1. OBJECTIVE

To perform the product evaluation Polyskope One modular multiplex pathogen Detection set used for the qualitative determination of ***Escherichia coli*** 0157, ***Salmonella*** spp and ***Listeria monocytogenes*** using the real-time PCR technique in environmental and food samples based on the AOAC PTM 041801 method.

2. SCOPE

Applies to the quality and performance evaluation of the Polyskope One Modular Multiplex Pathogen Detection Set Kits of the Polyskope Labs Brand in the following evaluated matrices, based on different categories contemplated in the ISO 16140-3 Standard: Raw meat and ready-to-cook meat products (except poultry); Milk and dairy products subjected to heat treatment; Fruits, vegetables and processed vegetables; Dairy products and raw milk; raw and ready-to-cook poultry products; Raw and ready-to-cook fish and shellfish; Environmental samples of food production for human or animal consumption.

3. RESPONSIBILITY

It is the responsibility of the Technical Manager and laboratory analysts to carry out the respective evaluation of quality control and performance of the tests received in the laboratory.

4. REFERENCES

- Catalog Number 04.00MX.096.QS5.01.01: Polyskope One Multiplex Pathogen Detection Assay
- AOAC Performance tested certificate No. 041801
- ISO 16140-3 Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory.
- Guide for the validation of microbiological methods (OAA) Argentine accreditation body.

5. BASIS OF THE METHOD

Polyskope One is a real-time PCR-based gene amplification and detection test. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific to the "big three" pathogens, as well as DNA polymerase and nucleotides.

PCR is a technique used to rapidly generate abundant copies of target DNA. During the PCR reaction, heating and cooling cycles promote denaturation of the DNA, followed by the binding of primers to specific target regions. DNA polymerase then recognizes these primers and uses deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA, called amplicons. Specific probes are then used to detect the DNA during amplification by hybridizing to the amplicons. These probes are attached to a fluorophore that emits fluorescence only when it hybridizes to the correct target sequence. In the absence of target DNA, no fluorescence will be detected. As the Amplicons increase with each round of amplification, the fluorescence intensity also increases. At the hybridization step of each PCR cycle, the detector measures this fluorescence and the associated software plots the fluorescence intensity versus the number of cycles. This method allows a simple determination of the presence or absence of up to five targets in a single reaction. An "internal control" of unrelated DNA is included in the reaction mixture. This control is amplified

with a specific probe at the same time as the other target DNA sequences of the probe and is detected by a specific fluorophore.

Polyskope One is specifically designed to detect pathogenic bacteria capable of infecting humans. The oligonucleotides target specific pathogen-related genes that are present in these bacteria and distinguish them from closely related non-pathogenic bacteria.

6. PRODUCT TRACEABILITY

Traceability information for the Polyskope One kit used during the performance tests can be found in Table 1.

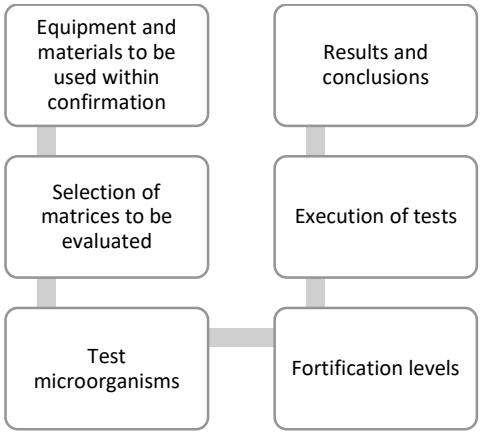
Table 1. Information products to evaluate Polyskope One

PRODUCT	LOT	EXPIRATION DATE	REF
Polyskope Multiplex Enrichment Media (PMEM)	100719	07/10/2022	PMEM-DM-2.5
Polyscope One	060121	01/11/2021	01.LM.96QS5

7. PERFORMANCE EVALUATIONS

The performance evaluations and tests performed were carried out assuming that there is no modification to the official method initially validated by POLYSKOPE LABS.

The Guide for the validation of microbiological methods published by the Argentinean Accreditation Organization was used for references in the guidelines for the expression and calculation of the results and the steps performed are referenced below:



7.1 REAGENTS, SUPPLIES AND EQUIPMENT USED

PolySkope Multiplex Enrichment media (PMEM)	PolySkope One modular multiplex Detection set
MacFarland Scale	5-50 µl micropipette
0.1% peptone water	1-10 µl micropipette
Plate Count Agar	10-100µl micropipette
Analytical balance	Magnetic Stirrer
100-1000 µl micropipettes	Light Cycler Thermocycler
Integral Mesh Filter Bags	PRC Light Cycler-LP Tubes
stomacher	Thermomixer 65±5°C 14000 RPM
37± 1° incubator	Thermomixer 95±5°C 14000 RPM
Sterile Filter Tips	1.5 ml sterile Eppendorf tubes

7.2 EVALUATED MATRICES

The samples evaluated for the methodology are established in accordance with current regulations and customer requirements, with a minimum of 3 or 2 parameters to be evaluated, as shown in Table 2. According to the categories, type and article based on ISO 16140-3.

Table 2. Articles or matrices evaluated in the Performance test

CATEGORY	TYPE	ARTICLE
Raw meat and ready-to-cook meat products (except poultry)	Fresh unprocessed meat	Frozen burger
Fruits and fresh products	Ready-to-cook cut vegetables and greens	Fresh lettuce
Raw and ready-to-cook unprocessed fish and shellfish	Unprocessed seadfood	Mussels, Oysters, Clams
Milk and dairy products subjected to heat treatment	Pasteurized dairy products	Pasteurized ice cream
Raw, ready-to-cook poultry products	Fresh unprocessed meat	Slaughtered chicken
Dairy products and raw milk	Raw milk-based products with high fat content and basal microflora	Fresh soft cheese
Environmental samples, food production for human and animal consumption	Equipment and production environment	Primary Production Areas

7.3 TEST MICROORGANISMS

The test microorganisms were selected according to the criteria established in the initial validation AOAC 041801 and according to the possible basal microbiological loads of the products evaluated.

The strains used correspond to reference material of which purity and viability can be guaranteed, acquired from recognized ATCC microbiologics collections and are listed in Table 3.

Table 3. Test microorganisms

TARGET	TCCA	INTERFERING	TCCA
Salmonella pullorum	13036	***Citrobacter freudii***	8090
Salmonella enteritidis	13076	***Proteus mirabilis***	7002
Salmonella typhimurium	14028	***Pseudomonas aeruginosa***	9027
Listeria monocytogenes	19111		
Escherichia coli O157:H7	43895		

7.4 FORTIFICATION LEVELS

7.4.1 INOCULUM PREPARATION AND STANDARDIZATION OF TEST MICROORGANISMS

The test microorganisms indicated in Table 3 were activated in nutrient culture media until their stationary phase was obtained, serial dilutions were made up to 101 and verification by plate count of the last three dilutions in order to be able to start from known concentrations for the fortification process, Table 4 shows the results obtained for each of the microorganisms.

Table 4. Results obtained checking Mac Farland Scale

MICROORGANISMS	103	102	101
Salmonella pullorum	>300	164	56
Salmonella enteritidis	>300	134	18
Salmonella typhimurium	>300	188	46
Listeria monocytogenes	>300	196	64
Escherichia coli O157:H7	>300	240	69
INTERFERENTS			
Interference pool*	>300	>300	124

*Interference pool: ***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***

7.4.2 SAMPLE FORTIFICATION LEVEL

After obtaining the results of the Mac Farland scale in the culture media, the samples were fortified as follows:

- **TARGET MICROORGANISMS**

Level 1 (Ultra Low): 0.3 CFU of the microorganism of interest in order to verify the feasibility of recovering lower quantities than the declared limit of detection.
Level 2 (Low): 1 CFU of the target microorganism, based on the declared method, corresponding to a detection limit of 1 CFU for the target microorganisms.
Level 3 (Medium): 3 times the sensitivity of the method, i.e. 3 CFU.

- **INTERFERING MICROORGANISMS**

Samples were inoculated into a Level 3 (Medium): 3 CFU of pool of interfering microorganisms

7.4.3 IDENTIFICATION OF PRODUCTS AND FORTIFICATIONS PERFORMED

Different types of fortifications of the microorganisms of interest were carried out in order to verify the performance of the kit in different process conditions. Table 5 lists the identification for each of the samples and the fortifications carried out for each of these with the microorganisms. indicated in point 7.3

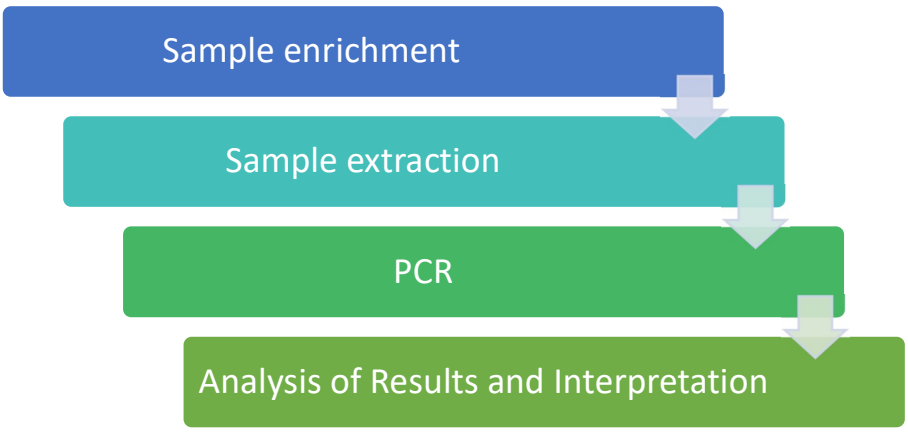
Table 5. Identification of Samples and Test Microorganisms

PRODUCT	ID	FORTIFIED MICROORGANISMS	FORTIFICATION LEVELS
Frozen burger	M1R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M1R1-2	***Escherichia coli O157:H7***	**Level 3**
		Listeria monocytogenes	**Level 2**
		Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M1R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 2**
	M1R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Fresh lettuce	M2R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M2R1-2	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M2R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 2**
	M2R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Mussels, Oysters, Clams	M3R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M3R1-2	***Escherichia coli O157:H7***	**Level 2**
		Listeria monocytogenes	**Level 2**
		Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M3R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 2**

	M3R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Pasteurized ice cream	M4R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M4R1-2	***Escherichia coli O157:H7***	**Level 2**
	M4R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 2**
	M4R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Slaughtered chicken	M5R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M5R1-2	***Escherichia coli O157:H7***	**Level 2**
		Listeria monocytogenes	**Level 3**
	M5R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella pullorum***	**Level 2**
	M5R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Fresh soft cheese	M6R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M6R1-2	***Escherichia coli O157:H7***	**Level 3**
		Listeria monocytogenes	**Level 2**
		Salmonella typhimurium	**Level 1**
	M6R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 2**
	M6R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella typhimurium***	**Level 3**
Production areas	M7R1-1	Interfering pool (***Citrobacter freudii, Proteus mirabilis, Pseudomonas aeruginosa***)	**Level 3**
	M7R1-2	***Escherichia coli O157:H7***	**Level 3**
	M7R1-3	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella enteritidis***	**Level 2**
	M7R1-4	***Escherichia coli O157:H7; Listeria monocytogenes; Salmonella enteritidis***	**Level 3**

7.5 TEST PROCEDURE

The steps were carried out for each of the samples to be evaluated without any type of modification in the processes, except for the configuration of the thermocycler in its Pre-incubation and Amplification programs, which is detailed below.



The changes made to the program configuration are due to the fact that the thermal cycler used for the tests performed did not have the same Fluorophores of the Quantstudio 5 thermal cycler. Image 1 shows the selected Fluorophores and Image 2 shows the template that was executed for the test.

Table 6 details the configuration of the program executed for the tests.

Image 1. Selected Fluorophores

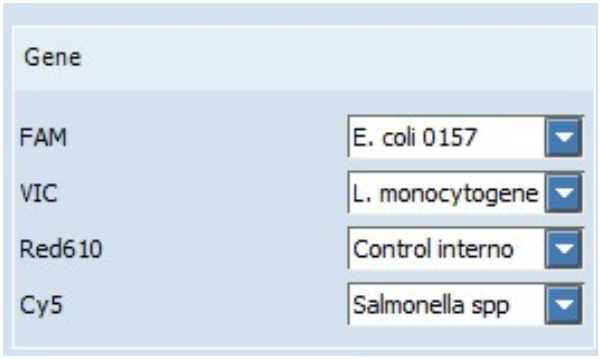


Image 2. Run Editor (Programs)

Image 2.1 Pre-incubation

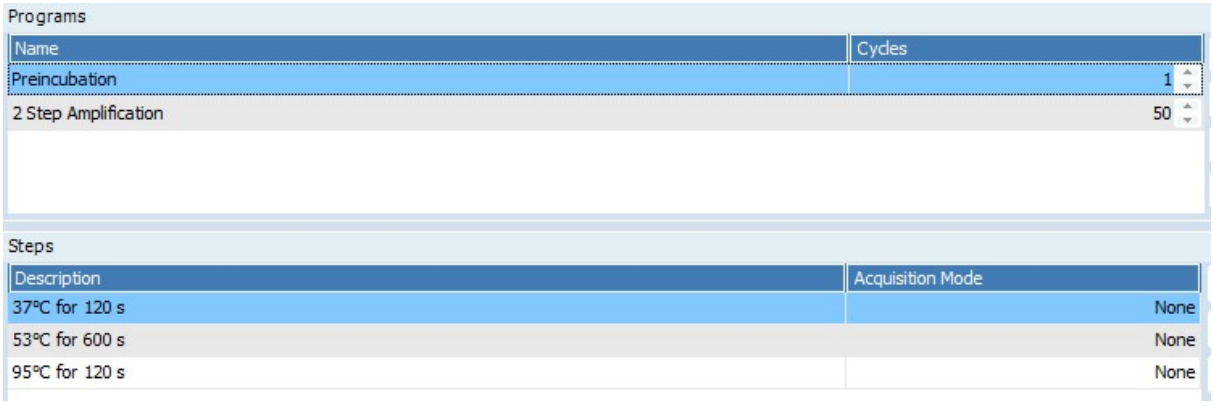


Image 2.2 Amplification

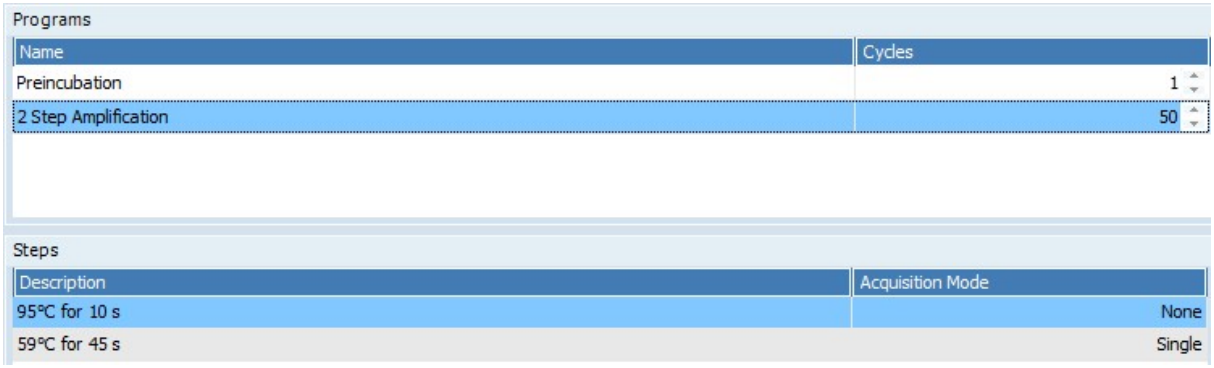


Table 6. Roche Light Cycler Thermocycler Program Configuration detail

Program	Terms
Pre-incubation	37°C for 120s
	52°C for 600s
	95°C for 120s
2-Step Amplification	95°C for 10s
50 cycles	59°C for 45s

8. RESULTS OBTAINED

The results obtained for each test are listed below. The primary data and DNA amplification graphs for each sample are listed in ANNEX 1.

Table 7. Results obtained interfering microorganisms

SAMPLE ID	PATHOGEN	INOCULUM LEVEL	NUMBER OF SAMPLES	cq	Result
M1R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	25.34/25.38	+
	Salmonella spp.	0	2	NA	-
M2R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	24.56/24.73	+
	Salmonella spp.	0	2	NA	-
M3R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	26.25/26.56	+
	Salmonella spp.	0	2	NA	-
M4R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	25.81/25.98	+
	Salmonella spp.	0	2	NA	-
M5R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	26.38/25.16	+
	Salmonella spp.	0	2	NA	-
M6R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	29.10/29.13	+
	Salmonella spp.	0	2	NA	-
M7R-1	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	26.56/25.58	+
	Salmonella spp.	0	2	NA	-

Table 7. Obtained Results Analyte Variables

SAMPLE ID	PATHOGEN	INOCULUM LEVEL	NUMBER OF SAMPLES	cq	Result
M1R-2	***E. coli O157***	3	2	31.45/36.96	+
	L. monocytogenes	1	2	32.55/31.27	+
	Internal control	0	2	25.07/25.19	+
	Salmonella spp.	0	2	NA	-
M2R-2	***E. coli O157***	0	2	NA	-
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	24.56/24.73	
	Salmonella spp.	0	2	NA	-
M3R-2	***E. coli O157***	1	2	32.22/32.15	+
	L. monocytogenes	1	2	29.91/29.31	+
	Internal control	0	2	25.00/25.13	+
	Salmonella spp.	0	2	NA	-
M4R-2	***E. coli O157***	3	2	33.28/30.56	+
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	24.48/25.09	+
	Salmonella spp.	0	2	NA	-
M5R-2	***E. coli O157***	1	2	29.35/29.53	+
	L. monocytogenes	3	2	30.03/30.64	+
	Internal control	0	2	24.59/25.02	+
	Salmonella spp.	0	2	NA	-
M6R-2	***E. coli O157***	3	2	36.64/36.23	+
	L. monocytogenes	1	2	32.02/32.01	+
	Internal control	0	2	25.10/25.11	+
	Salmonella spp.	0.1	2	NA	-
M7R-2	***E. coli O157***	3	2	33.66(33.65	+
	L. monocytogenes	0	2	NA	-
	Internal control	0	2	25.31/25.30	+
	Salmonella spp.	0	2	NA	-

Table 8. Results microorganisms Target Level 2

SAMPLE ID	PATHOGEN	INOCULUM LEVEL	NUMBER OF SAMPLES	cq	Result
M1R-2	***E. coli O157***	1	2	26.02/25.68	+
	L. monocytogenes	1	2	23.16/22.76	+
	Internal control	1	2	26.64/24.46	+
	Salmonella spp.	1	2	24.66/24.64	+
M2R-2	***E. coli O157***	1	2	24.38/24.42	+
	L. monocytogenes	1	2	25.29/24.13	+
	Internal control	1	2	28.60/28.29	+
	Salmonella spp.	1	2	24.65/24.34	+
M3R-2	***E. coli O157***	1	2	26.77/26.50	+
	L. monocytogenes	1	2	23.62/23.77	+
	Internal control	1	2	25.21/24.79	+
	Salmonella spp.	1	2	23.34/23.29	+
M4R-2	***E. coli O157***	1	2	25.67/25.64	+
	L. monocytogenes	1	2	22.92/23.03	+
	Internal control	1	2	24.68/24.70	+
	Salmonella spp.	1	2	23.99/24.02	+
M5R-2	***E. coli O157***	1	2	26.90/26.86	+
	L. monocytogenes	1	2	22.37/22.33	+
	Internal control	1	2	24.82/24.75	+
	Salmonella spp.	1	2	27.90/27.18	+
M6R-2	***E. coli O157***	1	2	27.59/28.00	+
	L. monocytogenes	1	2	17.85/18.34	+
	Internal control	1	2	19.80/19.83	+
	Salmonella spp.	1	2	28.08/28.10	+
M7R-2	***E. coli O157***	1	2	26.54/24.57	+
	L. monocytogenes	1	2	16.91/16.63	+
	Internal control	1	2	24.70/24.89	+
	Salmonella spp.	1	2	19.94/20.33	+

Table 9. Results microorganisms Target Level 3

SAMPLE ID	PATHOGEN	INOCULUM LEVEL	NUMBER OF SAMPLES	cq	Result
M1R-2	***E. coli O157***	3	2	30.68/30.36	+
	L. monocytogenes	3	2	20.94/20.76	+
	Internal control	3	2	24.23/24.18	+
	Salmonella spp.	3	2	24.72/24.65	+
M2R-2	***E. coli O157***	3	2	28.74/28.69	+
	L. monocytogenes	3	2	22.59/21.76	+
	Internal control	3	2	25.50/25.60	+
	Salmonella spp.	3	2	26.11/26.14	+
M3R-2	***E. coli O157***	3	2	25.73/25.52	+
	L. monocytogenes	3	2	20.85/20.48	+
	Internal control	3	2	24.84/24.92	+
	Salmonella spp.	3	2	19.49/19.45	+
M4R-2	***E. coli O157***	3	2	23.40/23.29	+
	L. monocytogenes	3	2	20.96/20.81	+
	Internal control	3	2	24.24/24.18	+
	Salmonella spp.	3	2	21.75/21.72	+
M5R-2	***E. coli O157***	3	2	25.84/26.54	+
	L. monocytogenes	3	2	22.20/22.90	+
	Internal control	3	2	24.62/24.64	+
	Salmonella spp.	3	2	26.11/26.10	+
M6R-2	***E. coli O157***	3	2	22.68/22.55	+
	L. monocytogenes	3	2	17.01/16.89	+
	Internal control	3	2	25.44/25.32	+
	Salmonella spp.	3	2	19.45/19.35	+
M7R-2	***E. coli O157***	3	2	20.80/20.71	+
	L. monocytogenes	3	2	15.95/15.98	+
	Internal control	3	2	23.48/23.30	+
	Salmonella spp.	3	2	20.03/20.21	+

8.1 STATISTICAL CALCULATIONS FOR QUALITATIVE METHODS

The statistical package was developed based on the Guide for the validation of microbiological methods published by the Argentine Accreditation Body for references in the guidelines for the expression and calculation of results.

Matrix	Variable	*Escherichia coli* O157	*Listeria monocytogenes*	*Salmonella* sp
Frozen burger	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1
Fresh lettuce	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1
Mussels, Oysters, Clams	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1

Pasteurized ice cream	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1
Slaughtered chicken	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1
Fresh soft cheese	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0
	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1
Production area	Sensitivity	100%	100%	100%
	Specificity	100%	100%	100%
	Relative accuracy	100%	100%	100%
	False negative rate	0	0	0

	False positive rate	0	0	0
	Intermediate precision (Kappa Index)	1	1	1

9. DISCUSSION OF THE PERFORMANCE STUDY

The PolySkope multiplex pathogen detection assay met the Performance Test performed by providing a qualitative detection of pathogens such as ***Escherichia coli*** 0157; ***Listeria monocytogenes*** and ***Salmonella*** spp in a single reaction in a reliable manner, allowing to improve productivity within the laboratory by decreasing times starting from a single reaction, lysis and the same pre-enrichment in PolySkope Multiplex Enrichment media (PMEM) at a temperature of 37°C during an incubation period of 20 hours for the evaluated products.

With reference to the specificity demonstrated, it was 100%, allowing the recovery of the target microorganism from low concentrations of 1 cfu onwards for each of the test microorganisms ***Escherichia coli*** 0157; ***Listeria monocytogenes*** and ***Salmonella*** spp, which allows us to guarantee the reliability of the method.

The results obtained for non-target interfering microorganisms give us a selectivity and exclusivity of the PolySkope multiplex pathogen detection assay of 100%, giving us negative results for all the samples evaluated.

The results obtained for the positive and negative controls of the run yielded the expected results, which allows us to guarantee the reliability of the data obtained with the samples evaluated.

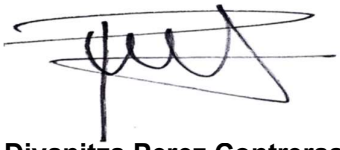
The evaluated samples do not present interferences in the methodology, this was verified with the curves obtained in the Red610 channel that corresponds to the internal control.

Robustness was determined since the changes made to the pre-incubation and amplification program for the LightCycler thermal cycler had no impact on the results obtained.

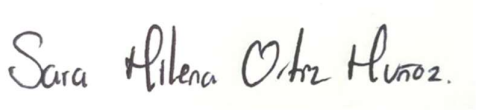
10. CONSIDERATIONS

After performing the performance test and executing each of the tests, we recommend improving the extraction system because the lysis solution is not completely homogeneous due to the formation of precipitate inside the beads, which does not guarantee the same transfer of these to all samples, as well as extending the useful life of 1 month after reconstitution.

Establish in the kit user manual the pre-incubation and amplification program in order to have the information in the case of using an open thermal cycler. Additionally, consider ranges in the times and temperatures of the programs according to what the different open thermal cyclers in the market have established.



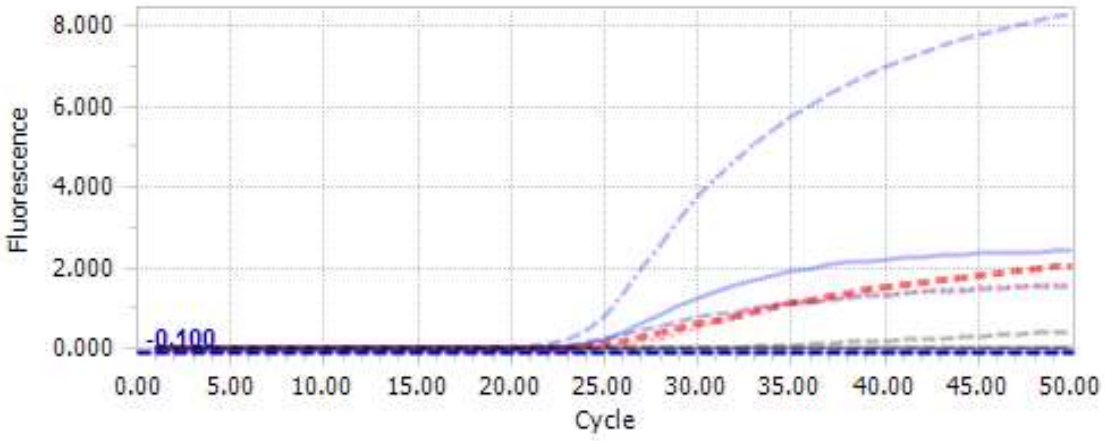
Diyanitza Perez Contreras
Technical Manager



Sara Milena Ortiz Munoz
Laboratory analyst

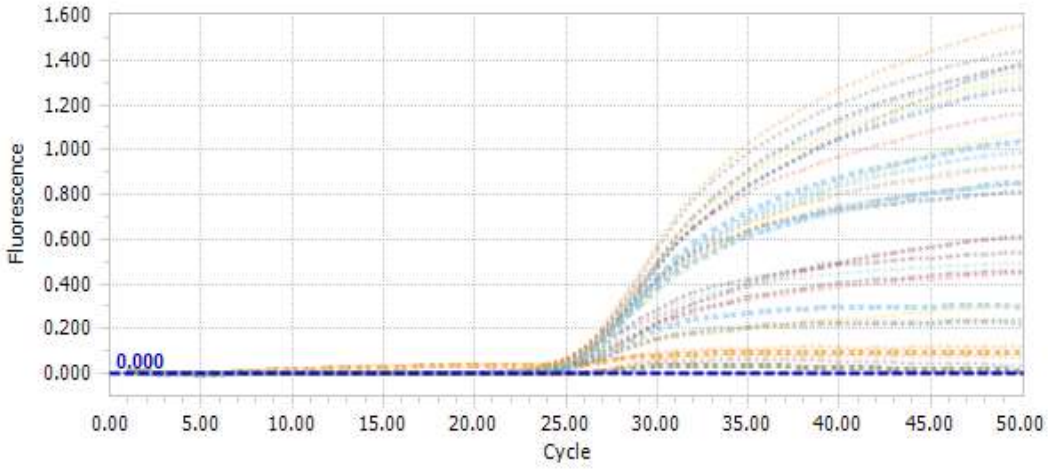
ANNEX I

1. Positive and negative control Run



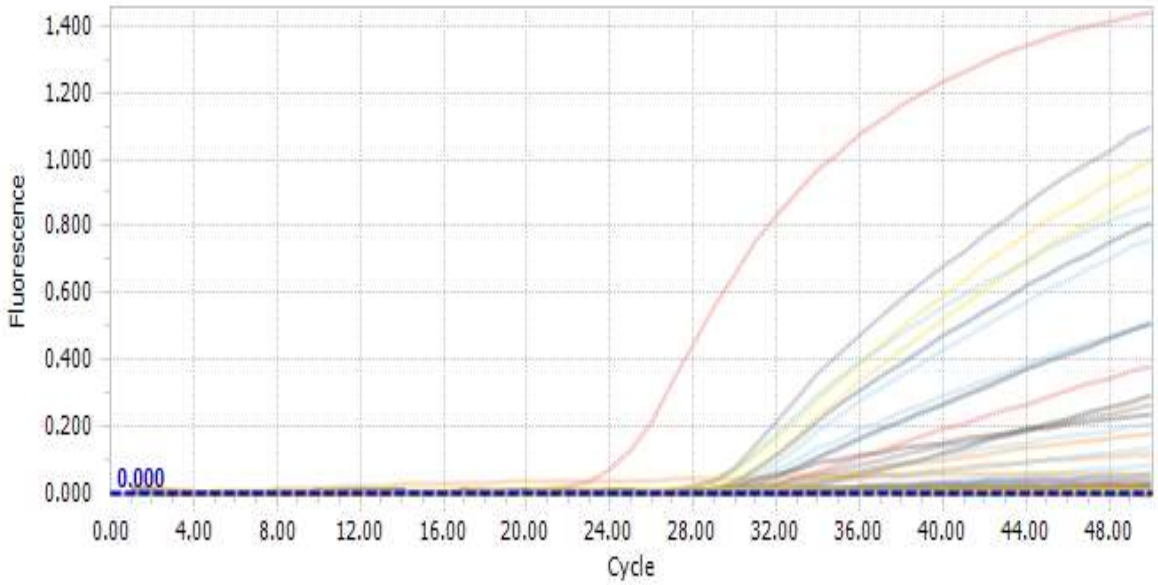
Sample Name	Gene Name	Gene Type	cq	call
NEGATIVE CONTROL KIT	***E. coli* STEC**	target	-	Negative
NEGATIVE CONTROL KIT	***L. monocytogenes***	target	-	Negative
NEGATIVE CONTROL KIT	Internal control	internal-control	24.84	Positive
NEGATIVE CONTROL KIT	***Salmonella*** spp	target	-	Negative
POSITIVE CONTROL	***E. coli* STEC**	target	22.91	Positive
POSITIVE CONTROL	***L. monocytogenes***	target	20.51	Positive
POSITIVE CONTROL	Internal control	internal-control	23.41	Positive
POSITIVE CONTROL	***Salmonella*** spp	target	21.79	Positive

2. Interfering Microorganisms and Analyte variables
RED 610 Fluorophore Chart (Internal Control)

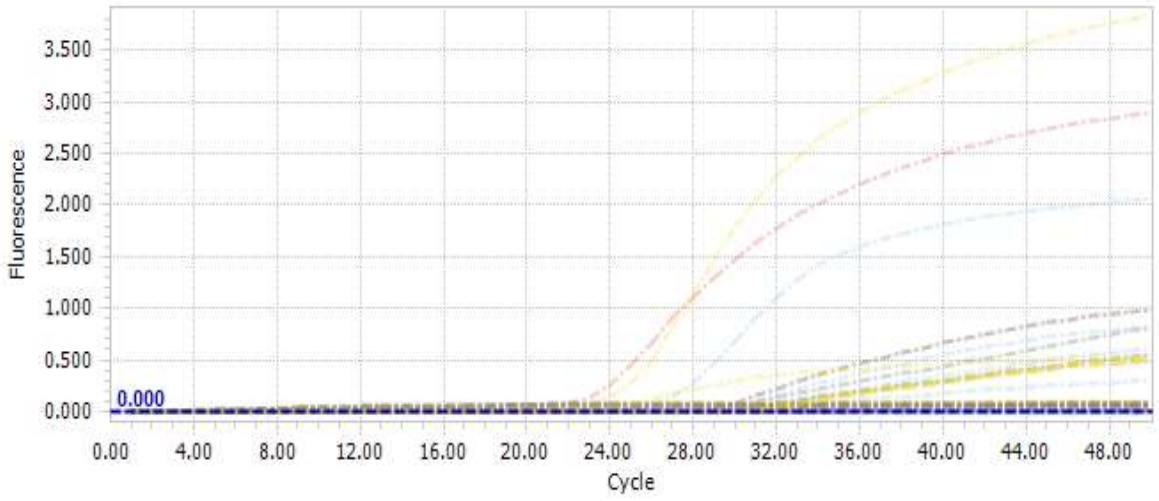


Note: The curves correspond to the RED610 Fluorophore internal control of the test, which indicates that there is no type of interference with the matrices.

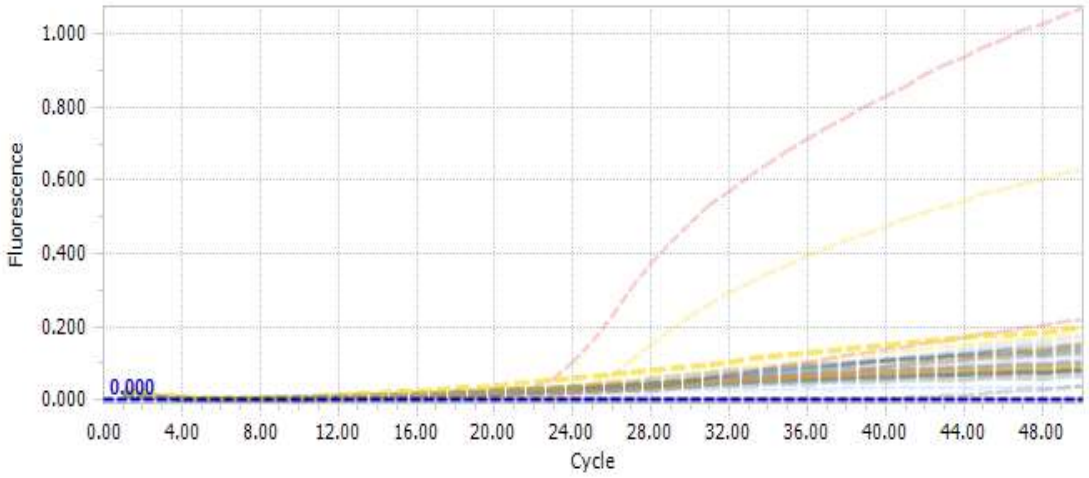
3. Results Variable Analyte: *Escherichia coli* 0157:H7
FAM Channel



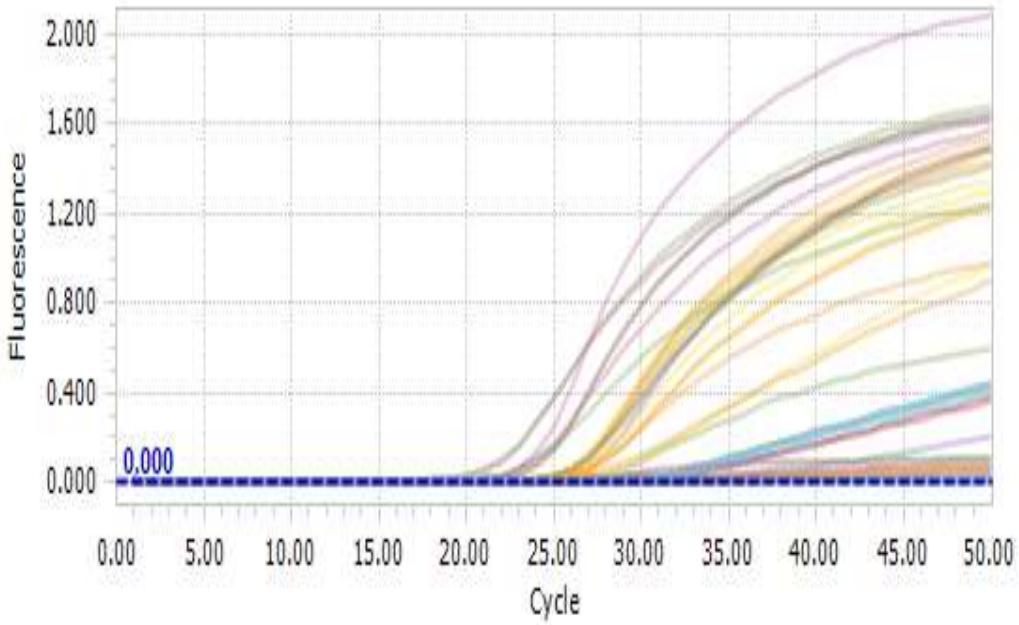
4. **Variable Results Analyte: *Listeria monocytogenes***
VIC Channel



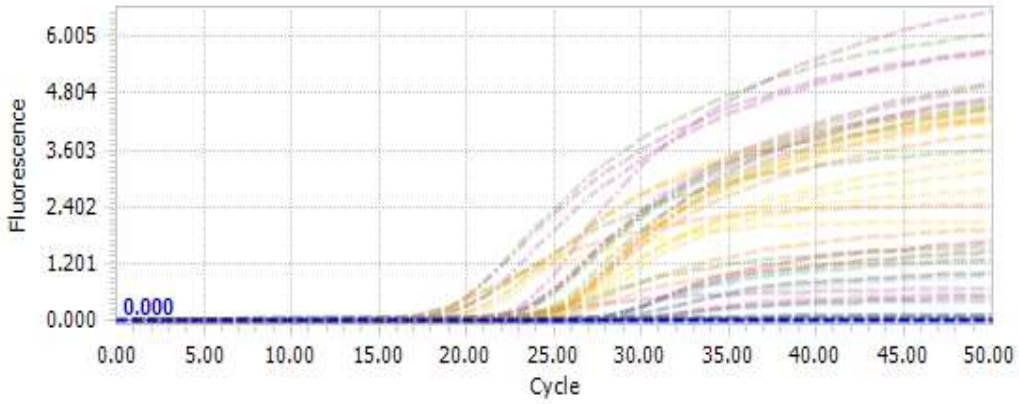
5. Variable results Analyte: *Salmonella* spp
CY5 Channel Graphics



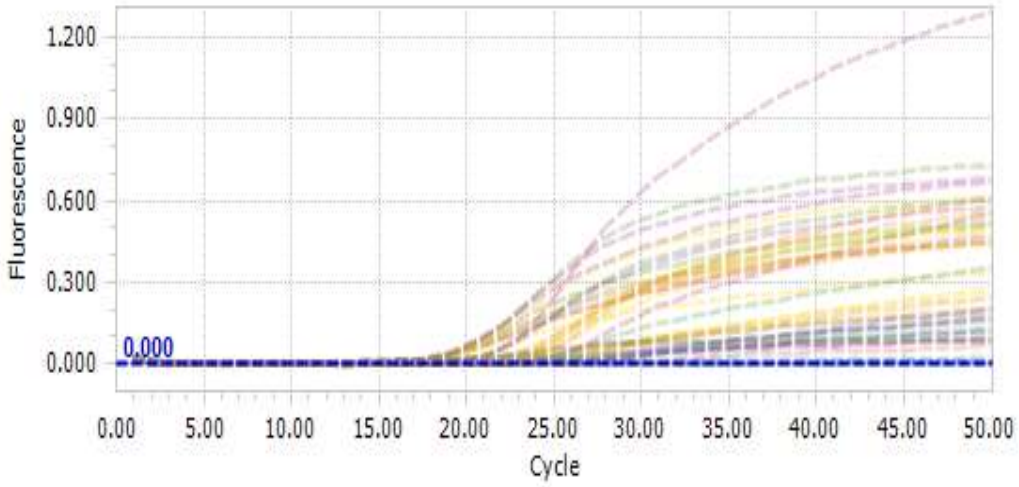
6. Results Level 2 and 3 *Escherichia coli* 0157:H7
FAM Channel



7. Results Level 2 and 3 *Listeria monocytogenes*
VIC Channel



8. Results Level 2 and 3 *Salmonella* spp
CY5 channel


1.200
0.900
0.600
0.300
0.000
Fluorescence
0.000
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Cycle