POLYSKOPE
LABS

AOAC Method Comparison Study

Deli Turkey

1 CFU/25g & 5 CFU/25g

AOAC Guidelines for Matrix Validation -

AOAC mandates two-tiered fractional recovery procedure
- 1 CFU/25g
  - Low level, recovery target between 25%-75%
  - Results <25% or >75% are considered invalid
- 5 CFU/25g
  - High Level
  - 100% recovery is required for 5 CFU inoculations
- Must be run in parallel with reference methods
  - *Escherichia coli* O157 STEC: FDA BAM 4A
  - *Salmonella enterica:* USDA MLG 4.08
  - *Listeria monocytogenes:* USDA MLG 8.09

Experimental Design

- All bacteria inoculated into matrix and stored at 4°C for 48 hours
    - As per AOAC guidelines
    - Replicates real-world conditions by inducing stress
    - All 3 target organisms inoculated, stressed and enriched simultaneously
        - *E. coli* O157:H7 (ATCC:43895)
        - *S. enterica* (ATCC:13076)
        - *L. monocytogenes* (ATCC: 13932)

- Low-level Inoculation: 20 replicates of 1 CFU/25g per matrix
    - 1 CFU inoculation is AOAC-required lower limit of detection
    - Inoculated into 1500 g matrix

- High-level Inoculation: 5 replicates of 5 CFU/25g per matrix
    - 5 CFU inoculation is AOAC-required high level of detection
    - Inoculated into 500 g matrix

Experimental Design

- After 48 hours, samples enriched at 37°C for 24 hours
  - Enriched in PolySkope proprietary enrichment media (PMEM)
  - Aerobic plate count (APC) performed on uninoculated control to determine native bacteria per gram

- Proprietary lysis procedure
  - Maximizes recovery and detection of bacterial DNA

- PolySkope 1.0 assay run on lysate
  - Real Time PCR detection of nucleic acid targets
  - Run on ThermoFisher ABI QuantStudio 5 (96-well format)

QuantStudio® 5

AOAC Method Comparison Study

Experimental Design

- Low-level Inoculation: 20 replicates of 1 CFU/25g deli turkey

  - 1 CFU inoculation is AOAC-required lower limit of detection

  - All 3 target organisms inoculated and incubated simultaneously

    - 0.68 CFU/25g *E. coli* O157:H7 (ATCC:43895)

    - 0.86 CFU/25g *S. enterica* (ATCC:13076)

    - 1.1 CFU/25g *L. monocytogenes* (ATCC: 13932)

- High Level Inoculation: 5 replicates of 5 CFU/25g deli turkey

  - All 3 target organisms inoculated and incubated simultaneously

    - 3.42 CFU/25g *E. coli* O157:H7 (ATCC:43895)

    - 4.3 CFU/25g *S. enterica* (ATCC:13076)

    - 5.5 CFU/25g *L. monocytogenes* (ATCC: 13932)

- All bacteria inoculated into turkey and stored at 4°C for 48 hours

  - As per AOAC guidelines

FDA BAM and USDA MLG Method Comparisons

**Study was carried out simultaneously using FDA BAM and USDA MLG reference methods**

- *E. coli* O157 STEC method comparison (FDA BAM 4A) 25 grams deli turkey enriched in 225 ml Buffered Peptone Water with pyruvate (BPWp) at 37°C for 24 hours
- After 24 hours, replicates were streaked on Biolog Rainbow chromogenic agar and incubated at 37°C for 24 hours

- *S. enterica* method comparison (USDA MLG 4.08) 25 grams deli turkey enriched in 225 ml Buffered Peptone Water (BPW) at 37°C for 24 hours
- After 24 hours, replicates were streaked on Hektoen chromogenic agar and incubated at 37°C for 24 hours

- *L. monocytogenes* method comparison (USDA MLG 8.09) 25 grams deli turkey enriched in 225 ml University of Vermont modified listeria enrichment broth (UVM) at 30°C for 24 hours
- After 24 hours, replicates were streaked on Modified Oxford Medium (MOX) chromogenic plates and incubated at 35°C for 24 hours. Additionally, supernatant from each enrichment was added to 10 ml of Fraser Broth (with supplements) and incubated at 35°C for 24 hours as an additional confirmation test
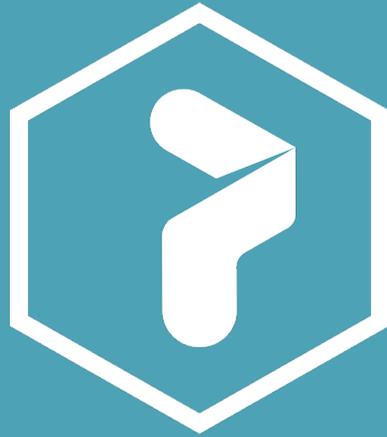
AOAC Method Comparison Study

Deli Turkey

1 CFU/25g

Amplification Plot
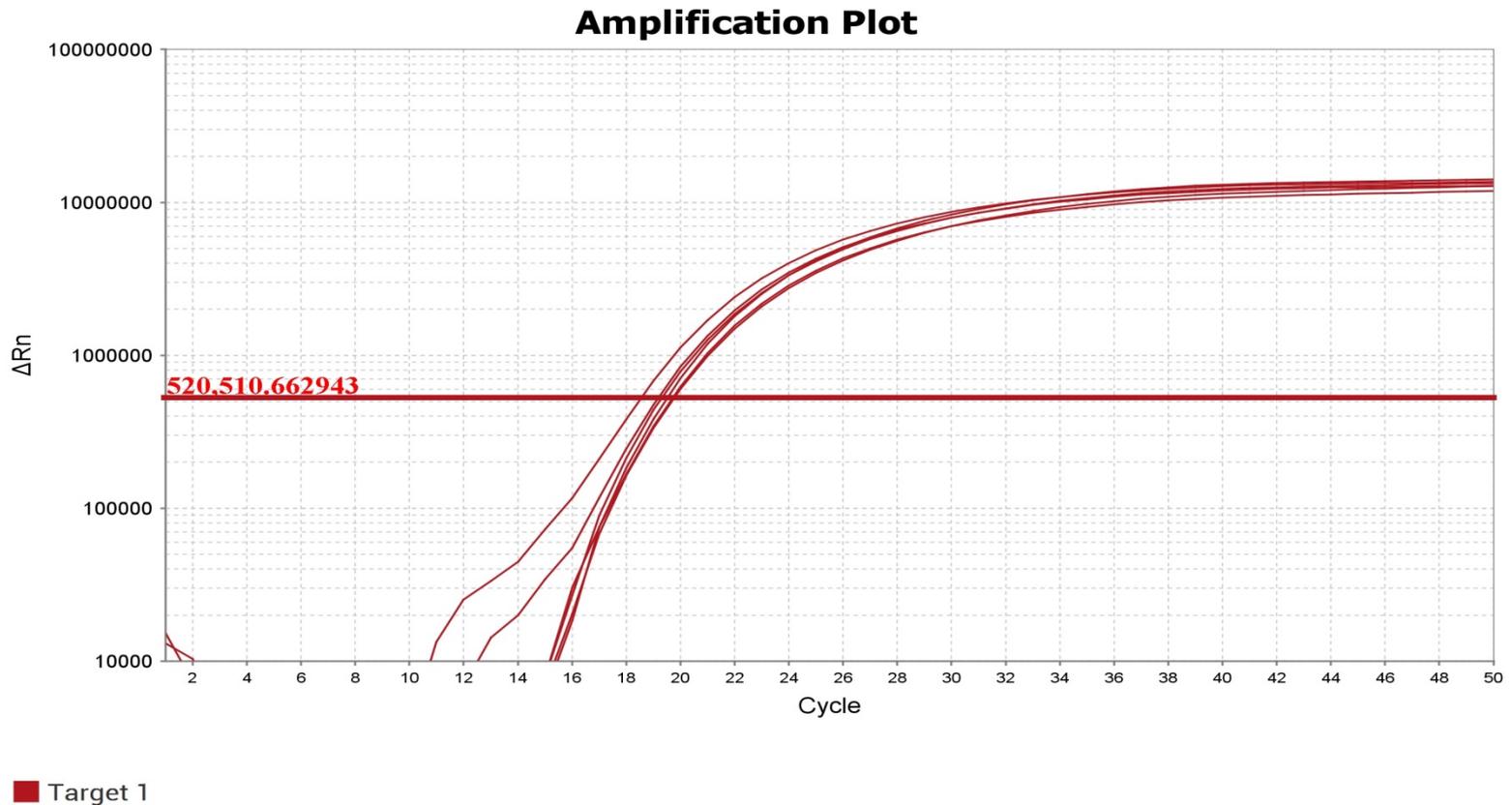
Polyskope 1.0 detected STX-1 and STX-2 targets in 6/20 replicates (30% recovery)

Polyskope 1.0 detected *E. coli* EAE in 6/20 replicates (30% recovery)

Amplification Plot
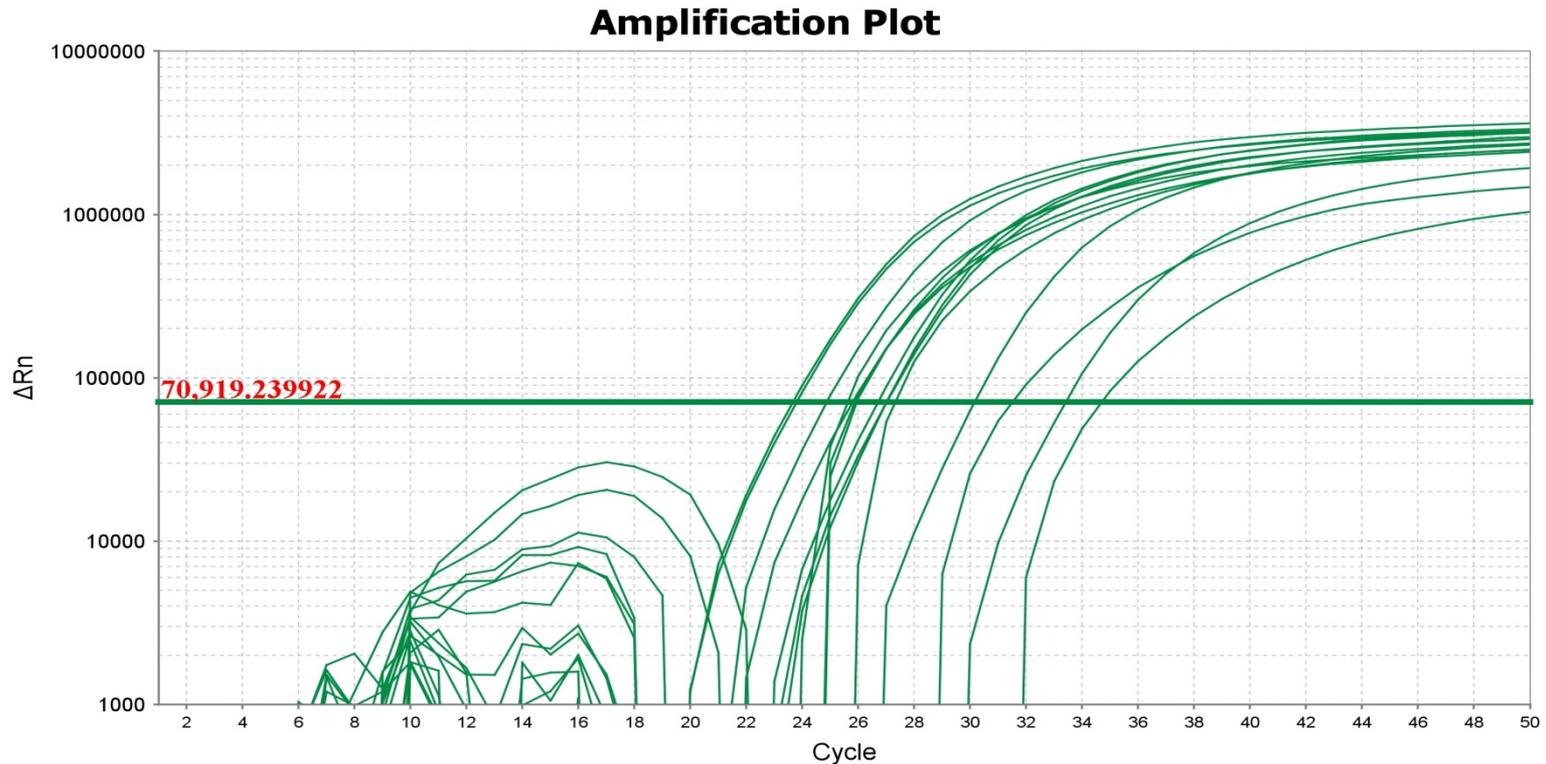
Polyskope 1.0 detected the *L. monocytogenes* target in 15/20 replicates (75% recovery)

Amplification Plot
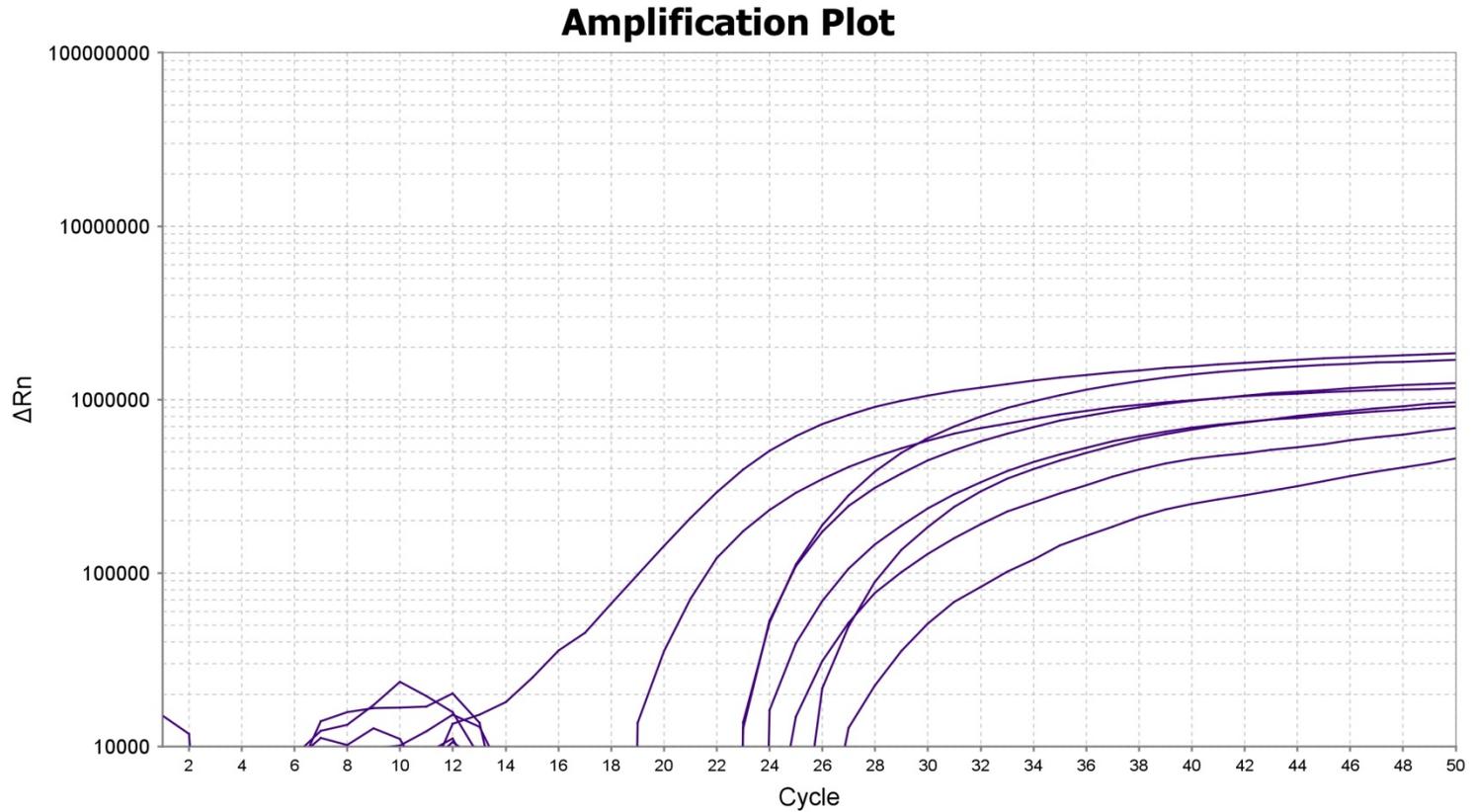
Polyskope 1.0 detected the *S. enterica* target in 8/20 replicates (40% recovery)

Amplification Plot
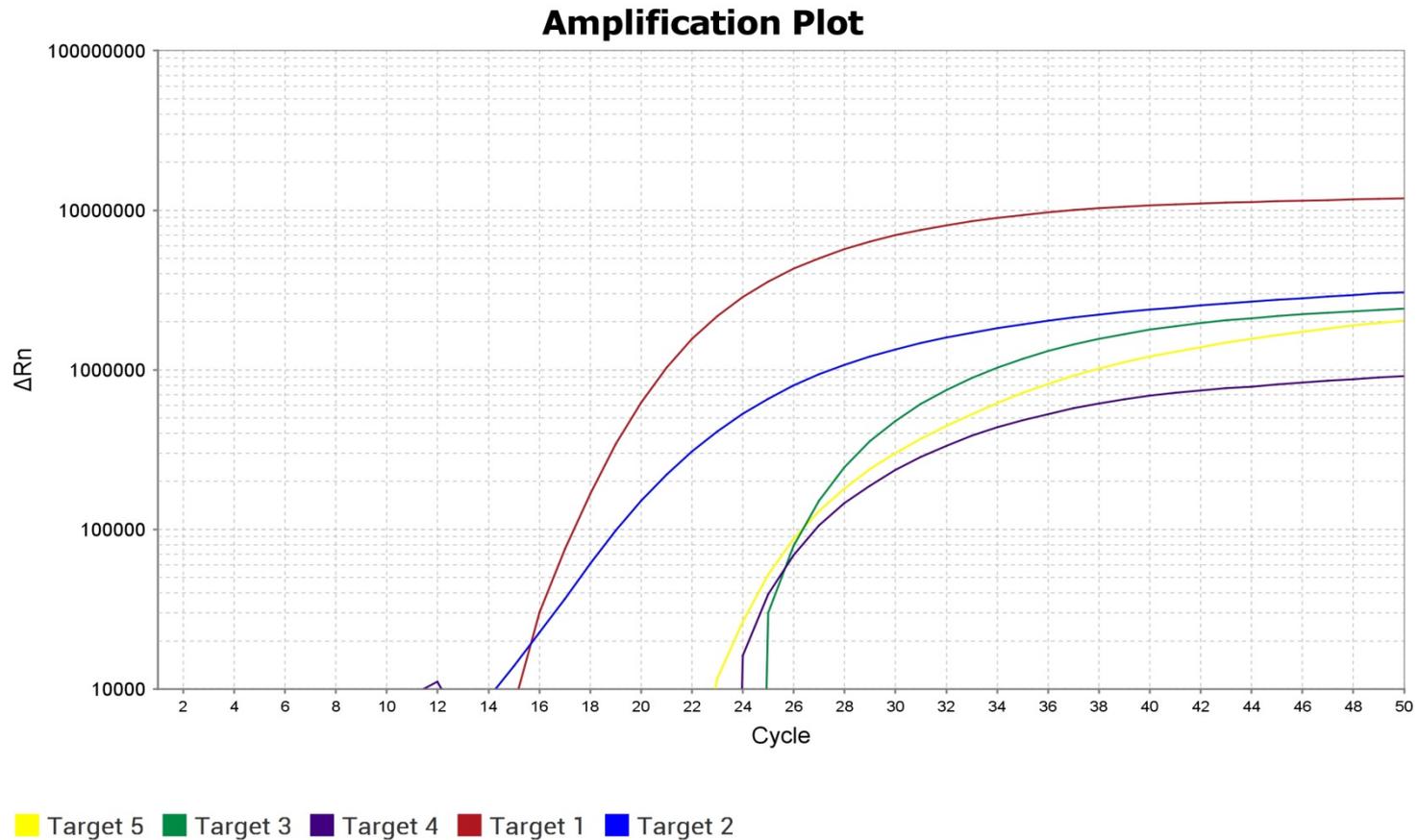
Example of Polyskope 1.0 detecting all three pathogens in a single enrichment and PCR reaction.
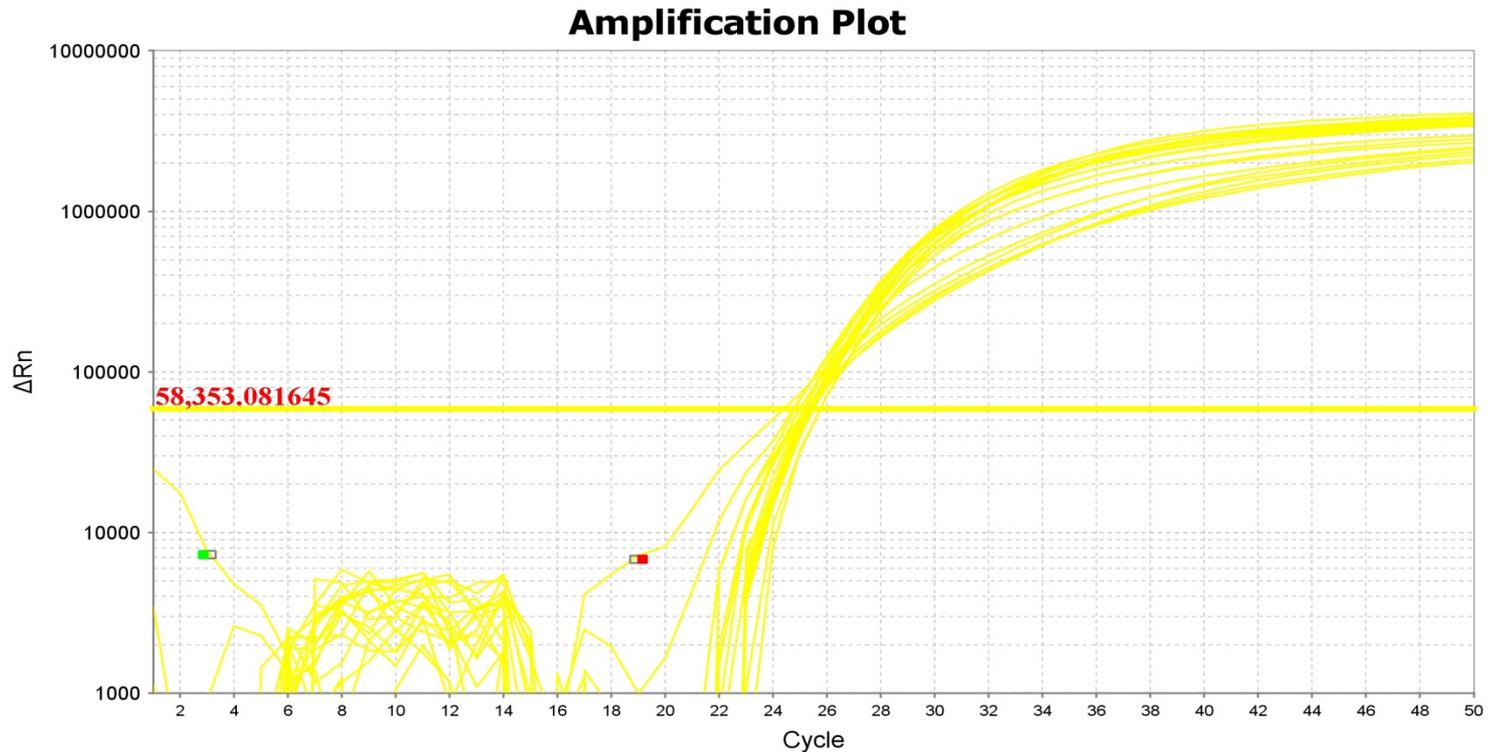
Polyskope 1.0 detected the internal control in 20/20 replicates (100% recovery)

Deli Turkey – 1 CFU/25g – Polyskope PCR Results

Replicate	STX-1/ STX-2 "+/-"	L. monocytogenes "+/-"	S. enterica "+/-"	EAE "+/-"	Internal Control "+/-"
1	+	+	-	+	+
2	-	-	-	-	+
3	-	+	+	-	+
4	+	+	-	+	+
5	+	+	+	+	+
6	-	+	+	-	+
7	-	-	-	-	+
8	-	+	+	-	+
9	-	+	-	-	+
10	-	-	+	-	+
11	-	+	+	-	+
12	+	+	-	+	+
13	-	-	-	-	+
14	+	+	+	+	+
15	-	-	-	-	+
16	-	+	-	-	+
17	+	+	+	+	+
18	-	+	-	-	+
19	-	+	-	-	+
20	-	+	-	-	+

Deli Turkey – 1 CFU/25g AOAC BAM and MLG Method Comparison Results

	E. coli	L. monocytogenes	S. enterica
Replicate	"+/-"	"+/-"	"+/-"
1	-	-	+
2	-	+	+
3	-	+	-
4	-	+	-
5	-	+	-
6	-	+	-
7	+	+	+
8	+	+	-
9	+	+	+
10	+	-	-
11	-	+	-
12	+	-	-
13	-	+	+
14	-	+	-
15	+	+	-
16	+	-	-
17	-	+	+
18	-	+	-
19	-	+	+
20	-	-	-

15

Polyskope PCR and Method Comparison Results* - 1 CFU/25g

Polyskope 1.0	% Positive	USDA/FDA Methods	% Positive
STX-1/STX-2 and EAE targets	30%	*E. coli*	35%
L. monocytogenes target	75%	*L. monocytogenes*	75%
S. enterica target	40%	*S. enterica*	35%

*Please note that since this was conducted as an **unpaired** study, the positive replicates will not be numerically matched when comparing the Polyskope PCR data to the BAM/MLG methods

Polyskope 1.0 PCR Results

- ***E. coli* (STEC) targets (3 targets)**
  - **STX-1/STX-2**: Polyskope 1.0 identified 6/20 (30%) replicates as positive for STX-1/STX-2
  - **EAE:** Polyskope 1.0 identified 6/20 (30%) replicates as positive for EAE
- ***S. enterica* (1 target):** Polyskope 1.0 identified 8/20 (40%) replicates as positive for *S. enterica*.
- ***L. monocytogenes* (1 target):** Polyskope 1.0 identified 15/20 (75%) replicates as positive for *L. monocytogenes*
- Internal control detected on all replicates

Methods Comparison Results

- ***E. coli* O157**: FDA BAM 4A identified 7/20 (35%) of replicates as positive for *E. coli* O157
- ***S. enterica***: USDA MLG 4.08 identified 7/20 (35%) of replicates as positive for *S. enterica*
- ***L. monocytogenes***: USDA MLG 8.09 identified 15/20 (75%) of replicates as positive for *L. monocytogenes*

### *30-75% detection of all targets at 1 CFU/25g meeting AOAC requirements*
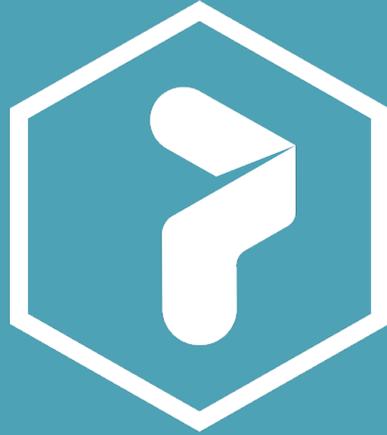
AOAC Method Comparison Study

Deli Turkey

5 CFU/25g

RTE Deli Meat (Deli Turkey) Validation (5 CFU/25g)

Experimental Design

- High-level Inoculation: 5 replicates of 5 CFU/25g deli turkey
    - 5 CFU inoculation is AOAC-required higher level of inoculation
    - All 3 target organisms inoculated, stressed and enriched simultaneously
        - 3.42 CFU/25g *E. coli* O157:H7 (ATCC:43895)
        - 4.30 CFU/25g *S. enterica* (ATCC:13076)
        - 5.50 CFU/25g *L. monocytogenes* (ATCC: 13932)

- All bacteria inoculated into deli turkey and stored at 4°C for 48 hours
    - As per AOAC guidelines

- After 48 hour incubation, samples enriched at 37°C for 24 hours
    - Enriched in PolySkope proprietary enrichment media (PMM)
    - Aerobic plate count (APC) performed on uninoculated control indicated 2.7 X10^3 native bacteria per gram

- Proprietary lysis procedure
    - Maximizes recovery and detection of bacterial DNA

- PolySkope 1.0 run on lysate (ThermoFisher Quantstudio 5 instrument)
   - Maximizes recovery and detection of bacterial DNA

Amplification Plot
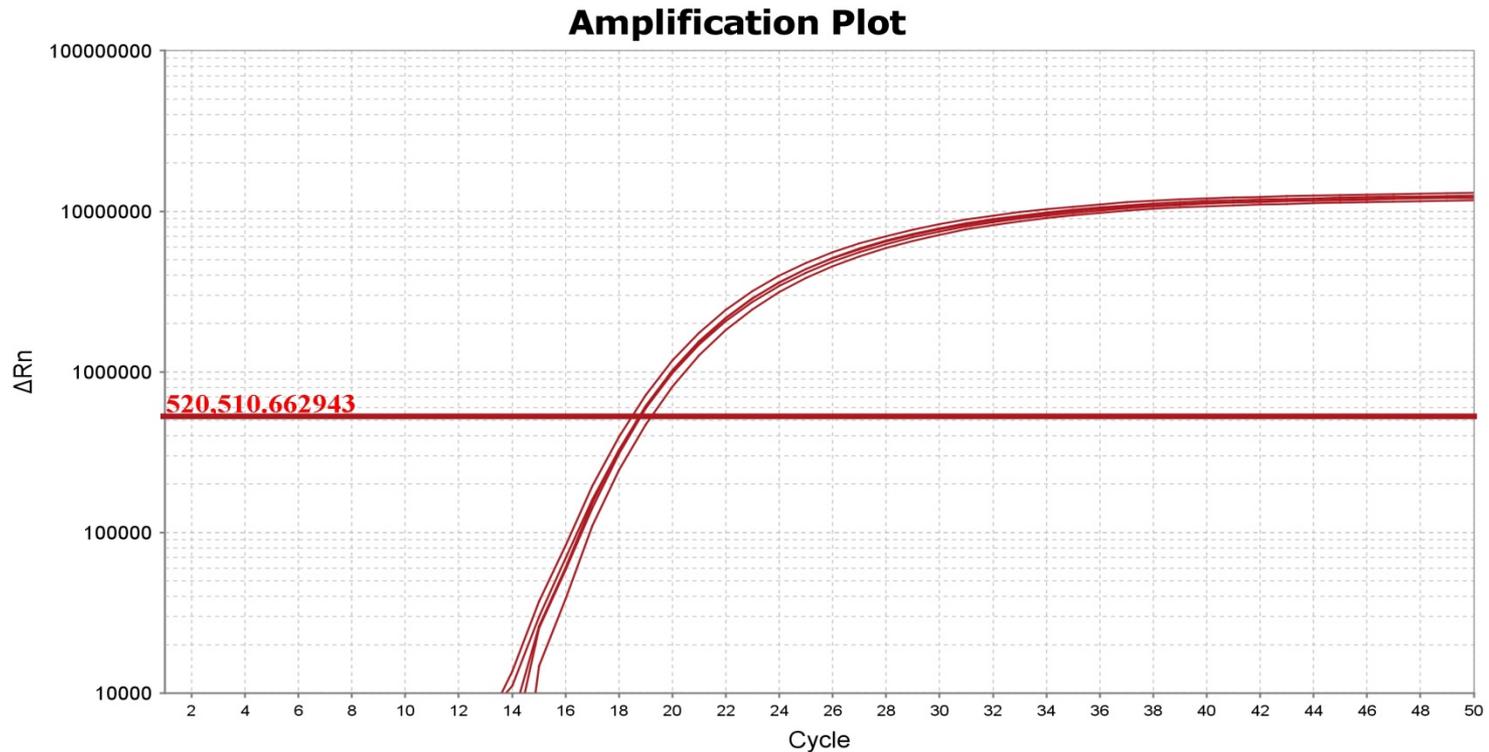
**Polyskope 1.0 detected STX-1 and STX-2 in 5/5 replicates (100% recovery)**

Amplification Plot
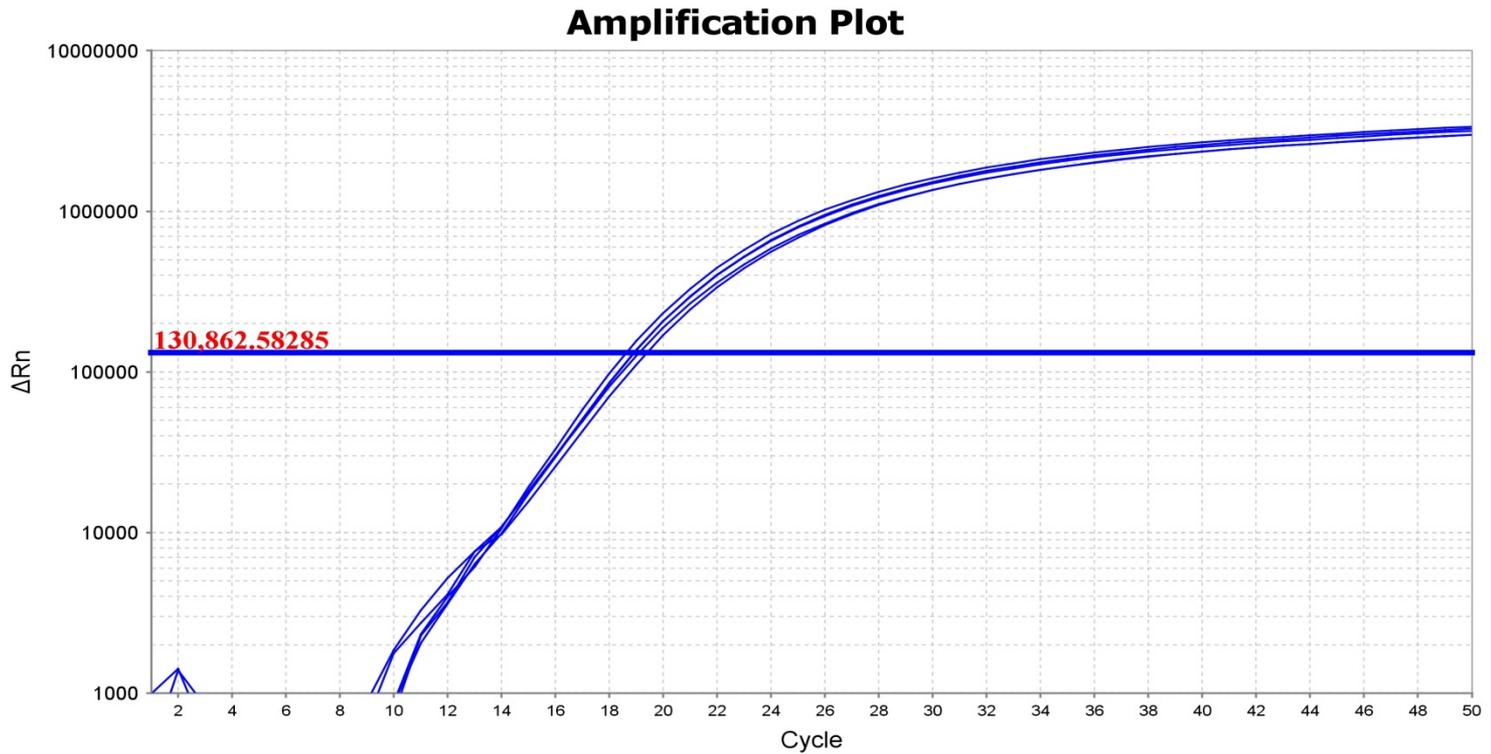
Polyskope 1.0 detected *E. coli* EAE in 5/5 replicates (100% recovery)
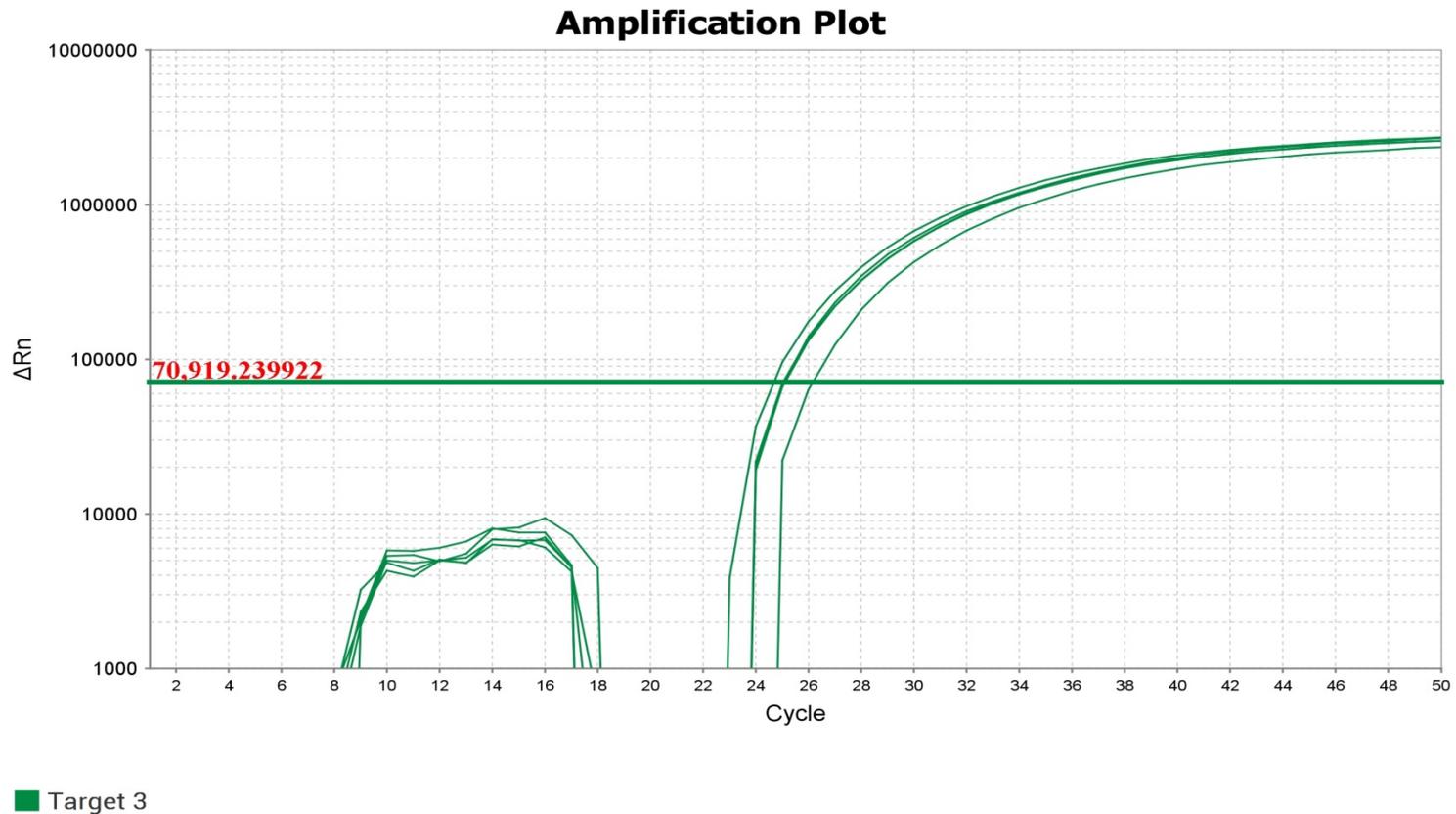
Polyskope 1.0 detected *L. monocytogenes* in 5/5 replicates (100% recovery)
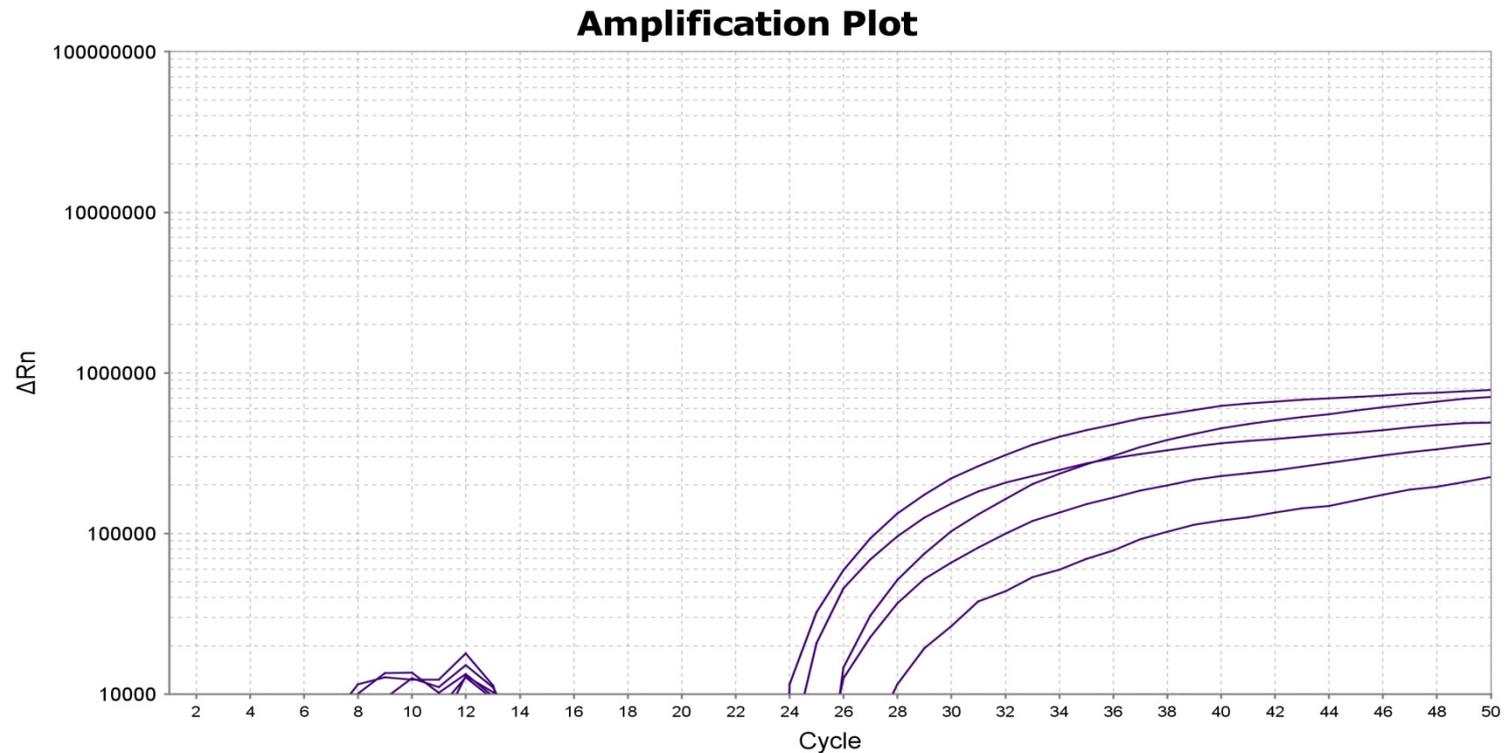
Polyskope 1.0 detected *S. enterica* in 5/5 replicates (100% recovery)
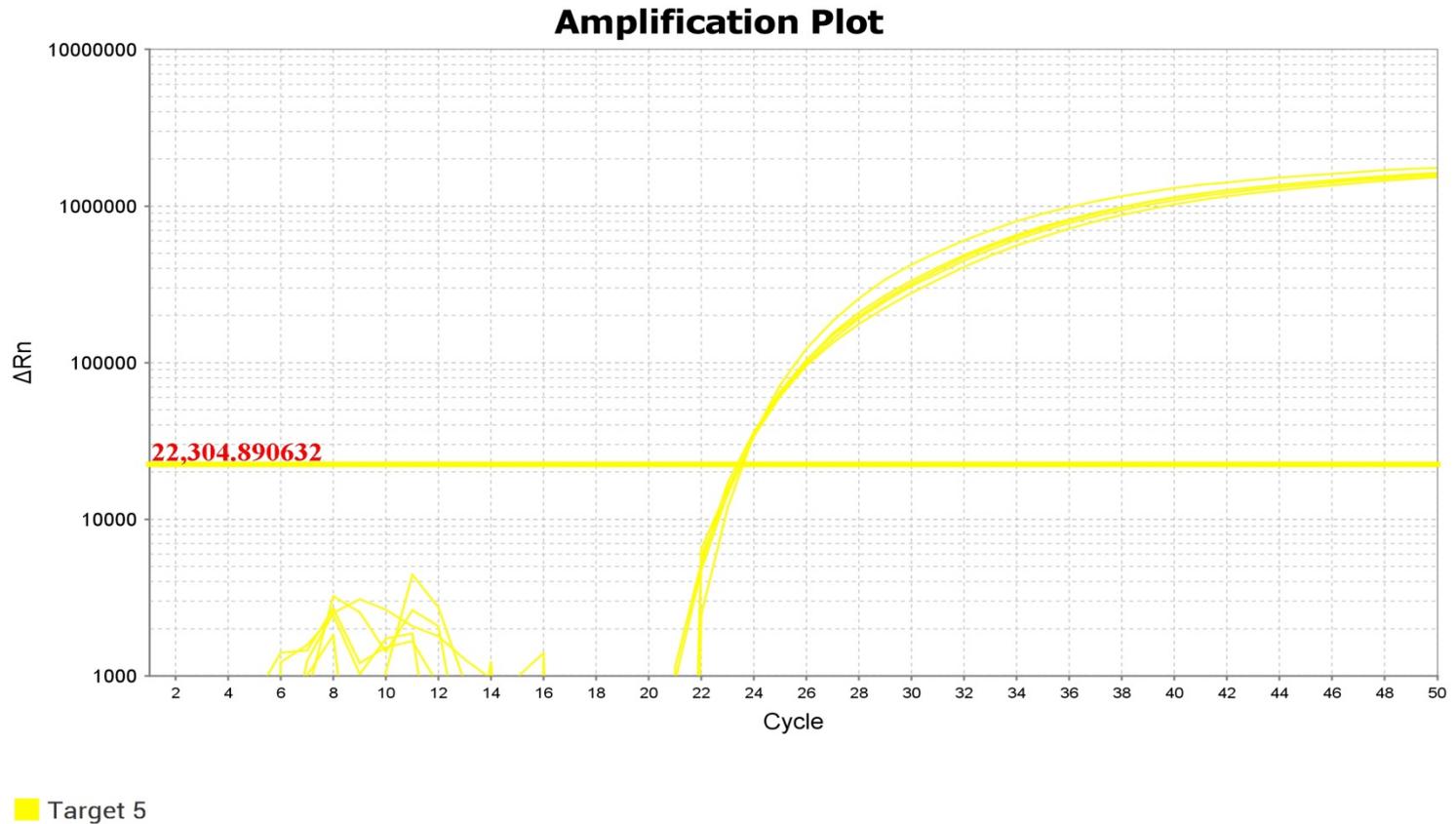
Polyskope 1.0 detected the internal control in 5/5 replicates (100% recovery)

Amplification Plot

Legend: Target 5, Target 3, Target 4, Target 1, Target 2
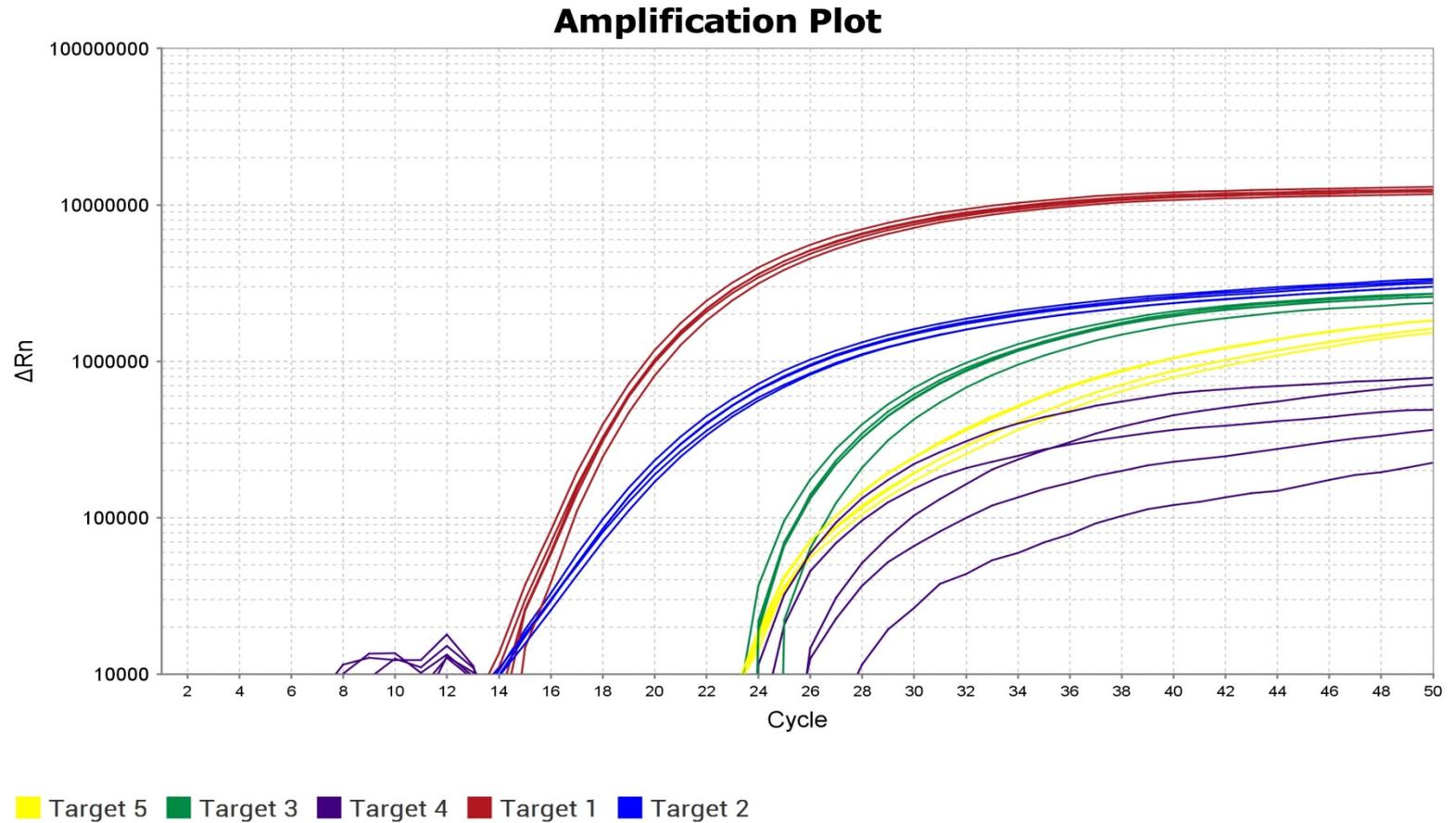
Polyskope 1.0 easily detects all targets when present in the same reaction

Deli Turkey - 5 CFU/25g – Polyskope PCR Results

	STX-1/STX-2	L. monocytogenes	S. enterica	EAE	Internal Control
Replicate	"+/-"	"+/-"	"+/-"	"+/-"	"+/-"
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+

Polyskope 1.0 detected all targets in 100% of replicates at a 5 CFU inoculation level

Deli Turkey - 5 CFU/25g – AOAC BAM and MLG Method Comparison Results

	E. coli	*L. monocytogenes*	*S. enterica*
Replicate	"+/-"	"+/-"	"+/-"
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+

BAM and MLG methods detected the 5 CFU inoculation level in 100% of replicates for all pathogens

Polyskope PCR and Method Comparison Results - 5 CFU/25g

Polyskope 1.0	% Positive	USDA/FDA Methods	% Positive
STX-1/STX-2 and EAE targets	100%	*E. coli*	100%
L. monocytogenes target	100%	*L. monocytogenes*	100%
S. enterica target	100%	*S. enterica*	100%

Conclusion – Deli Turkey - 5 CFU/25g

- ***E. coli* (STEC) targets (3 targets)**
  - **STX-1/STX-2**: Polyskope 1.0 identified 5/5 (100%) replicates as positive for STX-1/STX-2
  - **EAE:** Polyskope 1.0 identified 5/5 (100%) replicates as positive for EAE
- ***S. enterica* (1 target):** Polyskope 1.0 identified 5/5 (100%) replicates as positive for *S. enterica*.
- ***L. monocytogenes* (1 target):** Polyskope 1.0 identified 5/5 (100%) replicates as positive for *L. monocytogenes*
- Internal control detected in all replicates

Methods Comparison Results
- ***E. coli* O157**: FDA BAM 4A identified 5/5 (100%) of replicates as positive for *E. coli* O157
- ***S. enterica***: USDA MLG 4.08 identified 5/5 (100%) of replicates as positive for *S. enterica*
- ***L. monocytogenes***: USDA MLG 8.09 identified 5/5 (100%) of replicates as positive for *L. monocytogenes*

100% detection of all pathogens at 5 CFU/25g

Method Agreement: Polyskope 1.0 vs Reference Methods

Pathogen	Innoculum Level	MPN/25g	Number of Samples	PolySkope Positive	Reference Positive	Method Agreement	Sensitivity	Specificity
L. mono	Control	0	5	0	0	100%	NA	100%
	High	5.6	5	5	5	100%	100%	
	Low	1.12	20	15	15	100%	100%	
E. coli STEC	Control	0	5	0	0	100%	NA	100%
	High	4.0	5	5	5	100%	100%	
	Low	0.8	20	6	7	95%	86%	
S. enterica	Control	0	5	0	0	100%	NA	100%
	High	4.4	5	5	5	100%	100%	
	Low	0.88	20	8	7	105%	114%	

*95-100% method agreement in detection of all targets
with sensitivity and specificity meeting AOAC requirements*