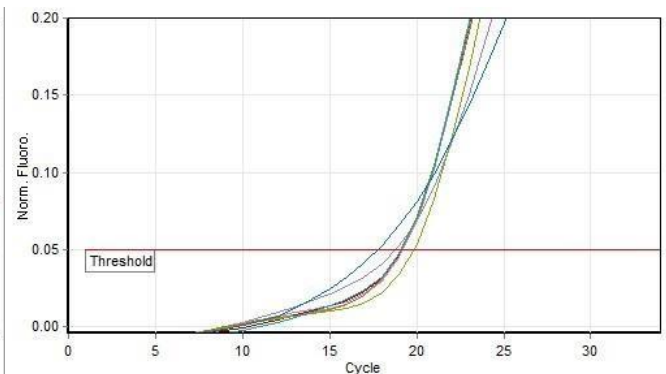
Run Name	BioControl Assay 36 Wells 2022-04-08 (1)
Run Start	08/04/2022 9:48:31 am
Run Finish	08/04/2022 11:14:56 am
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519









Assay: *Salmonella*

Salmonella



Internal Control



No.	C	Name	<i>Salmonella</i>	<i>Salmonella</i> result	Ct	Assay	Kit
17		63	Negative	-		<i>Salmonella</i>	011321-03
18		64	Negative	-		<i>Salmonella</i>	011321-03
19		65	Negative	-		<i>Salmonella</i>	011321-03
20		66	Negative	-		<i>Salmonella</i>	011321-03
21		67	Negative	-		<i>Salmonella</i>	011321-03
22		68	Negative	-		<i>Salmonella</i>	011321-03
23		Control -	Negative	-		<i>Salmonella</i>	011321-03
24		Control +	Positive	+	11.48	<i>Salmonella</i>	011321-03

BIOCONTROL

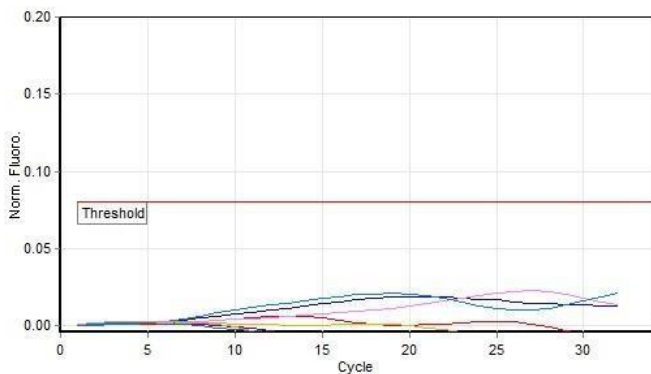
Assurance GDS Results Report

Run Information

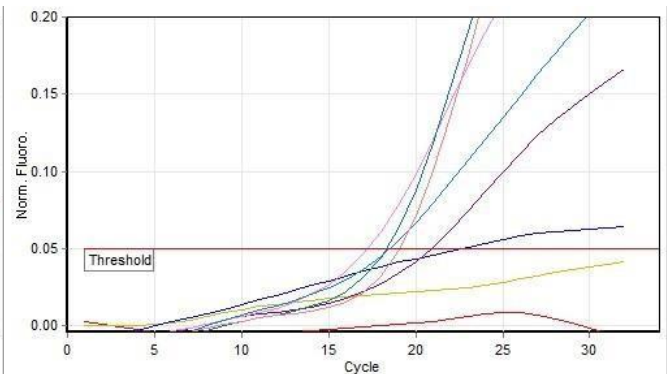
Run Name	BioControl Assay 36 Wells 2022-05-02 (1)
Run Start	02/05/2022 12:35:19 pm
Run Finish	02/05/2022 2:00:05 pm
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Salmonella*

Salmonella



Internal Control



No.	C	Name	Salmonella	Salmonella result	Ct	Assay	Kit
1		103	No Amp	-		Salmonella	011321-03
2		104	No Amp	-		Salmonella	011321-03
3		105	Negative	-		Salmonella	011321-03
4		80	Negative	-		Salmonella	011321-03
5		81	Negative	-		Salmonella	011321-03
6		82	Negative	-		Salmonella	011321-03
7		83	Negative	-		Salmonella	011321-03
8		84	Negative	-		Salmonella	011321-03

Las dos muestras no amplificadas (EF103 y 104) se volvieron a analizar en la siguiente qPCR.

BIOCONTROL

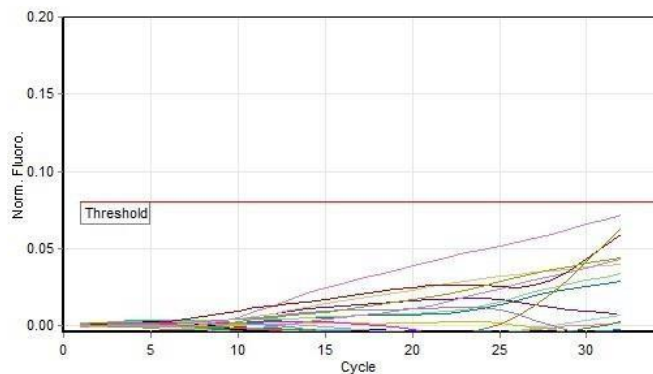
Assurance GDS Results Report

Run Information

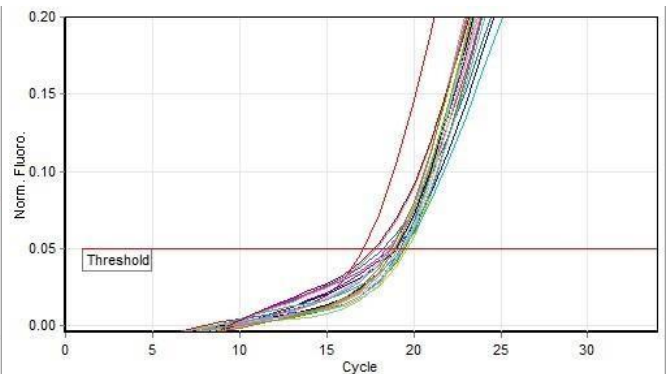
Run Name	BioControl Assay 36 Wells 2022-05-11 (1)
Run Start	11/05/2022 10:49:49 am
Run Finish	11/05/2022 12:16:18 pm
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Salmonella*

Salmonella















Internal Control



No.	C	Name	<i>Salmonella</i>	<i>Salmonella</i> result	Ct	Assay	Kit
1	■	77	Negative	-		<i>Salmonella</i>	011321-03
2	■	78	Negative	-		<i>Salmonella</i>	011321-03
3	■	79	Negative	-		<i>Salmonella</i>	011321-03
4	■	85	Negative	-		<i>Salmonella</i>	011321-03
5	■	86	Negative	-		<i>Salmonella</i>	011321-03
6	■	87	Negative	-		<i>Salmonella</i>	011321-03
7	■	88	Negative	-		<i>Salmonella</i>	011321-03
8	■	89	Negative	-		<i>Salmonella</i>	011321-03
9	■	90	Negative	-		<i>Salmonella</i>	011321-03
10	■	91	Negative	-		<i>Salmonella</i>	011321-03

Continued on next page...

11		95	Negative	-		<i>Salmonella</i>	011321-03
12		96	Negative	-		<i>Salmonella</i>	011321-03
13		97	Negative	-		<i>Salmonella</i>	011321-03
14		98	Negative	-		<i>Salmonella</i>	011321-03
15		99	Negative	-		<i>Salmonella</i>	011321-03
16		103	Negative	-		<i>Salmonella</i>	011321-03
17		104	Negative	-		<i>Salmonella</i>	011321-03
18		106	Negative	-		<i>Salmonella</i>	011321-03
19		107	Negative	-		<i>Salmonella</i>	011321-03
20		108	Negative	-		<i>Salmonella</i>	011321-03
21		109	Negative	-		<i>Salmonella</i>	011321-03
22		110	Negative	-		<i>Salmonella</i>	011321-03

BIOCONTROL

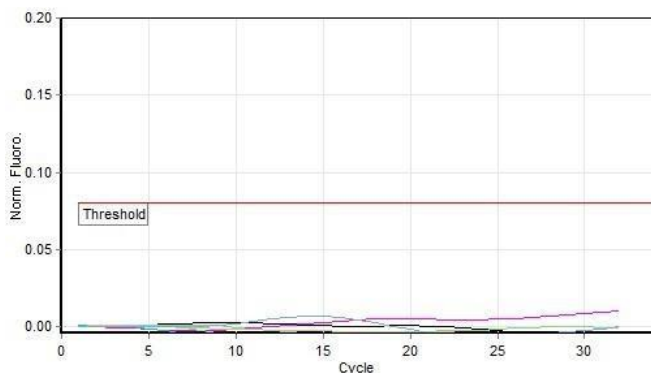
Assurance GDS Results Report

Run Information

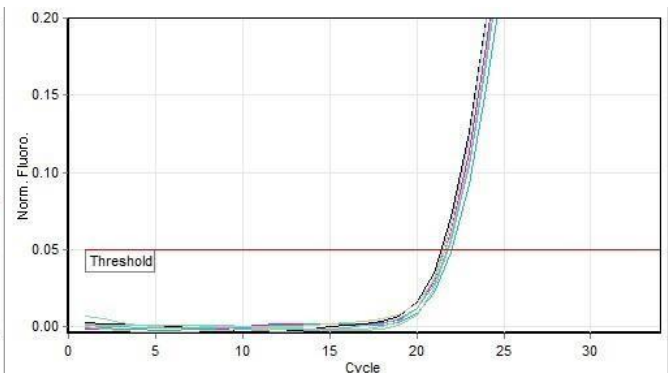
Run Name	BioControl Assay 36 Wells 2022-05-05 (1)
Run Start	05/05/2022 10:15:58 am
Run Finish	05/05/2022 11:41:48 am
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Listeria monocytogenes*

Listeria monocytogenes



Internal Control



No.	C	Name	<i>Listeria monocytogenes</i>	<i>L. mono.</i> result	Ct	Assay	Kit
10	■	95	Negative	-		<i>Listeria monocytogenes</i>	122719-04
11	■	96	Negative	-		<i>Listeria monocytogenes</i>	122719-04
12	■	97	Negative	-		<i>Listeria monocytogenes</i>	122719-04
13	■	98	Negative	-		<i>Listeria monocytogenes</i>	122719-04
14	■	99	Negative	-		<i>Listeria monocytogenes</i>	122719-04
15	■	106	Negative	-		<i>Listeria monocytogenes</i>	122719-04
16	■	107	Negative	-		<i>Listeria monocytogenes</i>	122719-04

BIOCONTROL

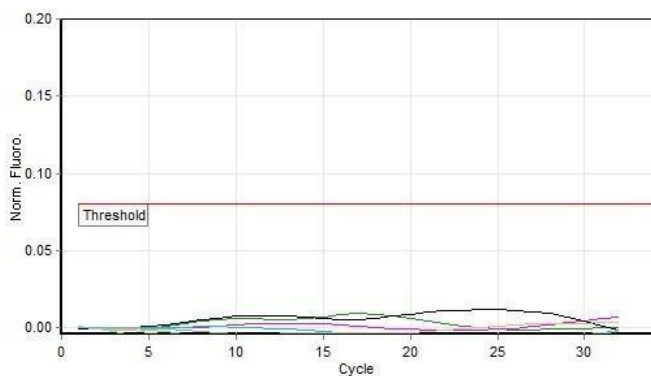
Assurance GDS Results Report

Run Information

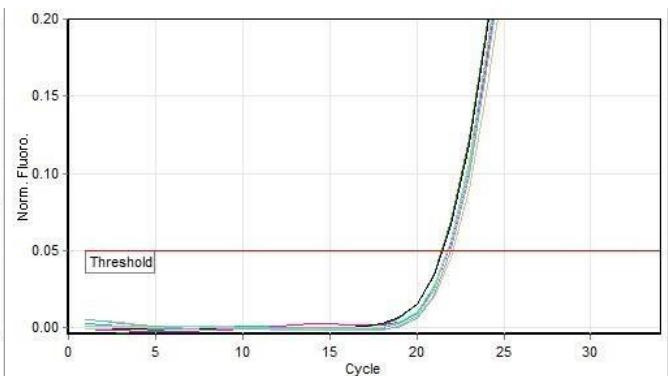
Run Name	BioControl Assay 36 Wells 2022-05-12 (1)
Run Start	12/05/2022 10:33:31 am
Run Finish	12/05/2022 11:59:24 am
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Listeria monocytogenes*

Listeria monocytogenes



Internal Control



No.	C	Name	<i>Listeria monocytogenes</i>	<i>L. mono.</i> result	Ct	Assay	Kit
9		86	Negative	-		<i>Listeria monocytogenes</i>	122719-04
10		89	Negative	-		<i>Listeria monocytogenes</i>	122719-04
11		92	Negative	-		<i>Listeria monocytogenes</i>	122719-04
12		96	Negative	-		<i>Listeria monocytogenes</i>	122719-04
13		100	Negative	-		<i>Listeria monocytogenes</i>	122719-04
14		101	Negative	-		<i>Listeria monocytogenes</i>	122719-04
15		103	Negative	-		<i>Listeria monocytogenes</i>	122719-04
16		105	Negative	-		<i>Listeria monocytogenes</i>	122719-04

BIOCONTROL

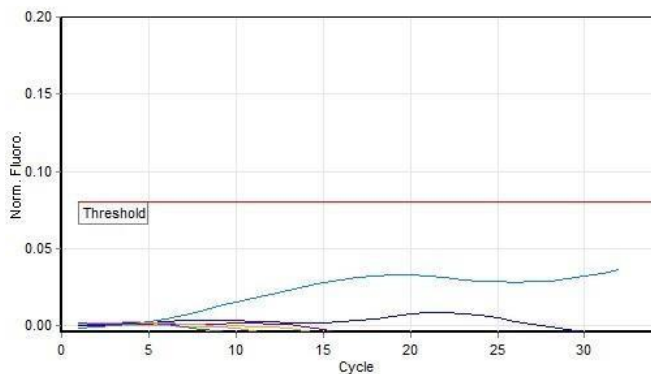
Assurance GDS Results Report

Run Information

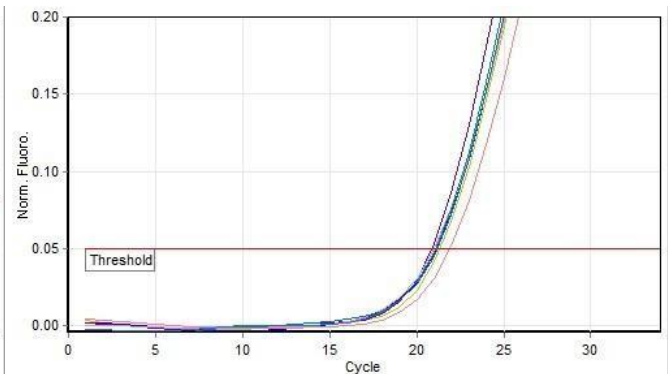
Run Name	BioControl Assay 36 Wells 2022-05-05 (1)
Run Start	05/05/2022 10:15:58 am
Run Finish	05/05/2022 11:41:48 am
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Listeria* spp.

Listeria spp.



Internal Control



No.	C	Name	<i>Listeria</i> spp.	<i>Listeria</i> spp. result	Ct	Assay	Kit
2		95	Negative	-		<i>Listeria</i> spp.	100520-03
3		96	Negative	-		<i>Listeria</i> spp.	100520-03
4		97	Negative	-		<i>Listeria</i> spp.	100520-03
5		98	Negative	-		<i>Listeria</i> spp.	100520-03
6		99	Negative	-		<i>Listeria</i> spp.	100520-03
7		106	Negative	-		<i>Listeria</i> spp.	100520-03
8		107	Negative	-		<i>Listeria</i> spp.	100520-03

BIOCONTROL

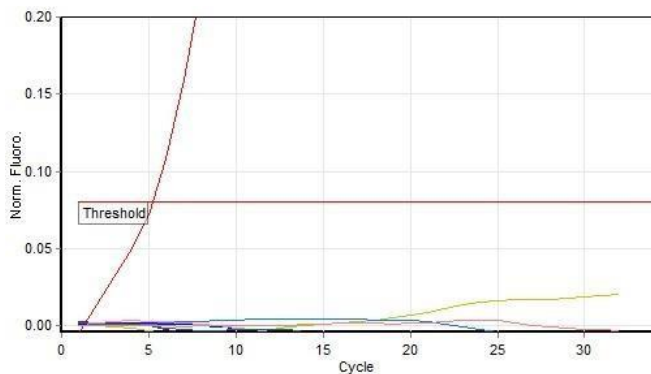
Assurance GDS Results Report

Run Information

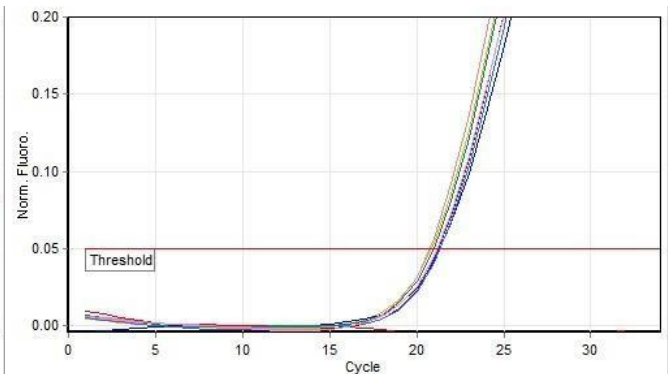
Run Name	BioControl Assay 36 Wells 2022-05-12 (1)
Run Start	12/05/2022 10:33:31 am
Run Finish	12/05/2022 11:59:24 am
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Listeria* spp.

Listeria spp.



Internal Control



No.	C	Name	<i>Listeria</i> spp.	<i>Listeria</i> spp. result	Ct	Assay	Kit
1	■	86	Positive	+	5.28	<i>Listeria</i> spp.	100520-03
2	■	89	Negative	-		<i>Listeria</i> spp.	100520-03
3	■	92	Negative	-		<i>Listeria</i> spp.	100520-03
4	■	96	Negative	-		<i>Listeria</i> spp.	100520-03
5	■	100	Negative	-		<i>Listeria</i> spp.	100520-03
6	■	101	Negative	-		<i>Listeria</i> spp.	100520-03
7	■	103	Negative	-		<i>Listeria</i> spp.	100520-03
8	■	105	Negative	-		<i>Listeria</i> spp.	100520-03

BIOCONTROL

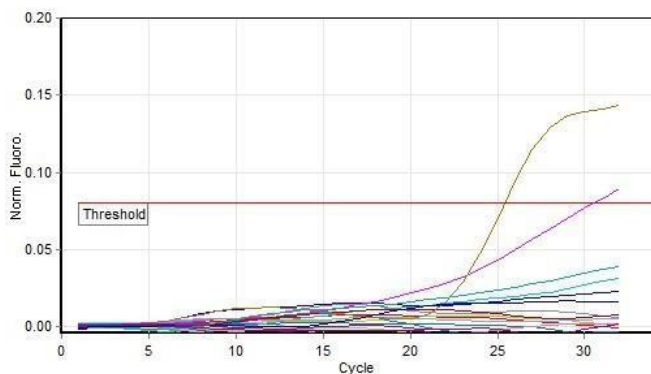
Assurance GDS Results Report

Run Information

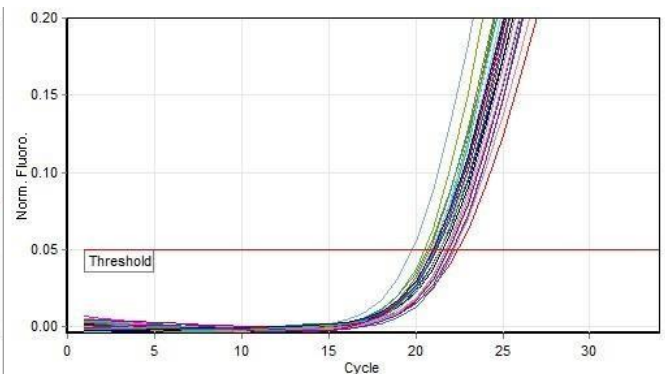
Run Name	BioControl Assay 36 Wells 2022-05-18 (1).rex listeria spp + ASO
Run Start	18/05/2022 10:32:57 am
Run Finish	18/05/2022 12:00:58 pm
Operator	Assurance GDS User
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.103
Run Signature	The Run Signature is valid.
Machine Serial No.	0819519

Assay: *Listeria* spp.

Listeria spp.




















Internal Control



No.	C	Name	<i>Listeria</i> spp.	<i>Listeria</i> spp. result	Ct	Assay	Kit
1	■	77	Negative	-		<i>Listeria</i> spp.	100520-03
2	■	78	Negative	-		<i>Listeria</i> spp.	100520-03
3	■	79	Negative	-		<i>Listeria</i> spp.	100520-03
4	■	90	Negative	-		<i>Listeria</i> spp.	100520-03
5	■	91	Negative	-		<i>Listeria</i> spp.	100520-03
6	■	92	Negative	-		<i>Listeria</i> spp.	100520-03
7	■	93	Negative	-		<i>Listeria</i> spp.	100520-03
8	■	94	Negative	-		<i>Listeria</i> spp.	100520-03
9	■	85	Negative	-		<i>Listeria</i> spp.	100520-03
10	■	86	Positive	+	30.53	<i>Listeria</i> spp.	100520-03

Continued on next page...

11		87	Negative	-		<i>Listeria</i> spp.	100520-03
12		88	Negative	-		<i>Listeria</i> spp.	100520-03
13		89	Negative	-		<i>Listeria</i> spp.	100520-03
14		80	Negative	-		<i>Listeria</i> spp.	100520-03
15		81	Negative	-		<i>Listeria</i> spp.	100520-03
16		82	Negative	-		<i>Listeria</i> spp.	100520-03
17		83	Negative	-		<i>Listeria</i> spp.	100520-03
18		84	Negative	-		<i>Listeria</i> spp.	100520-03
19		100	Negative	-		<i>Listeria</i> spp.	100520-03
20		101	Negative	-		<i>Listeria</i> spp.	100520-03
21		102	Positive	+	25.40	<i>Listeria</i> spp.	100520-03
22		103	Negative	-		<i>Listeria</i> spp.	100520-03
23		104	Negative	-		<i>Listeria</i> spp.	100520-03
24		105	Negative	-		<i>Listeria</i> spp.	100520-03
25		108	Negative	-		<i>Listeria</i> spp.	100520-03
26		109	Negative	-		<i>Listeria</i> spp.	100520-03
27		110	Negative	-		<i>Listeria</i> spp.	100520-03

Anexo 4: Resultados tras el análisis qPCR para la detección de *Salmonella* spp., *Listeria* spp. y *Listeria monocytogenes* de la mayoría de las muestras. Los resultados positivos aparecen en rojo, mientras que los resultados negativos aparecen en verde.