

POLYSKOPE L A B S

Preliminary Validation

Cannabis flower (Hemp surrogate)

2 CFU/1g & 15 CFU/1g

ABI QuantStudio5 & Agilent AriaMX



PolySkope One “Green Box”



- Reduced target set, Shiga Toxin *E. coli* & *Salmonella* spp.
- STEC & *Salmonella* testing required for the detection of microbial contaminants



Experimental Design - Inoculation

Experimental Design

- All bacteria inoculated into CBD-containing hemp strain as Cannabis surrogate (Suver Haze, Tweedle Farms) and incubated at 37°C.
 - All target organisms inoculated and enriched simultaneously
 - *E. coli* O157:H7 (ATCC:43895)
 - *S. enterica* (ATCC:13076)
- Low-level Inoculation: 15 replicates of 2 CFU/1g per matrix
 - 2 CFU inoculation is within 0.2-2.0 CFU range of lower limit of detection
 - Inoculated into 1g matrix
- High-level Inoculation: 15 replicates of 15 CFU/1g per matrix
 - 15 CFU inoculation to demonstrate efficacy in high-titer levels of pathogens
 - Inoculated into 1g matrix



Experimental Design – Recovery and Detection

Experimental Design

- Samples enriched at 37°C for 24 hours
 - Enriched in PolySkope proprietary enrichment media (PMEM)
- Proprietary lysis procedure
 - Maximizes recovery and detection of bacterial DNA
- PolySkope One assay run on lysate
 - Real Time PCR detection of nucleic acid targets
 - Run on ThermoFisher ABI QuantStudio 5 (96-well format)
& Agilent AriaMX (96-well format) in parallel



QuantStudio® 5



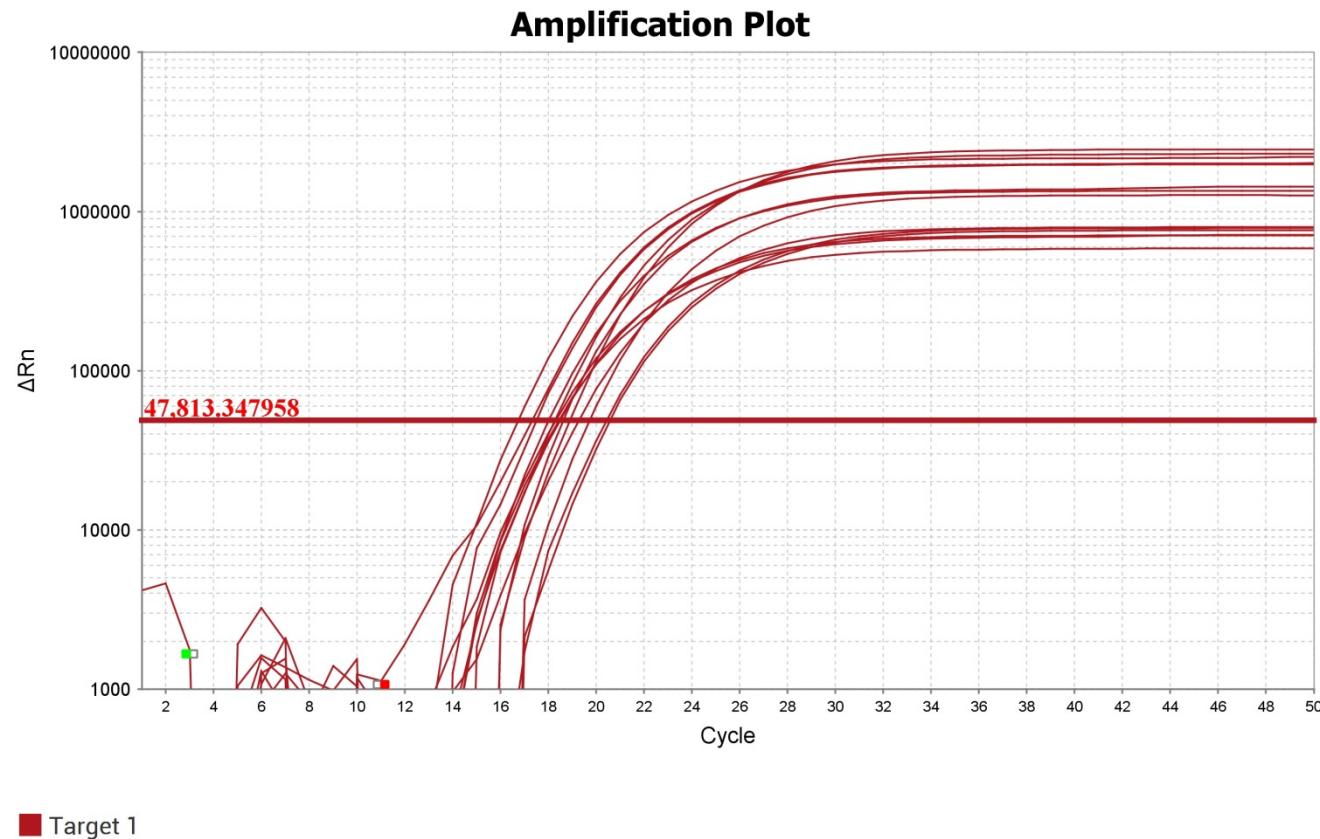
Study Specifics

Experimental Design

- Low-level Inoculation: 15 replicates of 2 CFU/1g Hemp
 - 0.2-2.0 CFU inoculation is AOAC-required lower limit of detection
 - All 3 target organisms inoculated and incubated simultaneously
 - 2.65 CFU/25g *E. coli* O157:H7 (ATCC:43895)
 - 3.3 CFU/25g *S. enterica* (ATCC:13076)
- High Level Inoculation: 15 replicates of 15 CFU/1g Hemp
 - All 3 target organisms inoculated and incubated simultaneously
 - 19.8 CFU/25g *E. coli* O157:H7 (ATCC:43895)
 - 24.75 CFU/25g *S. enterica* (ATCC:13076)



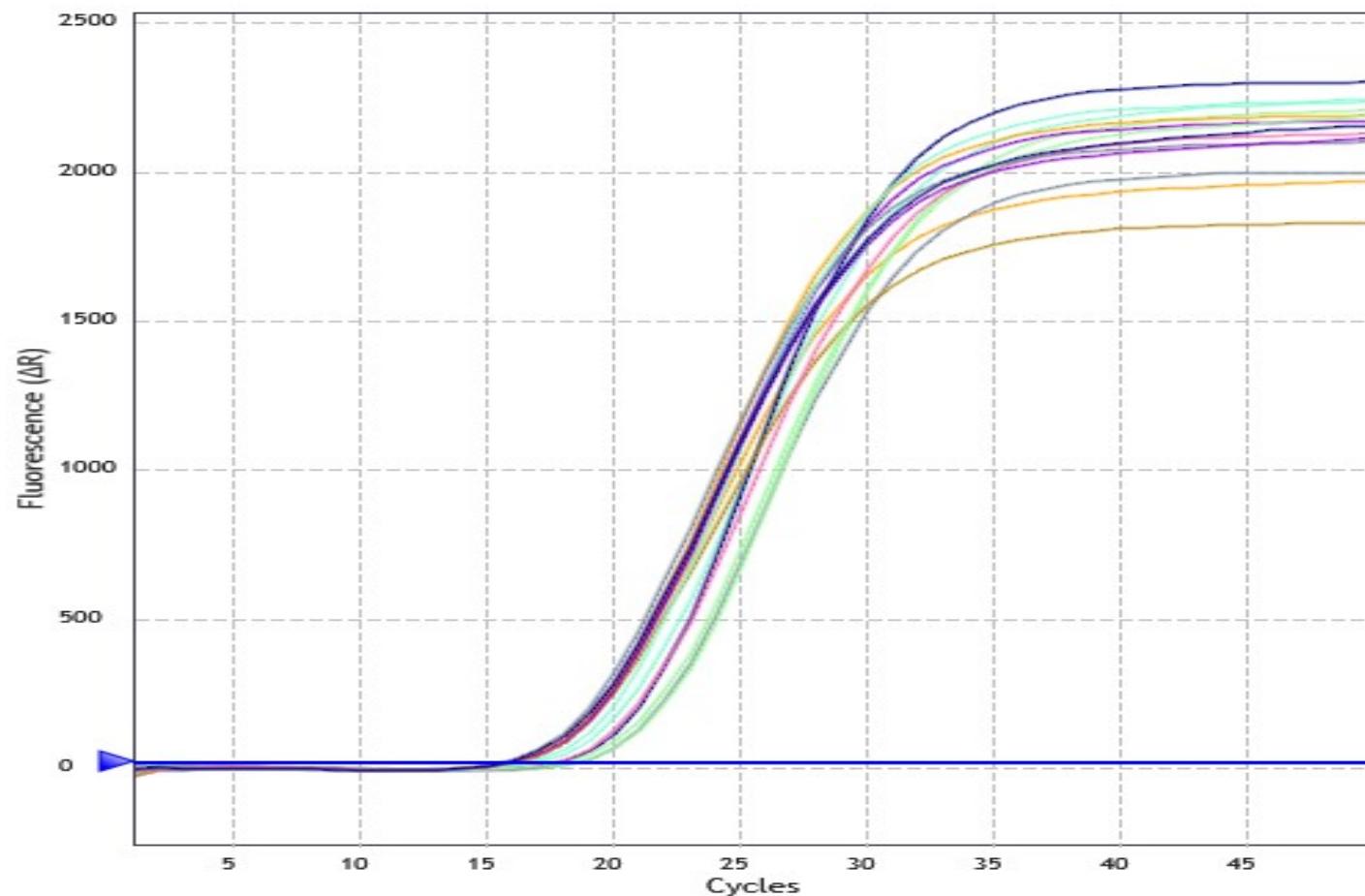
QuantStudio5, Hemp – 2 CFU *E. coli* O157:H7 STEC STX-1 and STX-2



Polyskope 1.0 detected STX-1 and STX-2 targets in 14/15 replicates (93% recovery)



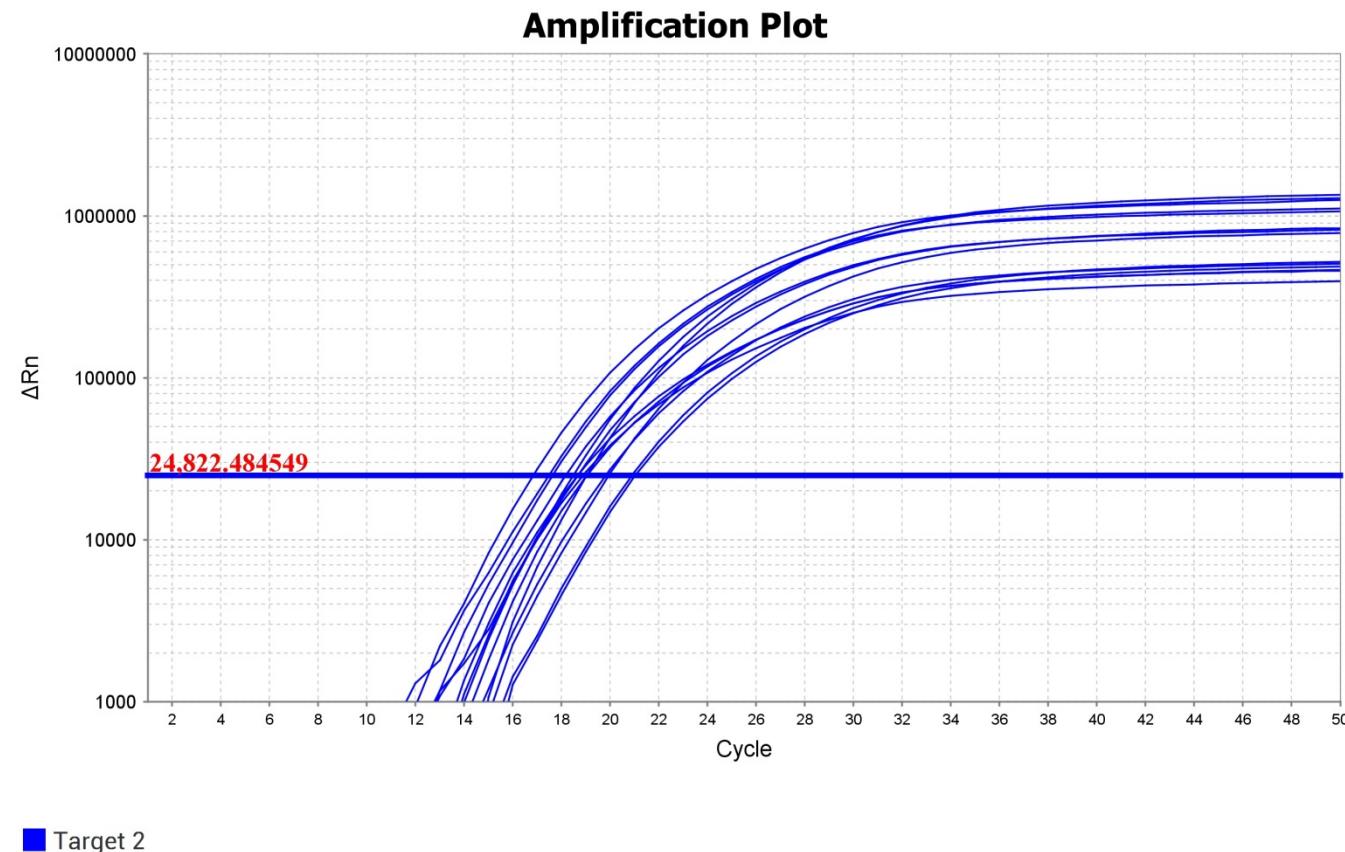
AriaMX, Hemp – 2 CFU *E. coli* O157:H7 STEC STX-1 and STX-2



Polyskope 1.0 detected STX-1 and STX-2 targets in 14/15 replicates (93% recovery)



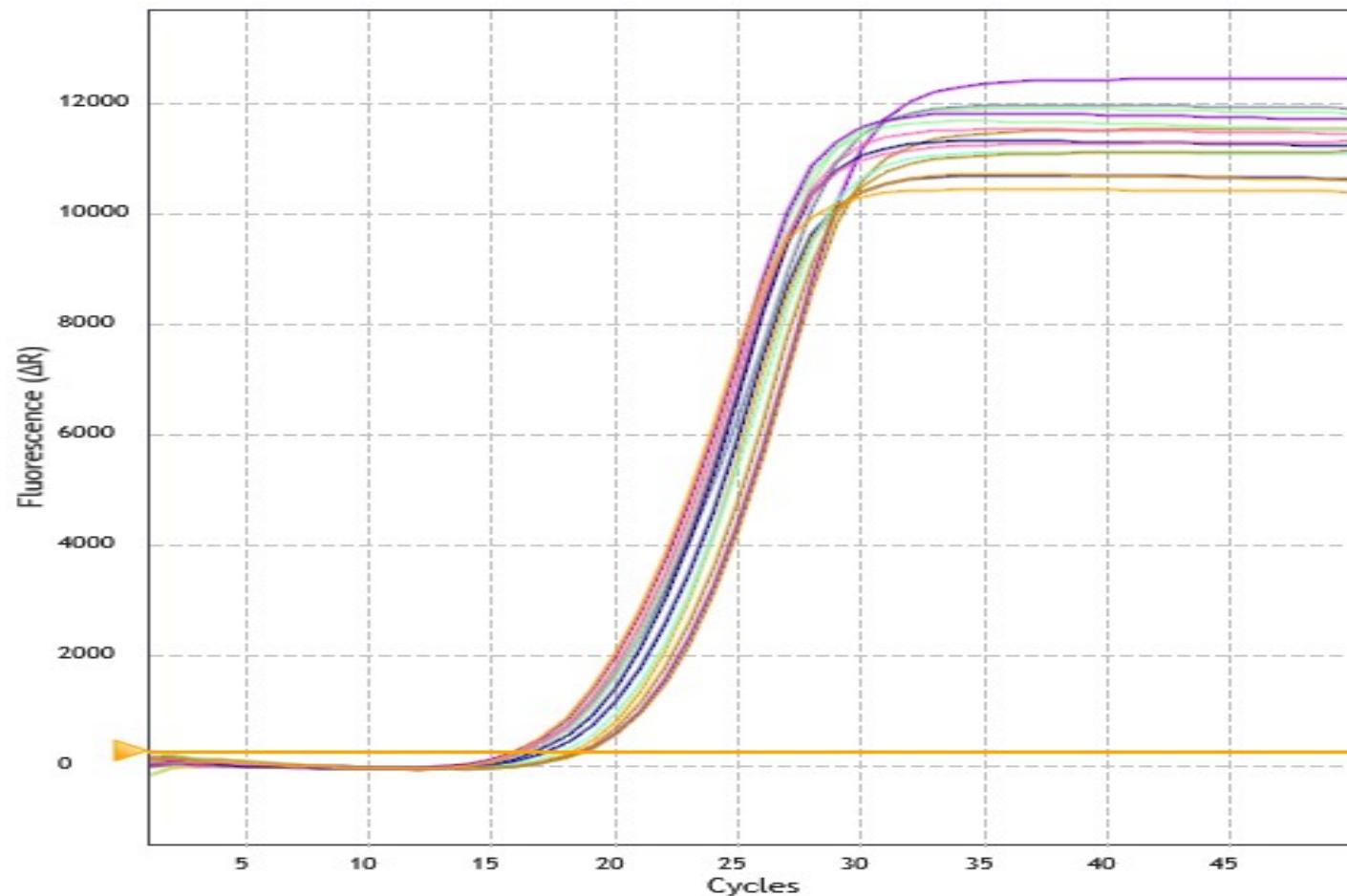
QuantStudio5, Hemp – 2 CFU *E. coli* O157:H7 STEC EAE target



Polyskope 1.0 detected *E. coli* EAE in 14/15 replicates (93% recovery)



AriaMX, Hemp – 2 CFU *E. coli* O157:H7 STEC EAE target

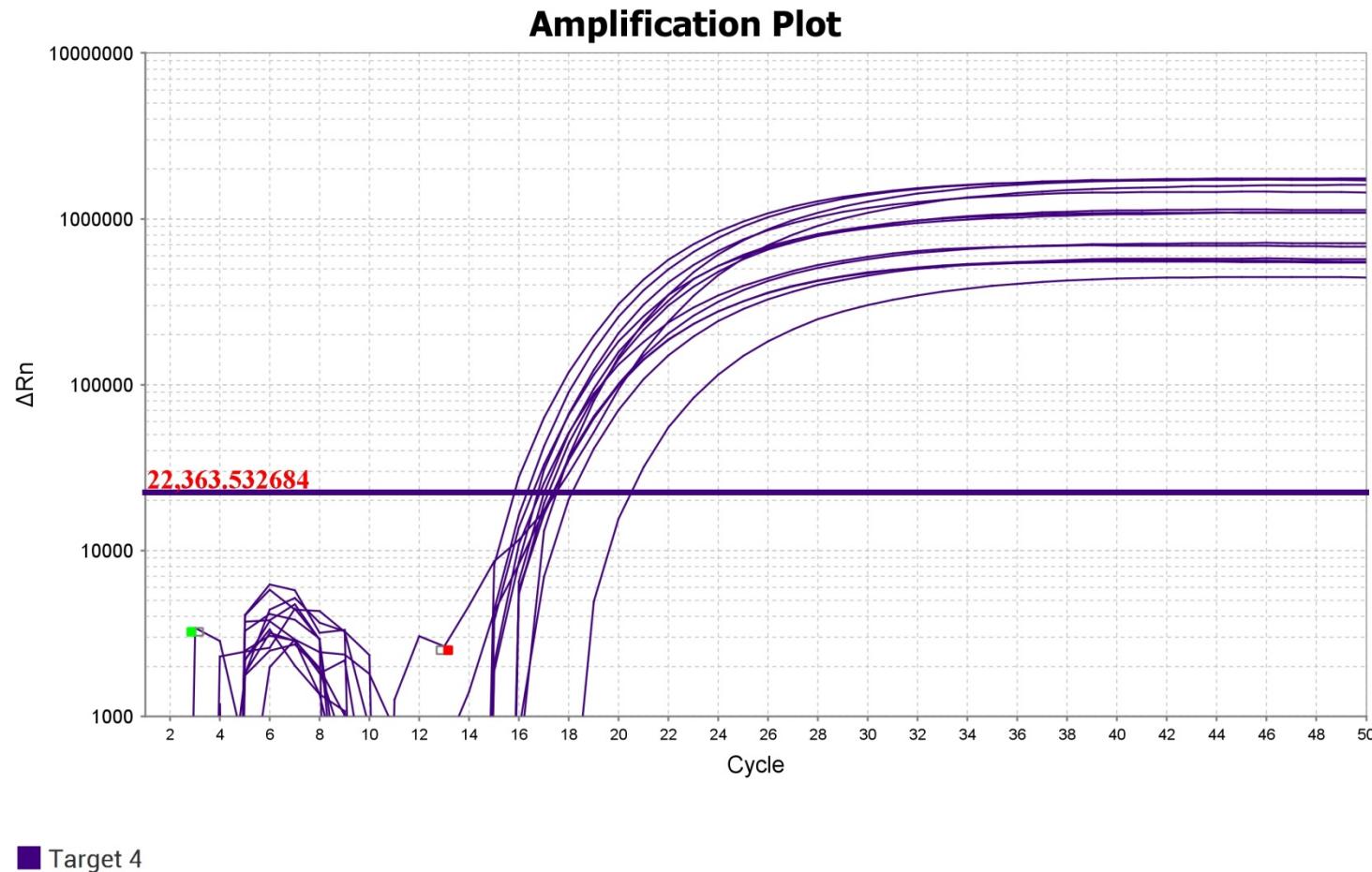


Polyskope 1.0 detected *E. coli* EAE in 14/15 replicates (93% recovery)



ABI QuantStudio5

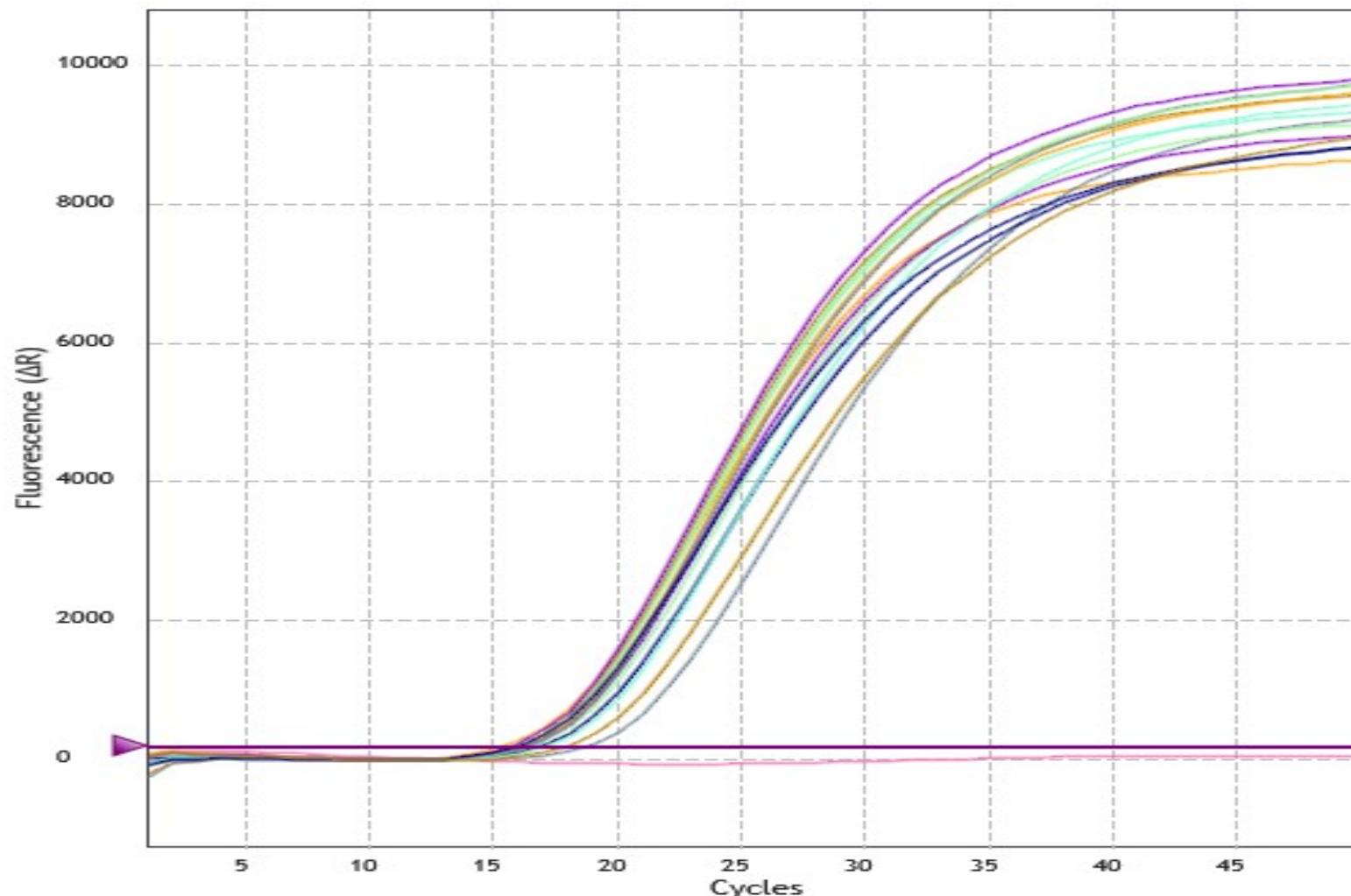
Hemp – 2 CFU *S. enterica* target



Polyskope 1.0 detected the *S. enterica* target in 14/15 replicates (93% recovery)



AriaMX, Hemp – 2 CFU *S. enterica* target

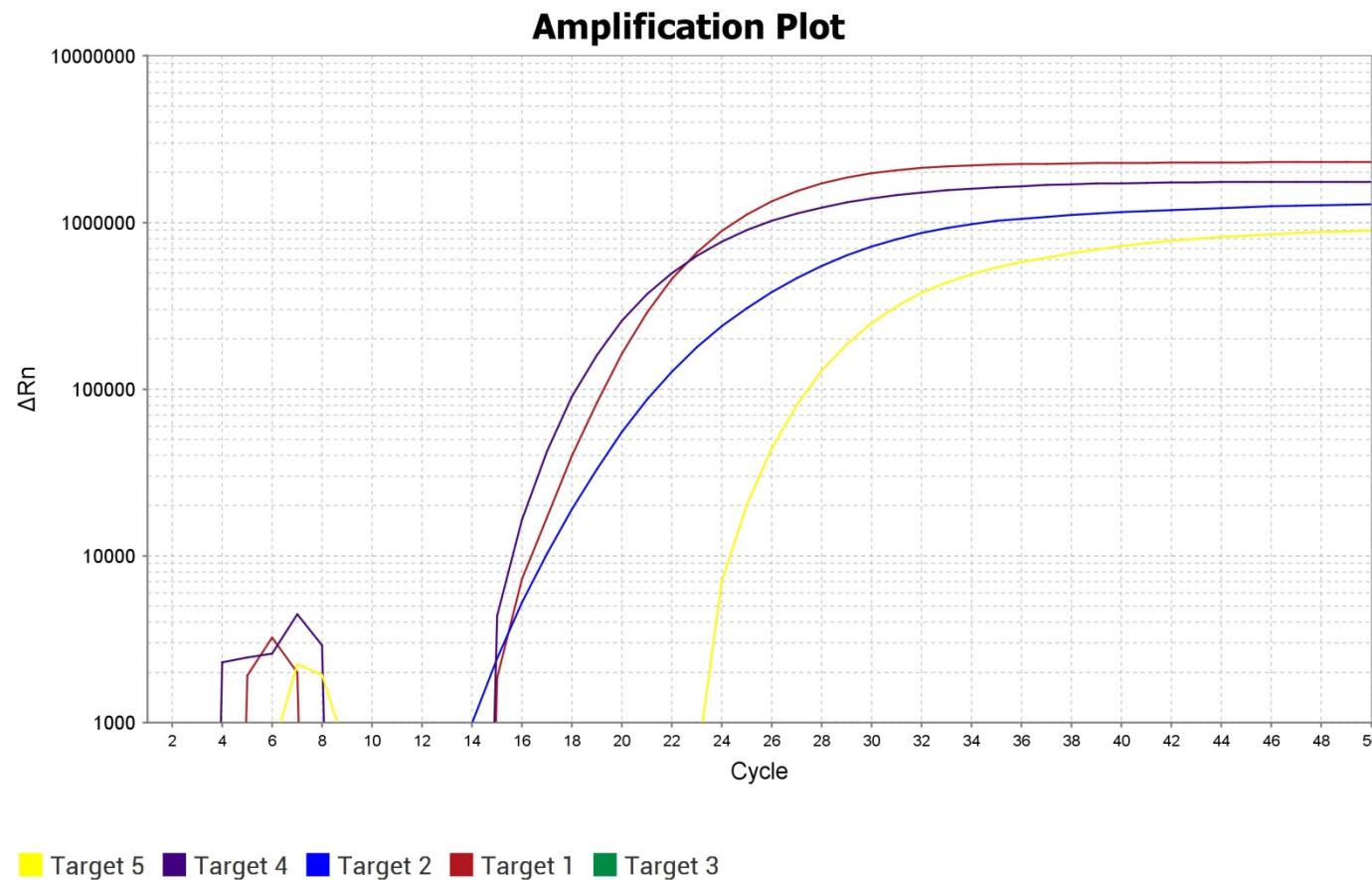


Polyskope 1.0 detected the *S. enterica* target in 14/15 replicates (93% recovery)



QuantStudio5

All Targets in a Single Reaction – 2 CFU

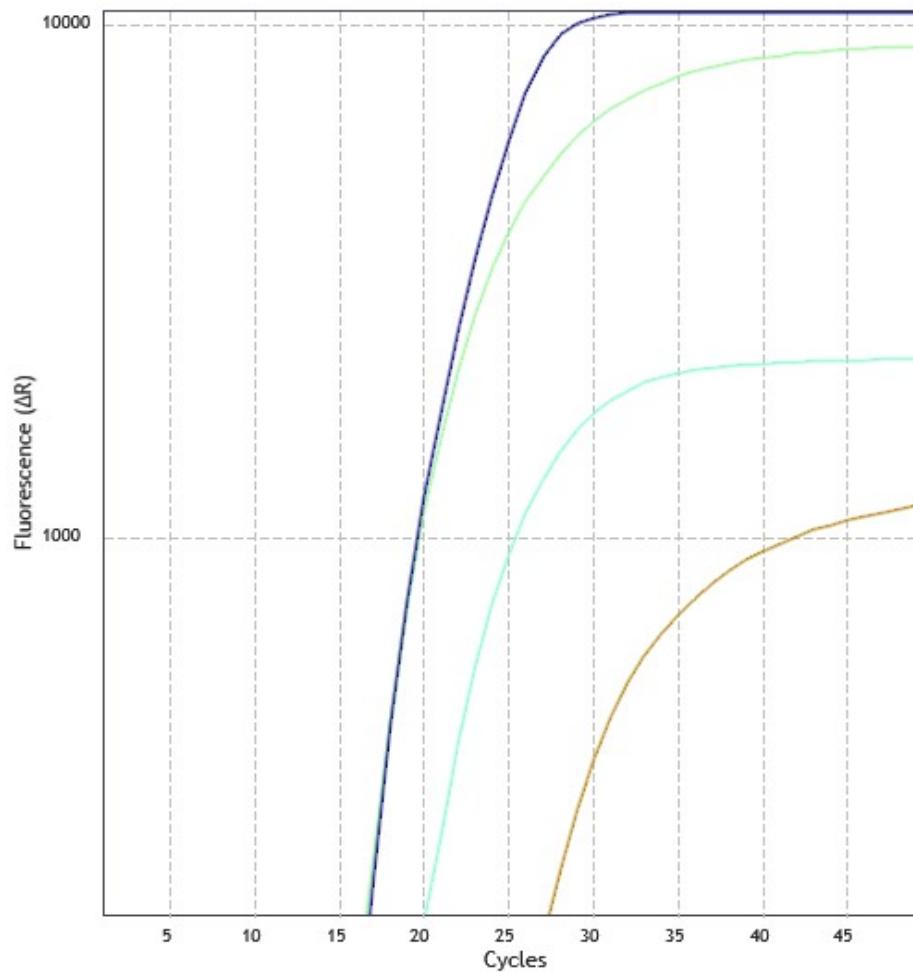


Example of Polyskope 1.0 detecting all pathogen targets in a single enrichment and PCR reaction.



AriaMX , Hemp

All Targets in a Single Reaction – 2 CFU



Example of Polyskope 1.0 detecting all pathogen targets in a single enrichment and PCR reaction.



Results & Conclusion – 2 CFU/1g

- ***Shiga Toxin E. coli (STEC) targets***
 - **STX-1/STX-2:** Polyskope One identified 93% of replicates as positive for STX-1/STX-2
 - **EAE:** Polyskope One identified 93% replicates as positive for EAE
- ***Salmonella enterica***
 - Polyskope One identified 93% of replicates as positive for *S. enterica*.
- Internal control detected on all replicates
- **QuantStudio5 & AriaMX Agreement**
100% agreement between QS5 and Aria results for all analytes

93% detection of all targets at 2 CFU/1g



Results Table

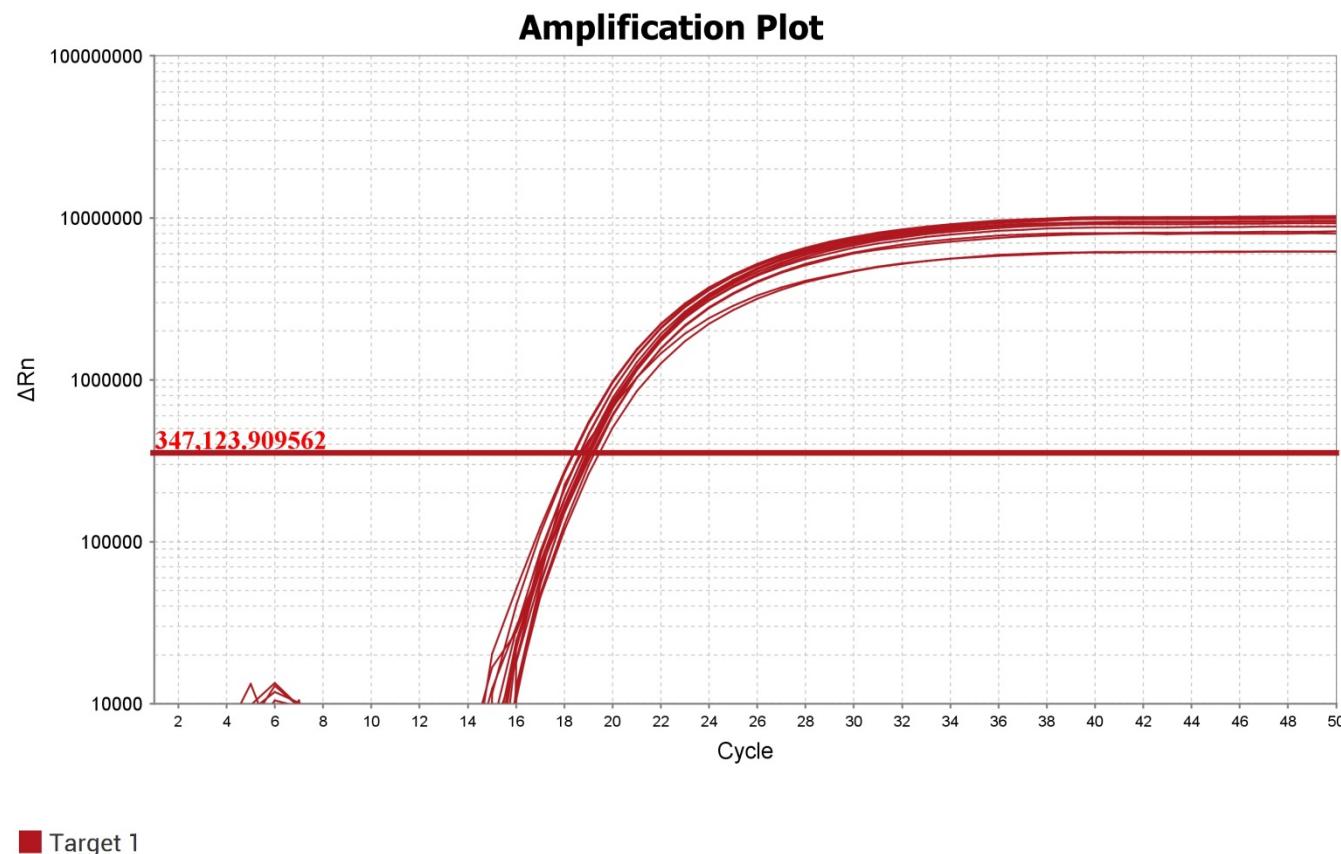
AriaMX and Quantstudio5, 2 CFU/ 1g

	AriaMX			Quantstudio 5		
	STX-1/ STX-2	EAE	<i>Salmonella</i> spp.	STX-1/ STX-2	EAE	<i>Salmonella</i> spp.
Replicate	"+/-"	"+/-"	"+/-"	"+/-"	"+/-"	"+/-"
1	-	-	+	-	-	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	+	+	-	+	+	-
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	+	+	+	+	+
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+	+	+	+

100% agreement on endpoint detection for all targets across both instruments



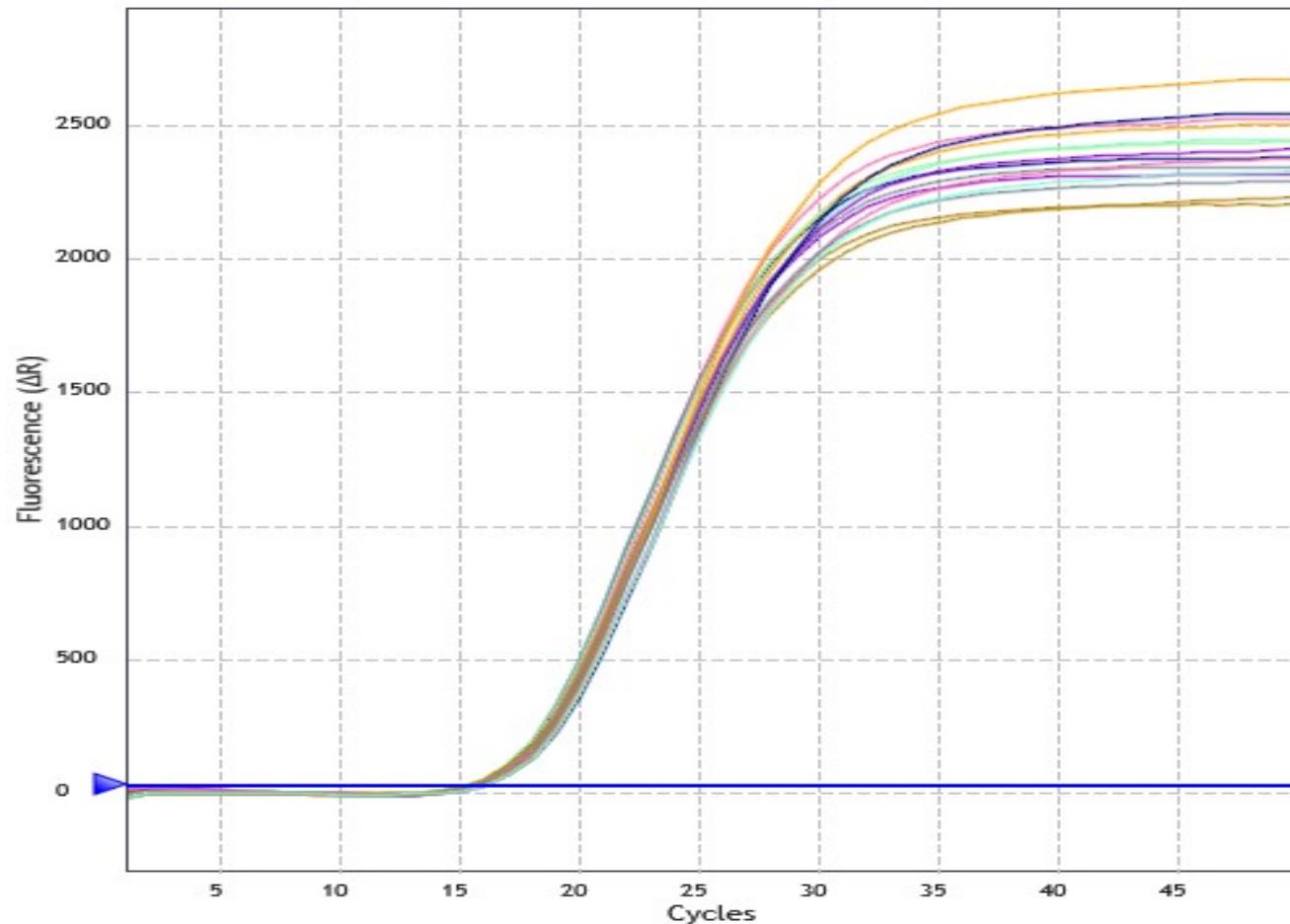
QuantStudio5, Hemp – 15 CFU *E. coli* O157:H7 STEC STX-1 and STX-2



Polyskope 1.0 detected STX-1 and STX-2 in 15/15 replicates (100% recovery)



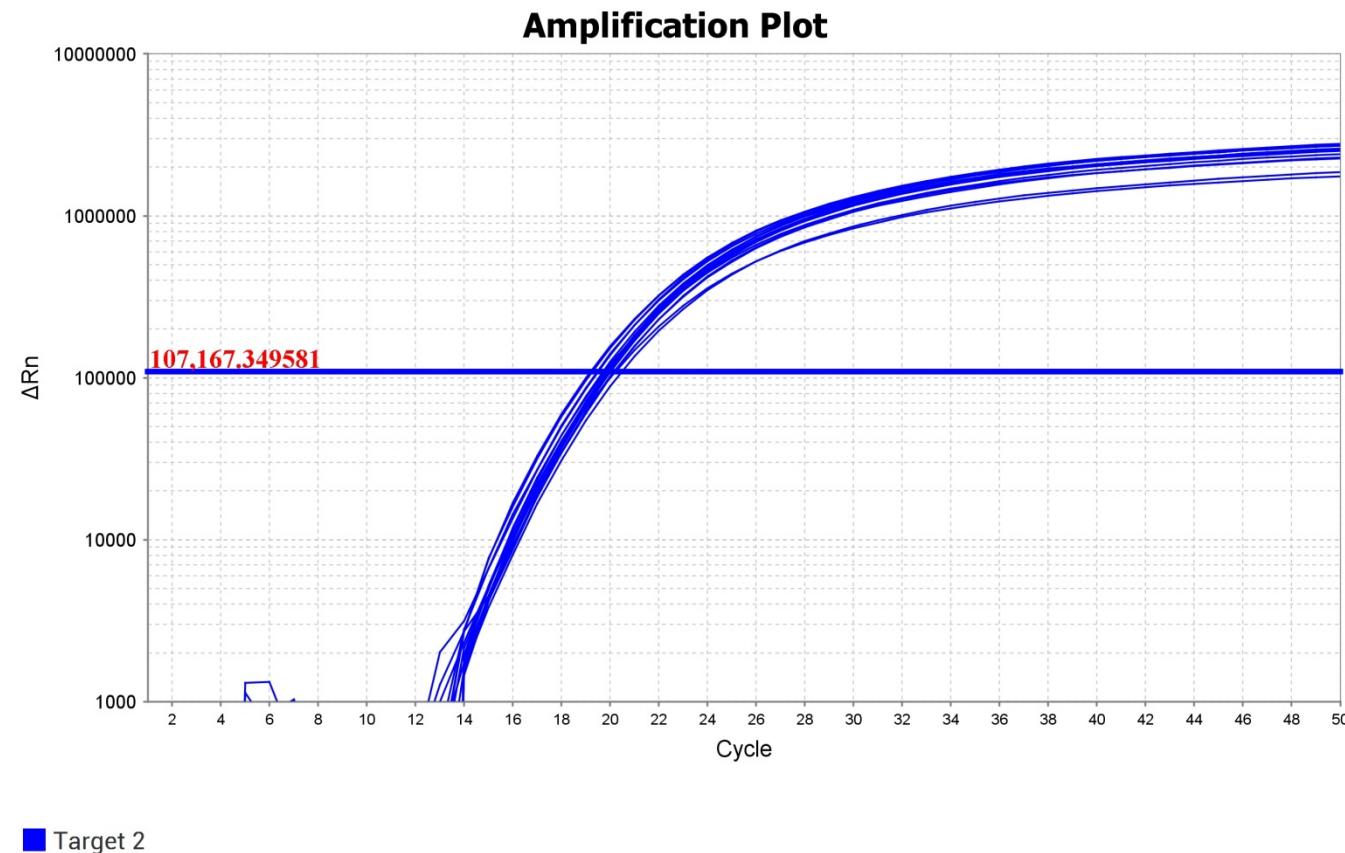
AriaMX, Hemp – 15 CFU *E. coli* O157:H7 STEC STX-1 and STX-2



Polyskope 1.0 detected STX-1 and STX-2 in 15/15 replicates (100% recovery)



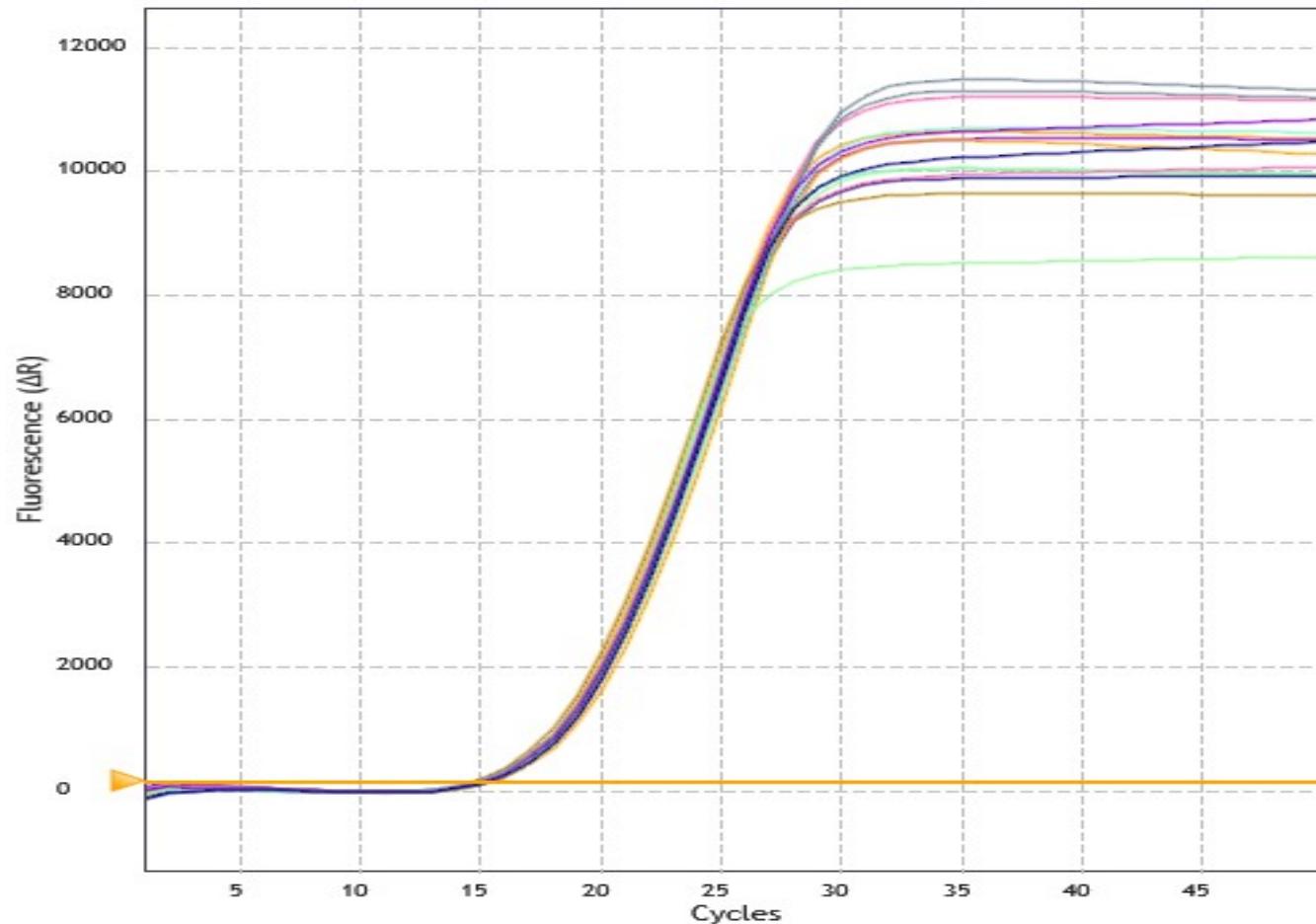
QuantStudio5, Hemp – 15 CFU *E. coli* O157:H7 STEC EAE target



Polyskope 1.0 detected *E. coli* EAE in 5/5 replicates (100% recovery)



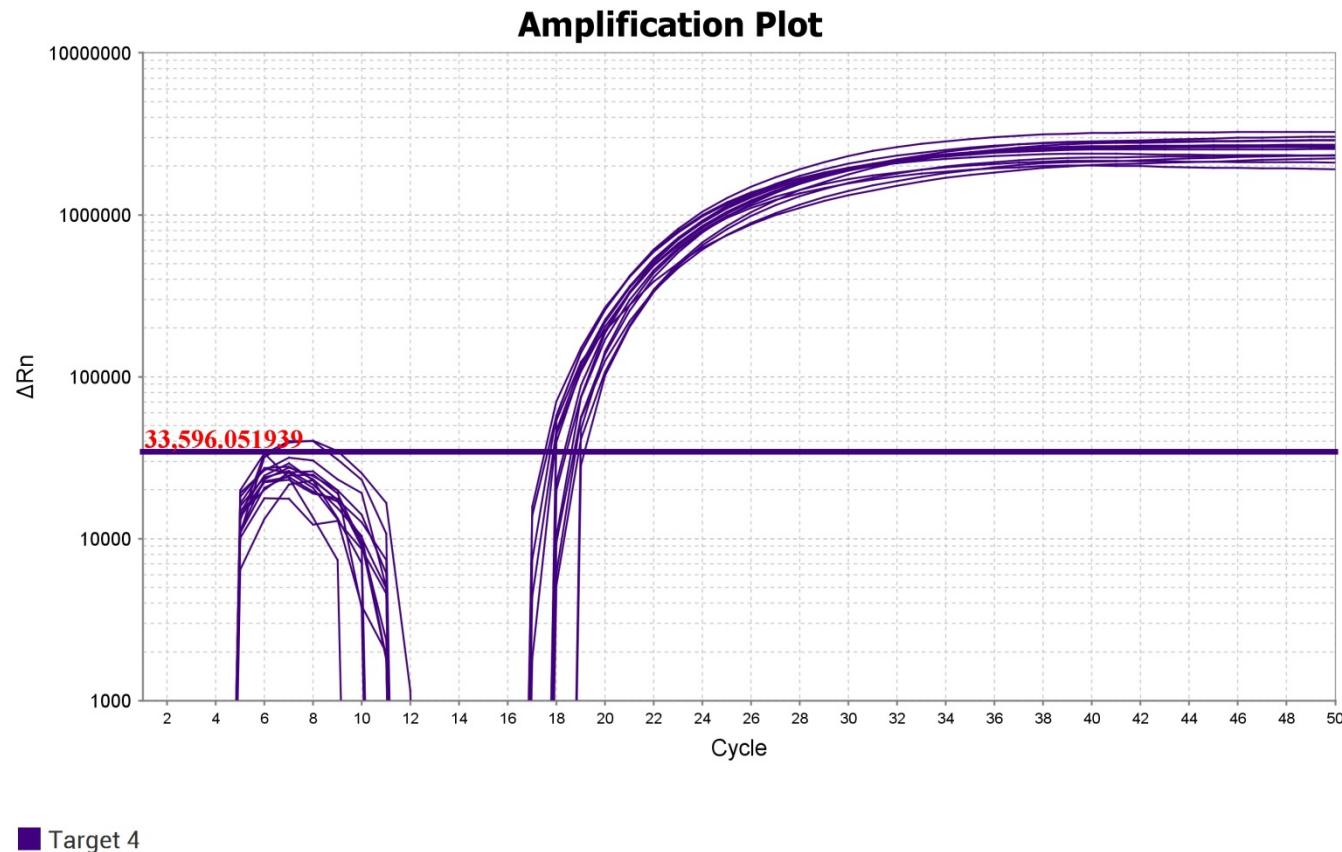
AriaMX, Hemp – 15 CFU *E. coli* O157:H7 STEC EAE target



Polyskope 1.0 detected *E. coli* EAE in 15/15 replicates (100% recovery)



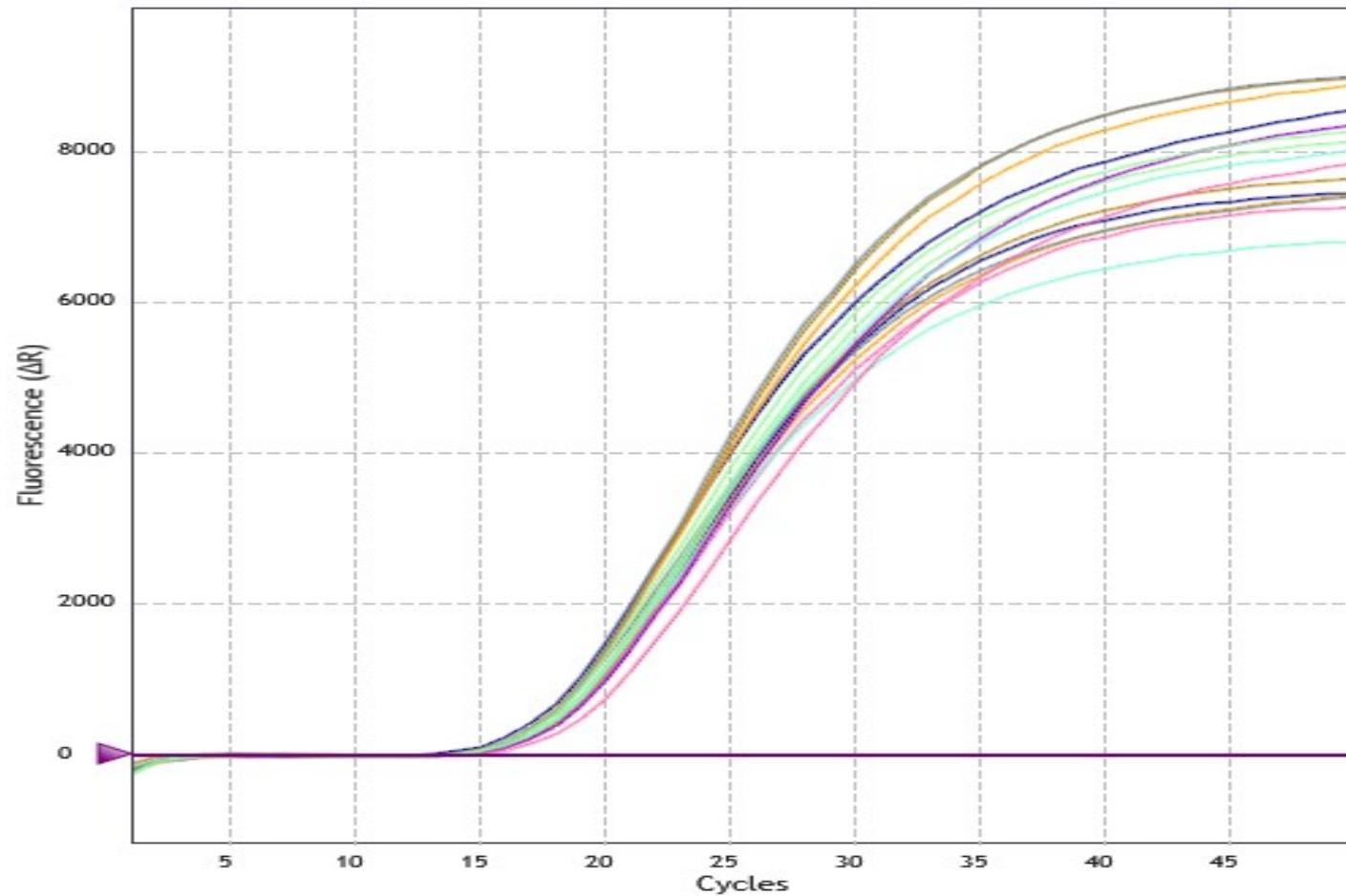
QuantStudio5, Hemp – 15 CFU *S. enterica* target



Polyskope 1.0 detected *S. enterica* in 15/15 replicates (100% recovery)



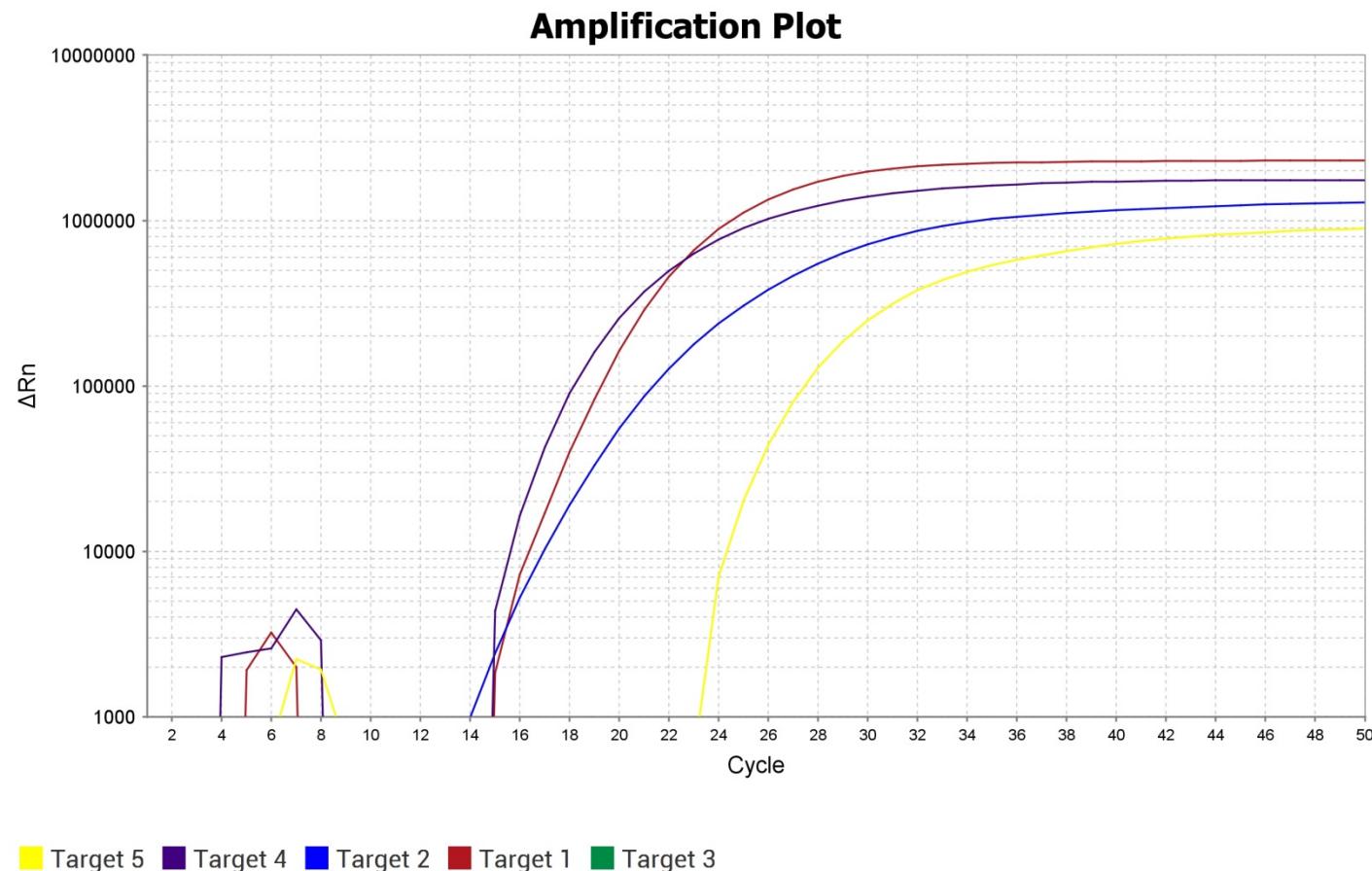
AriaMX, Hemp – 15 CFU *S. enterica* target



Polyskope 1.0 detected *S. enterica* in 15/15 replicates (100% recovery)



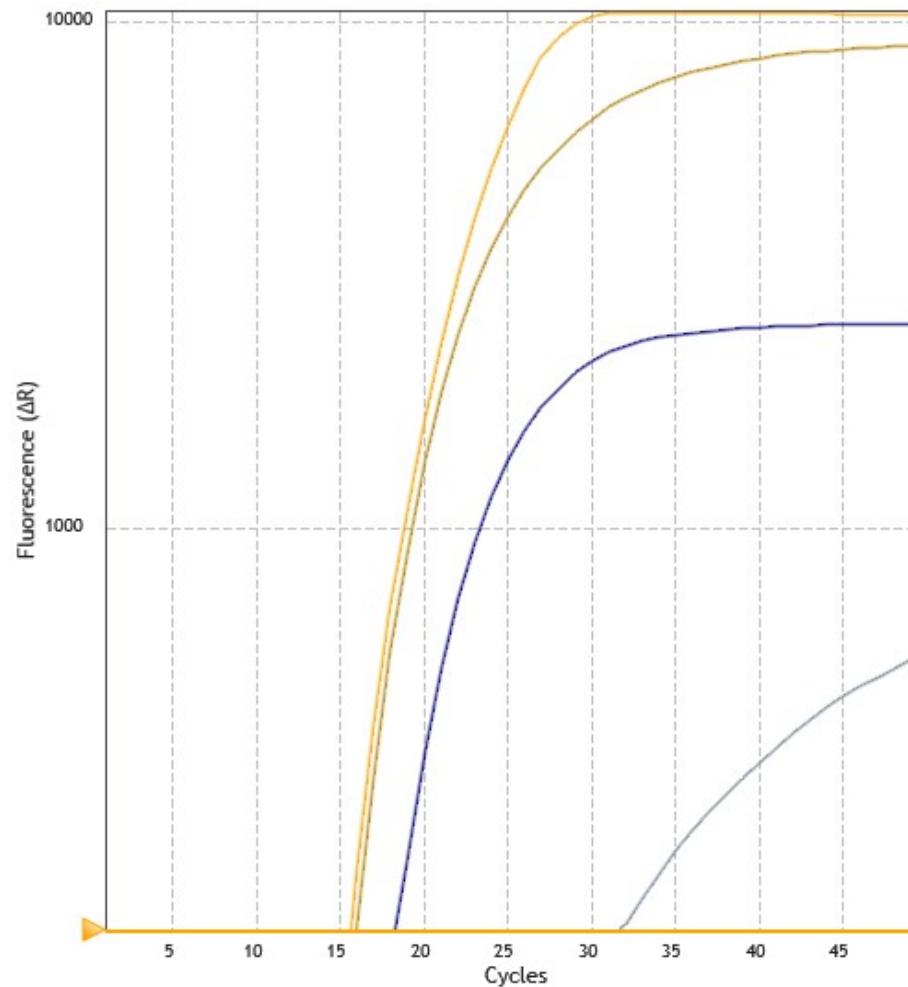
QuantStudio5, Hemp 15 CFU All Targets Present



Polyskope 1.0 easily detects all targets when present in the same reaction



AriaMX, Hemp 15 CFU All Targets Present



Polyskope 1.0 easily detects all targets when present in the same reaction



Results & Conclusion 15 CFU

- ***Shiga Toxin E. coli (STEC) targets***
 - **STX-1/STX-2:** Polyskope One identified 100% of replicates as positive for STX-1/STX-2
 - **EAE:** Polyskope One identified 100% replicates as positive for EAE
- ***Salmonella enterica***
 - Polyskope 1.0 identified 100% of replicates as positive for *S. enterica*.
- Internal control detected on all replicates
- **QuantStudio5 & AriaMX Agreement**
100% agreement between QS5 and Aria results for all analytes

100% detection of all targets at 15 CFU/1g



Results Table

AriaMx and Quantstudio5, 15 CFU/g

	AriaMX			Quantstudio 5		
	STX-1/ STX-2	EAE	<i>Salmonella</i> spp.	STX-1/ STX-2	EAE	<i>Salmonella</i> spp.
Replicate	"+/-"	"+/-"	"+/-"	"+/-"	"+/-"	"+/-"
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	+	+	+	+	+	+
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	+	+	+	+	+
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+	+	+	+

100% agreement on endpoint detection for all targets across both instruments



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