表 2　共通試料を用いる検査に使用したプライマー

	菌　　種	Tm 値	増幅部位		primer 名称	配列	文献
1	*Clostridium perfringens*	76.1	*cpe*	F	GAP-11	GGTTCATTAATTGAAACTGGTG	4
				R	GAP-12b	AACGCCAATCATATAAATTACCAGC	
2	*Campylobacter jejuni*	77.8	specific	F	AB-F	CTGAATTTGATACCTTAAGTGCAGC	5
			DNA	R	AB-R	AGGCACGCCTAAACCTATAGCT	
3	Emetic toxin producing *Bacillus cereus*	78.5	*ces*	F	ces-TM-F	GATGTTTGCGACGATGCAA	6
				R	ces-TM-R	CTTTCGGCGTGATACCCATT	
4	*eae* positive *Escherichia coli*	78.9	*eaeA*	F	eae-F2	CATTGATCAGGATTTTTCTGGTGATA	7
				R	eae-R	CTCATGCGGAAATAGCCGTTA	
5	*Staphylococcus aureus*	81.5	*femB*	F	FemB-fw	AATTAACGAAATGGGCAGAAACA	8
				R	FemB-rv	TGCGCAACACCCTGAACTT	
6	*tdh* positive *Vibrio parahaemolyticus*	80.5	*tdh*	F	tdh-F176	TCCATCTGTCCCTTTTCCTG	2
				R	R422	AGACACCGCTGCCATTGTAT	
7	*astA* positive *Escherichia coli*	83.7	*astA*	F	EAST-1-S	GCCATCAACACAGTATATCC	9
				R	EAST-AS	GAGTGACGGCTTTGTAGTCC	
8	*Salmonella* spp.	82.6	*invA*	F	invA2-F	GATTCTGGTACTAATGGTGATGATC	10
				R	invA2-R	GCCAGGCTATCGCCAATAAC	

表 3-1 各菌種の希釈濃度別増幅サイクル数(機器別)1

	菌種	希釈	菌量	A	B	C	D	
				TaKaRa Dice	ABI	ABI	ABI	STRATA
			cfu/mL	TP800	7500FAST	7500	7500FAST	Mx3000P
1	*Clostridium perfringens*	10^1	5.0×10^7	11	11	14	11	11
	GAP gene	10^2	5.0×10^6	14	15	17	14	15
		10^3	5.0×10^5	18	20	21	20	19
		10^4	5.0×10^4	23	25	22	23	23
		10^5	5.0×10^3	26	28	23	26	27
		10^6	5.0×10^2	28	30	25	–	–
2	*Campylobacter jejuni*	10^1	4.9×10^7	12	12	15	12	12
	specific gene	10^2	4.9×10^6	16	16	18	16	15
		10^3	4.9×10^5	19	22	21	21	20
		10^4	4.9×10^4	23	26	23	24	24
		10^5	4.9×10^3	26	29	24	28	27
		10^6	4.9×10^2	29	–	25	29	–
3	*Bacillus cereus*	10^1	3.3×10^6	17	18	18	18	17
	ces gene	10^2	3.3×10^5	21	23	23	21	21
		10^3	3.3×10^4	25	27	27	26	25
		10^4	3.3×10^3	27	–	29	30	30
		10^5	3.3×10^2	–	–	–	–	–
		10^6	3.3×10^1	–	–	–	–	–
4	*eae* positive *Escherichia coli*	10^1	1.5×10^8	14	14	15	13	13
	EHEC, EPEC	10^2	1.5×10^7	16	18	18	17	17
	eae gene	10^3	1.5×10^6	21	23	21	21	21
		10^4	1.5×10^5	25	27	23	26	25
		10^5	1.5×10^4	28	30	27	29	29
		10^6	1.5×10^3	–	–	28	–	–

下線付き赤字は増幅がみられたものの 30 サイクルまでに指数増幅期に移行しなかったもの

「－」は増幅がみられなかったもの

表 3-2 各菌種の希釈濃度別増幅サイクル数(機器別)2

	菌種	希釈	菌量	A	B	C	D	
				TaKaRa Dice	ABI	ABI	ABI	STRATA
			cfu/mL	TP800	7500FAST	7500	7500FAST	Mx3000P
5	*Staphylococcus aureus*	10^1	1.4×10^8	13	13	15	13	13
	FemB gene	10^2	1.4×10^7	17	18	19	17	17
		10^3	1.4×10^6	21	23	21	22	21
		10^4	1.4×10^5	24	26	25	25	24
		10^5	1.4×10^4	27	29	29	28	28
		10^6	1.4×10^3	–	–	–	–	–
6	*Vibrio parahaemolyticus*	10^1	4.1×10^7	16	12	13	11	12
	tdh gene	10^2	4.1×10^6	19	16	17	16	16
		10^3	4.1×10^5	23	20	18	20	19
		10^4	4.1×10^4	26	25	21	24	24
		10^5	4.1×10^3	30	27	23	27	27
		10^6	4.1×10^2	–	30	23	–	–
7	*astA* positive *Escherichia coli*	10^1	1.1×10^8	14	9	13	10	10
	EAEC	10^2	1.1×10^7	18	13	15	13	13
	astA gene	10^3	1.1×10^6	21	18	18	18	17
		10^4	1.1×10^5	25	21	20	21	20
		10^5	1.1×10^4	27	25	22	25	23
		10^6	1.1×10^3	–	28	26	28	28
8	*Salmonella* spp.	10^1	6.3×10^7	18	17	17	15	15
	invA gene	10^2	6.3×10^6	22	21	21	19	19
		10^3	6.3×10^5	25	23	23	24	–
		10^4	6.3×10^4	27	28	28	27	–
		10^5	6.3×10^3	–	–	–	–	–
		10^6	6.3×10^2	–	–	–	–	–

下線付き赤字は増幅がみられたものの 30 サイクルまでに指数増幅期に移行しなかったもの

「－」は増幅がみられなかったもの

表 4 リアルタイム PCR 法と培養法の比較

No.	検査時の日数経過	摂食者	原因食	患者数/総数	Real time PCR 陽性数/検査数	培養法 病原体	培養法 PCR 陽性数/検査数	培養法 陽性数/検査数	備考
1	2	仕事仲間	ちらし寿司	13/15	0/2	*Salmonella* Enteritidis	1/2	3/4	
2	6	市民	焼き肉	4/4	3/4	*C. jejuni*	3/4	3/4	
3	2	市民	不明	13/44	2/5	STEC O63 *(stx2f)*	1/5	1/5	*eaeA* and *astA* 1/5
4	13	市民	おにぎり	4/4	0/2	*Salmonella* Enteritidis	2/2	2/2	
5	3 〜 7	従業員	不明	−	3/7	EHEC O157	4/7	4/7	
6	3	グループ	弁当	171/296	7/7	*C. perfringens*	7/7	16/17	
7	1	投宿者	弁当	11/21	4/4	*S. aureus*	4/4	6/9	
8	4	市民	不明	9/16	4/4	*C. jejuni*	4/4	4/4	

表 5 リアルタイム PCR 法と培養法の比較

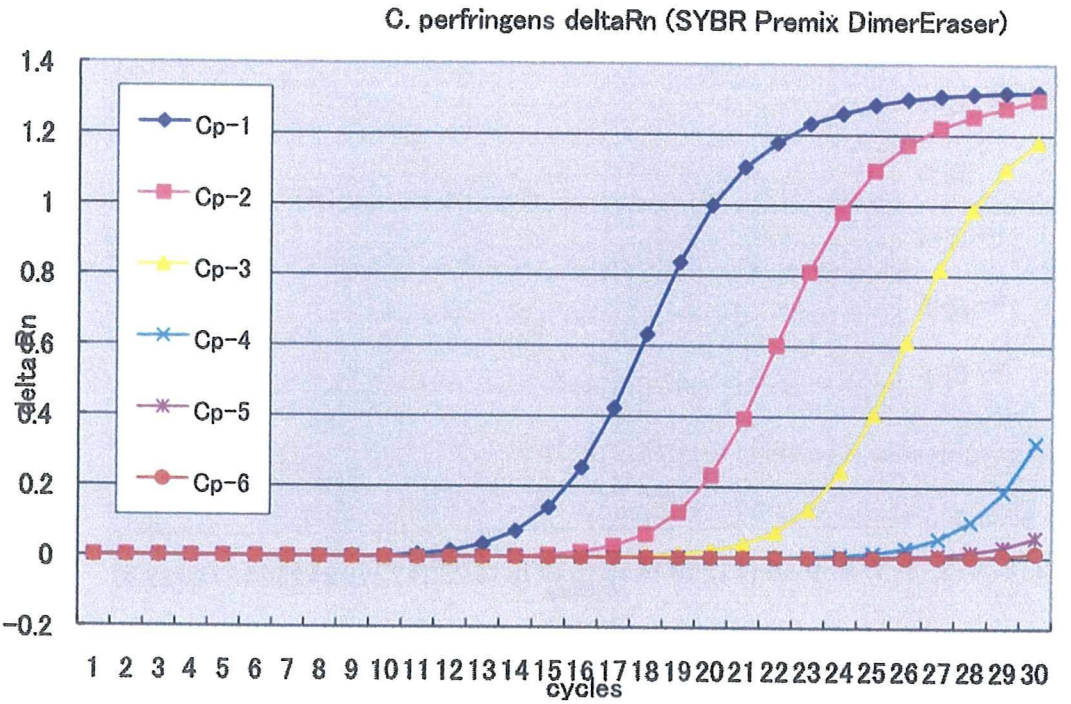
	菌種・菌群	事例数	SYBR		培養		SYBR が陽性多い			培養が陽性多い		
			陽性	合計	陽性	合計	事例数	SYBR 陽性/計	培養陽性/計	事例数	SYBR 陽性/計	培養陽性/計
1	*Campylobacter jejuni*	14	51	77	50	77	1	5/6	1/6	2	4/7	5/7
											4/7	6/7
2	*Clostridium perfringens*	5	25	34	28	34	0			2	4/7	6/7
											2/6	3/6
3	EPEC, EHEC, *astA* 陽性	6	18	40	20	40	0			2	4/7	5/7
											3/7	4/7
4	*Salmonella* spp.	3	3	11	7	11	0			3	0/2	1/2
											0/2	2/2
											3/7	4/7
5	*Staphylococcus aureus, Bacillus cereus*	3	6	13	8	13	0			2	1/5	2/5
											1/4	2/4
6	*Vibrio parahaemolyticus Plesiomonas shigelloides*	3	6	18	7	18	0			1	1/7	2/7
	計	34	109	193	120	193	1			12		

Fukushima et al[2] ならびに表 4 について本研究事業の結果のみを合算した。

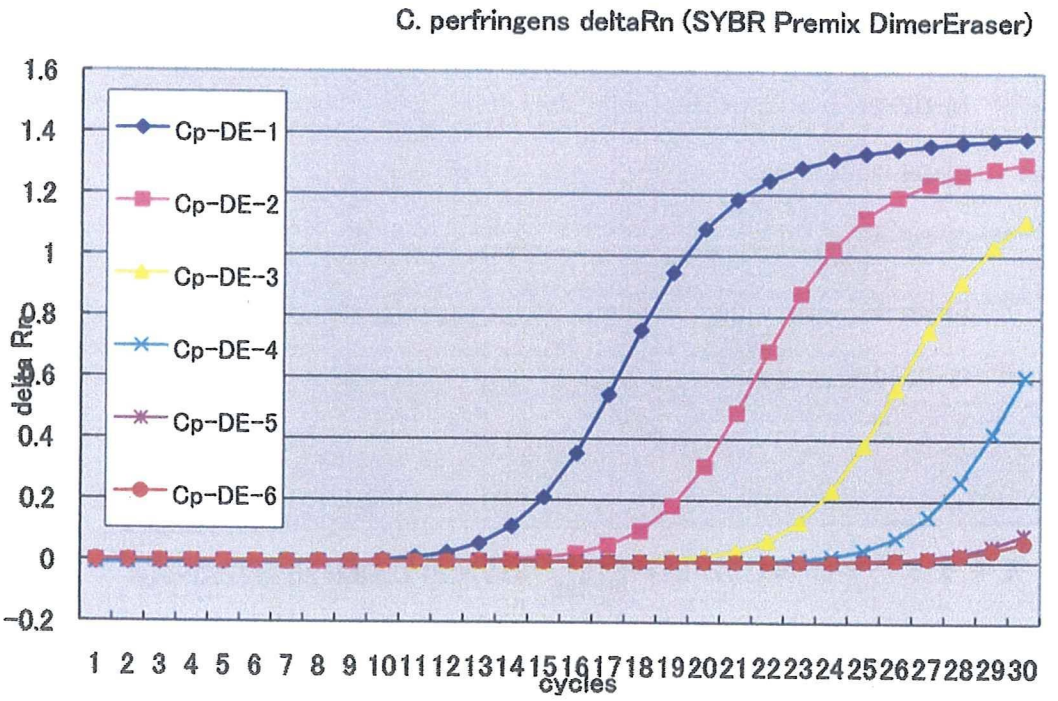
図 1　同一機種 2 台を用いたリアルタイム PCR　ABI7500FAST による

1-1 *C. perfringens*

施設 A

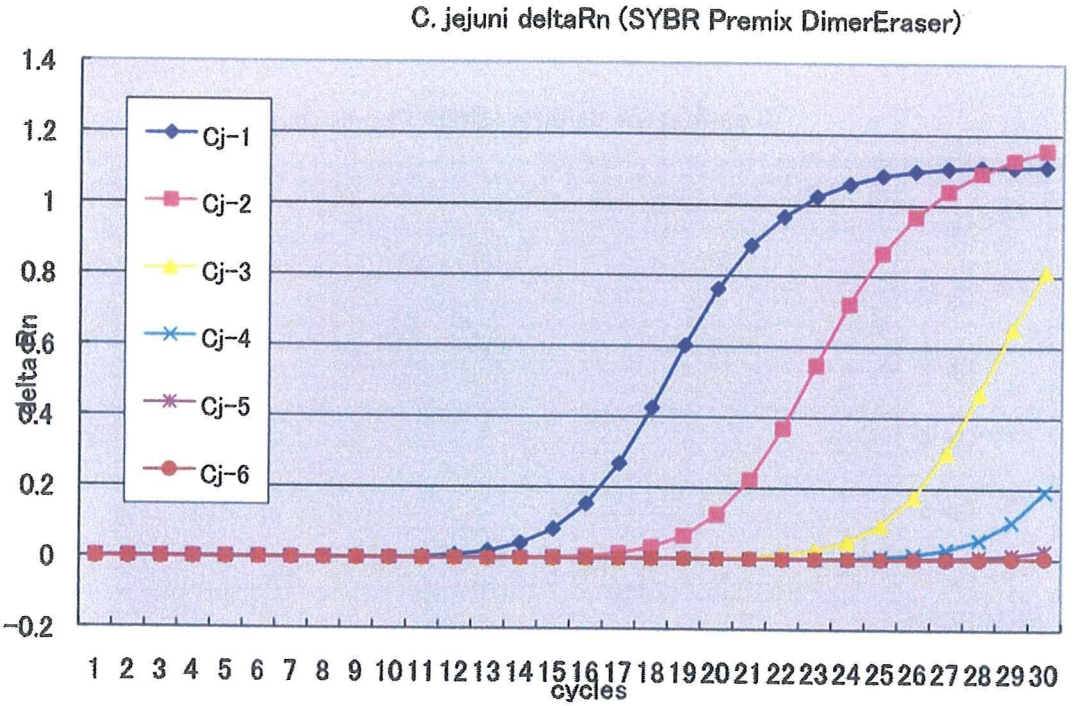


施設 B

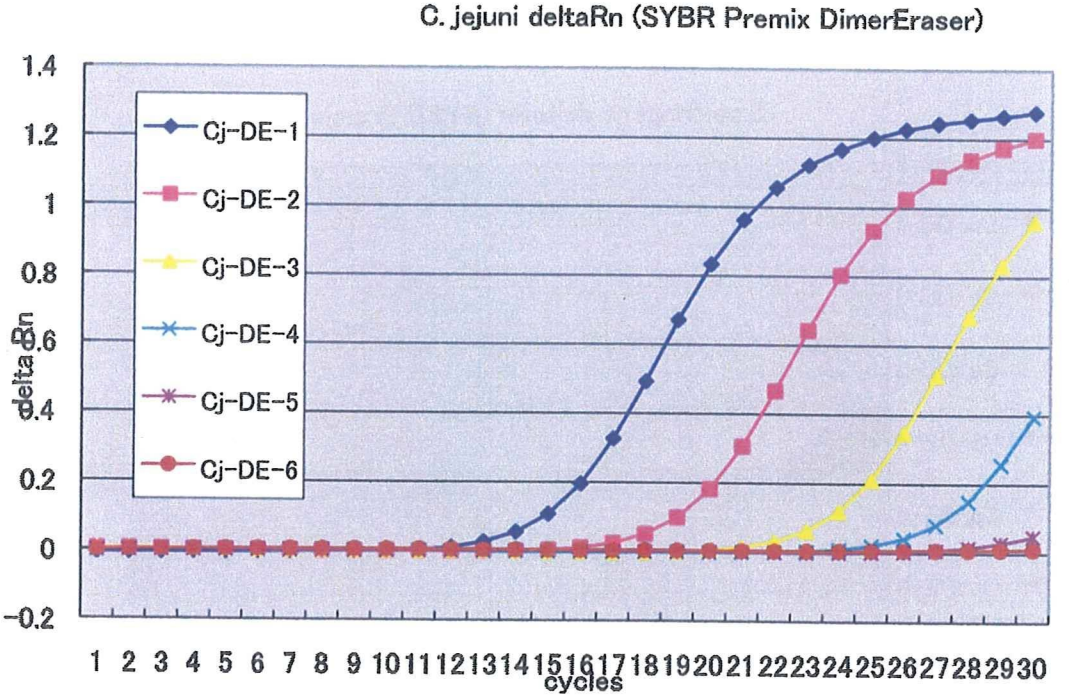


1-2 *Campylobacter jejuni*

施設 A

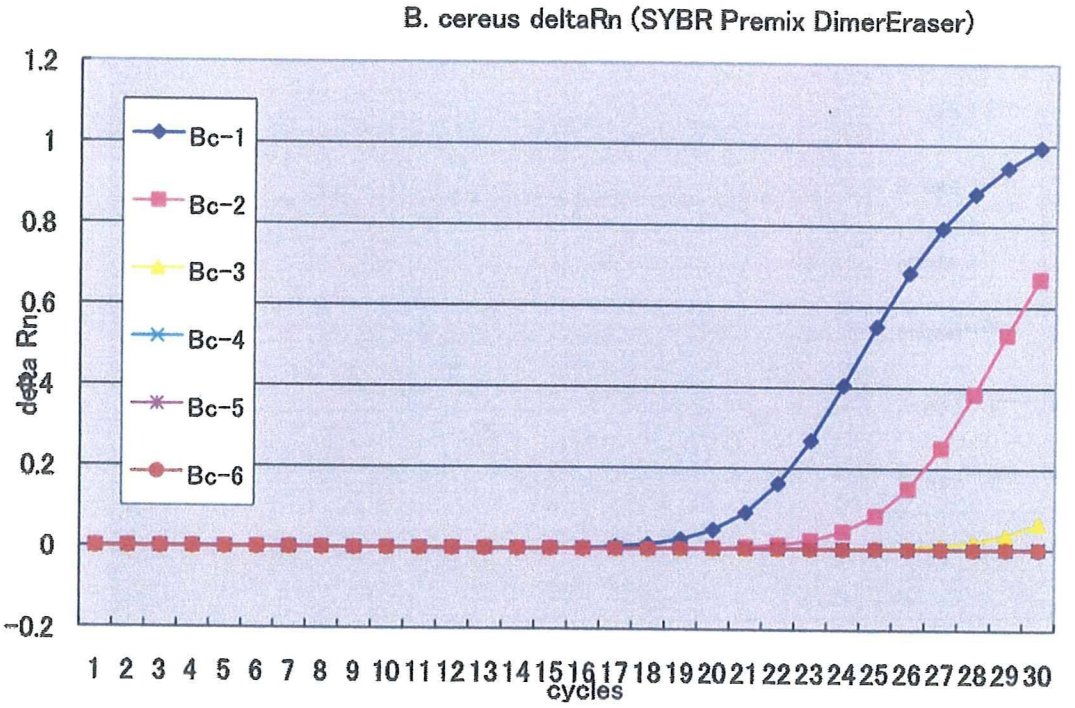


施設 B

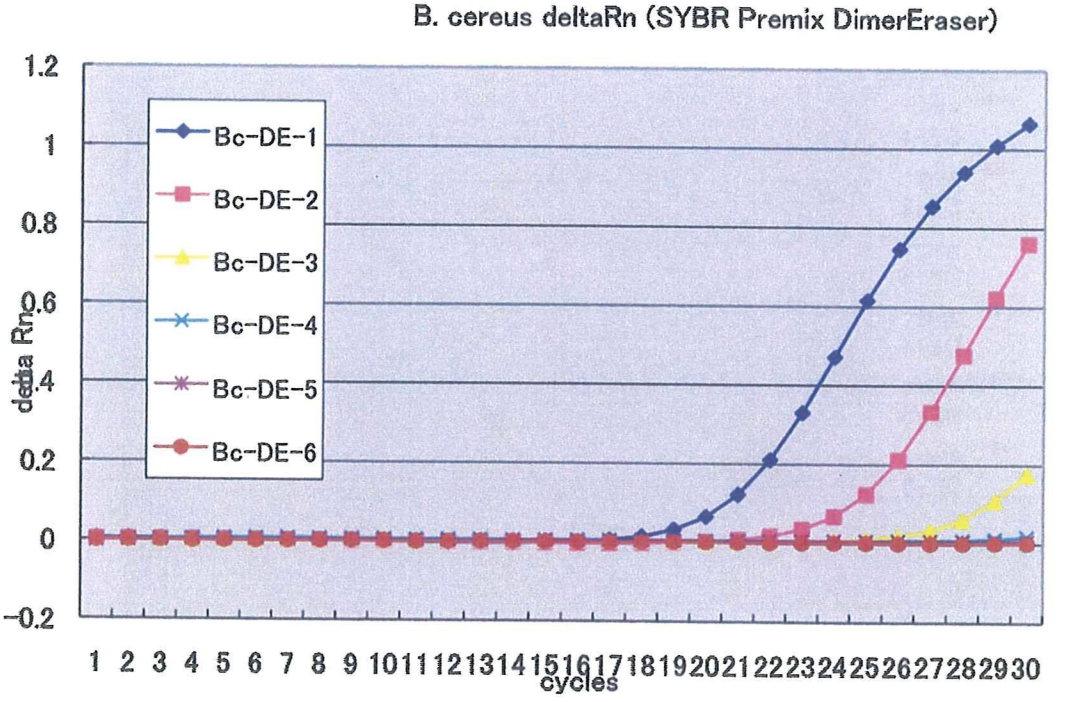


1-3 *Bacillus cereus*

施設 A

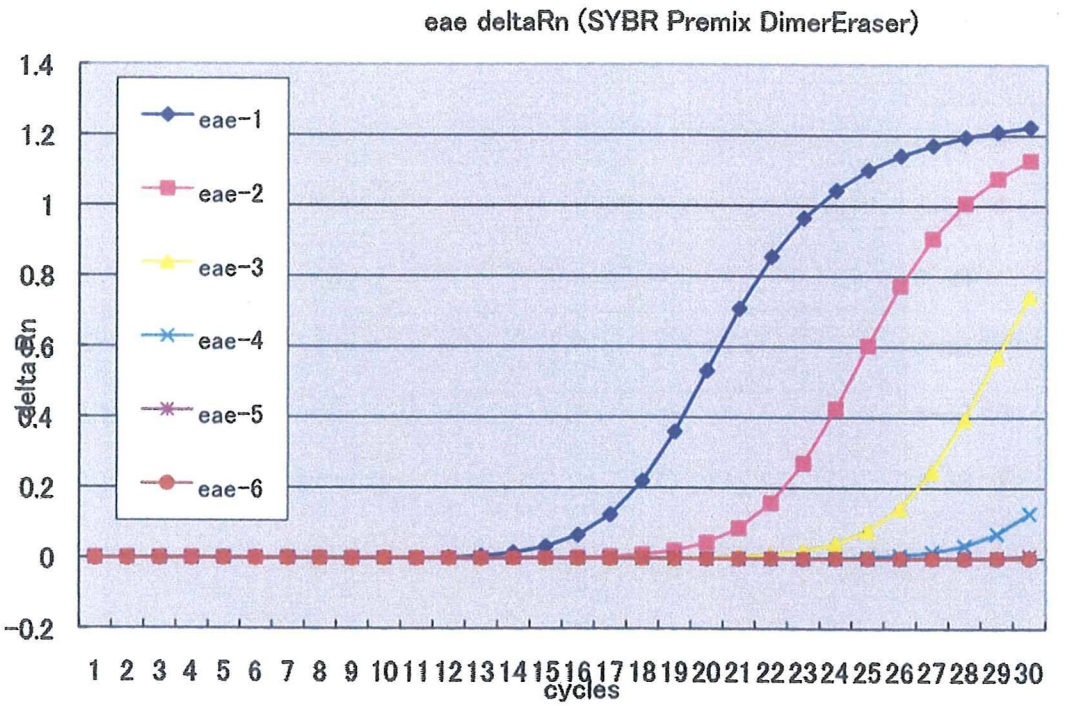


施設 B

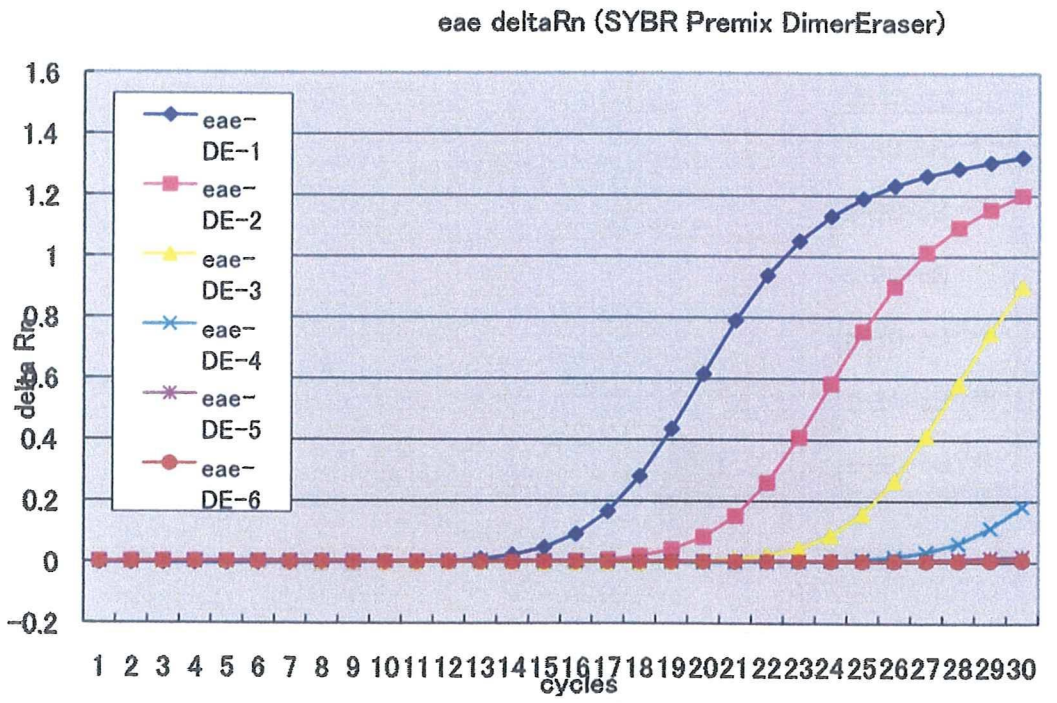


1-4　*eae* 陽性 *Escherichia coli*

施設 A

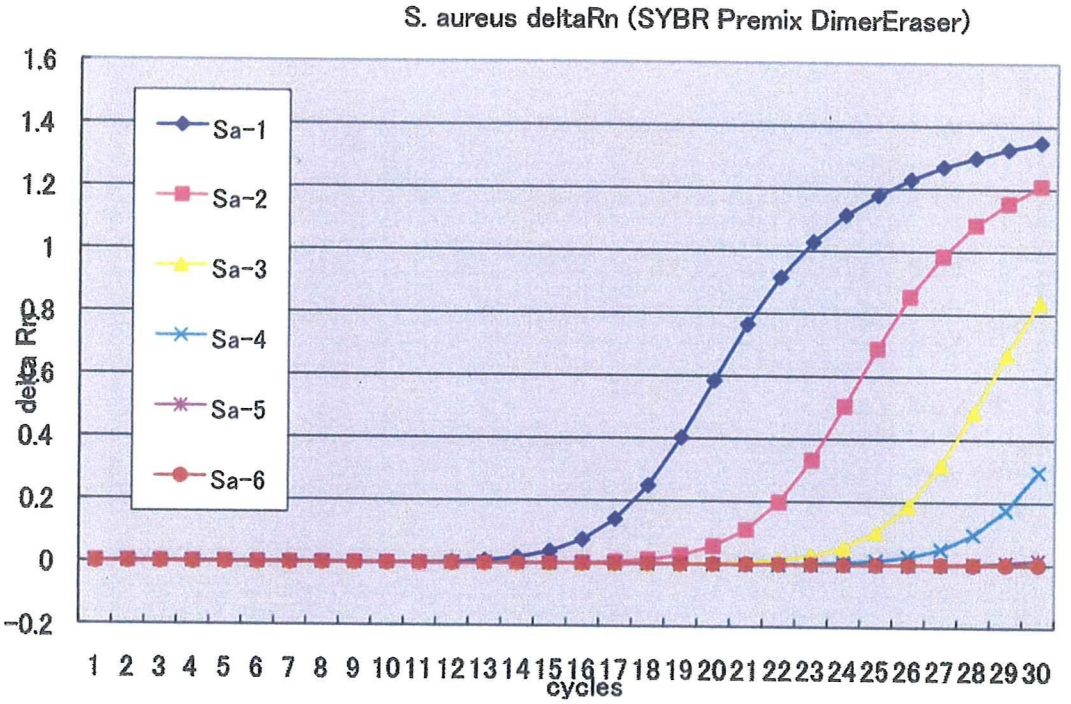


施設 B

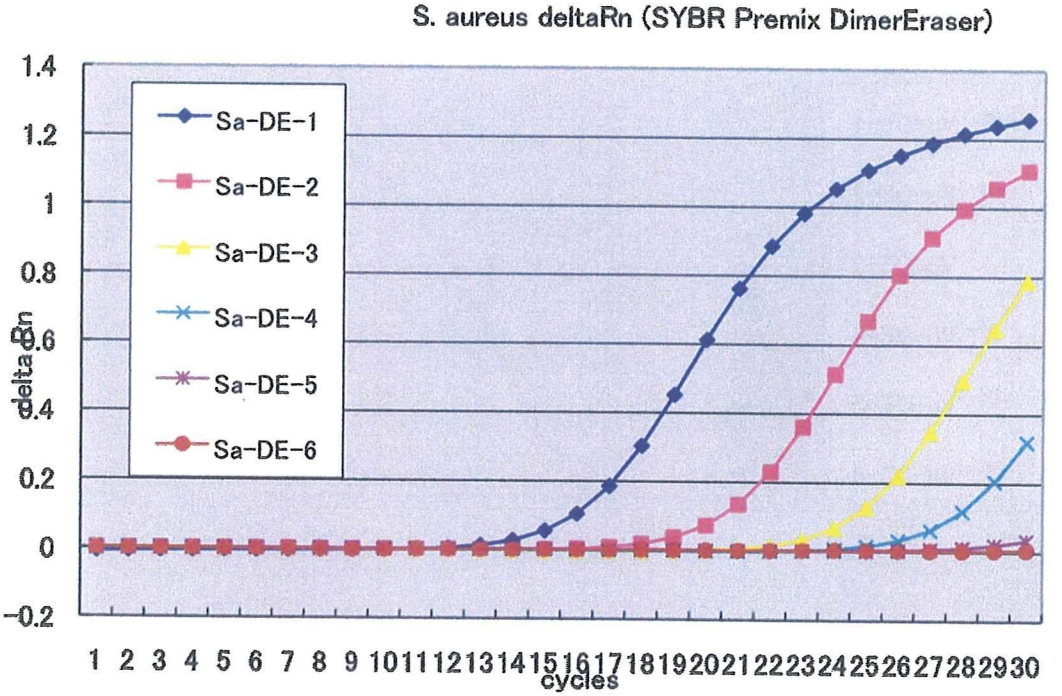


1-5 *Staphylococcus aureus*

施設 A

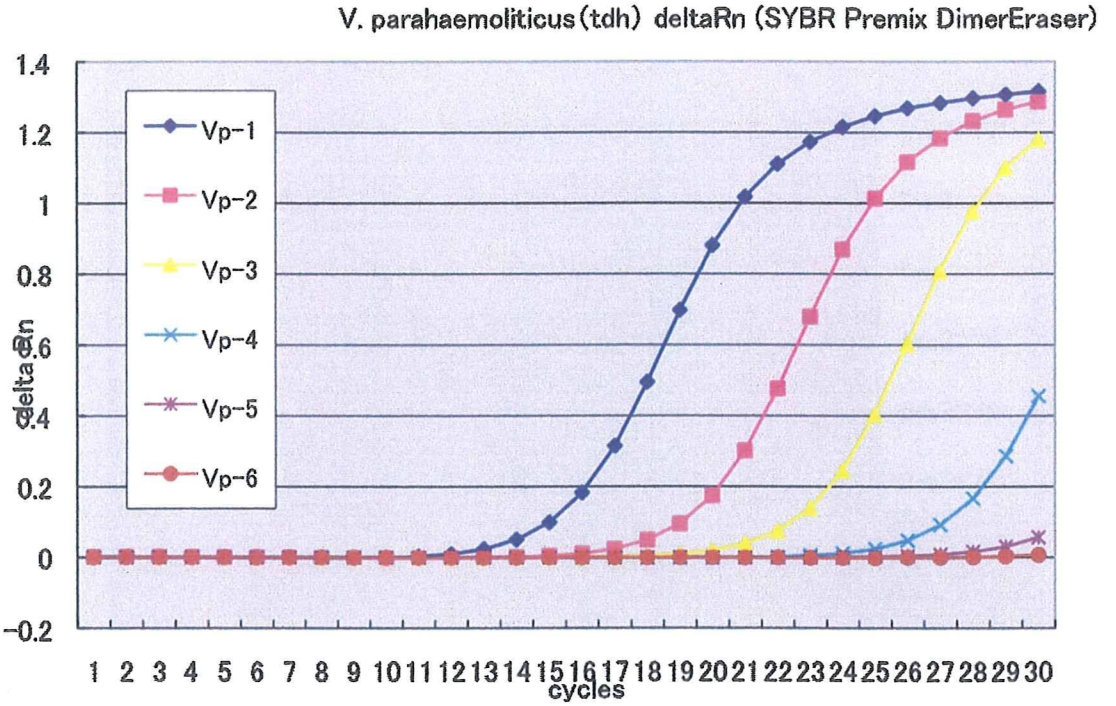


施設 B

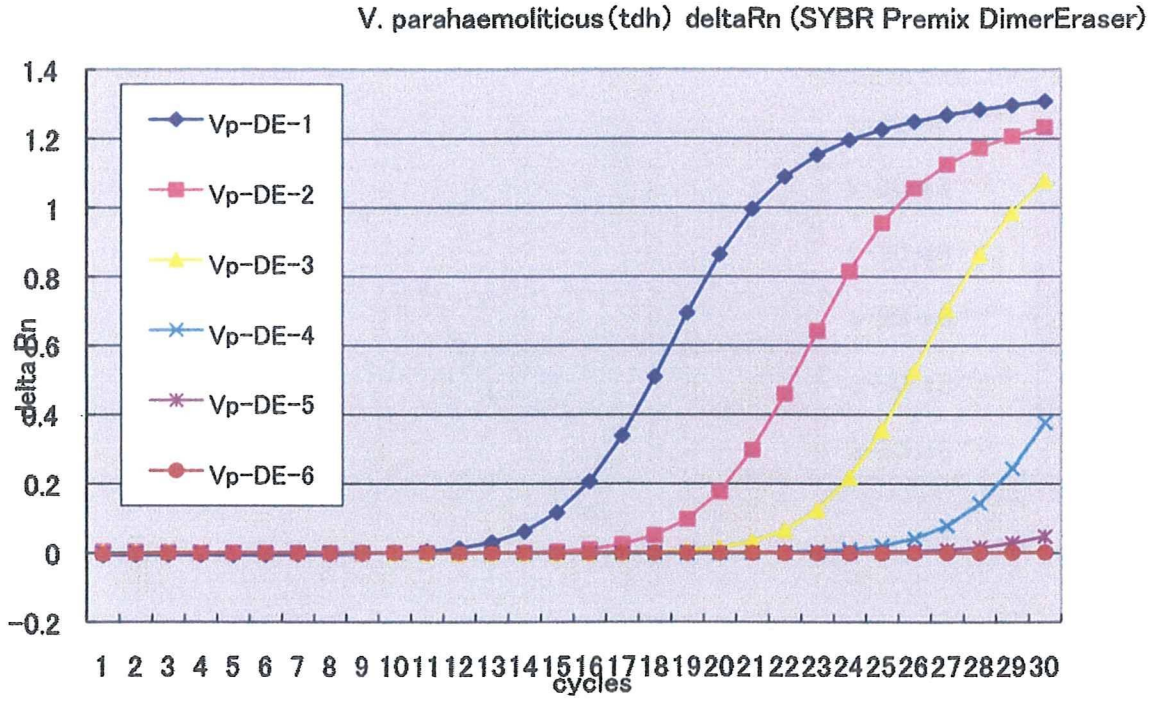


1-6 *Vibrio parahaemolyticus*

施設 A

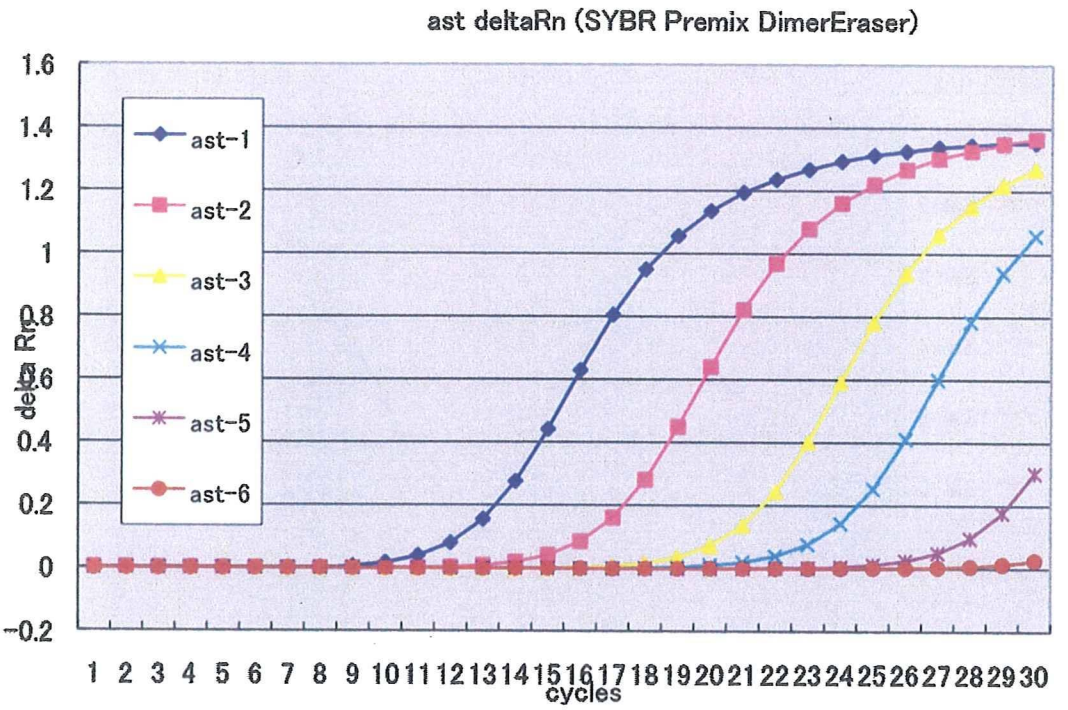


施設 B



1-7　*astA* 陽性 *Escherichia coli*

施設 A



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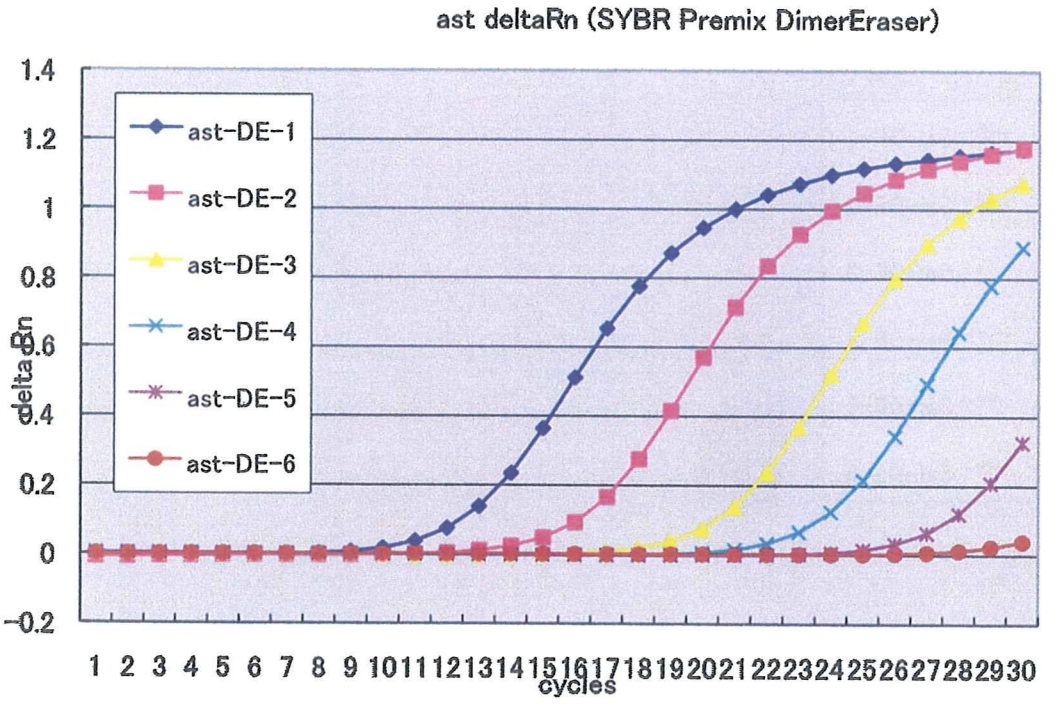
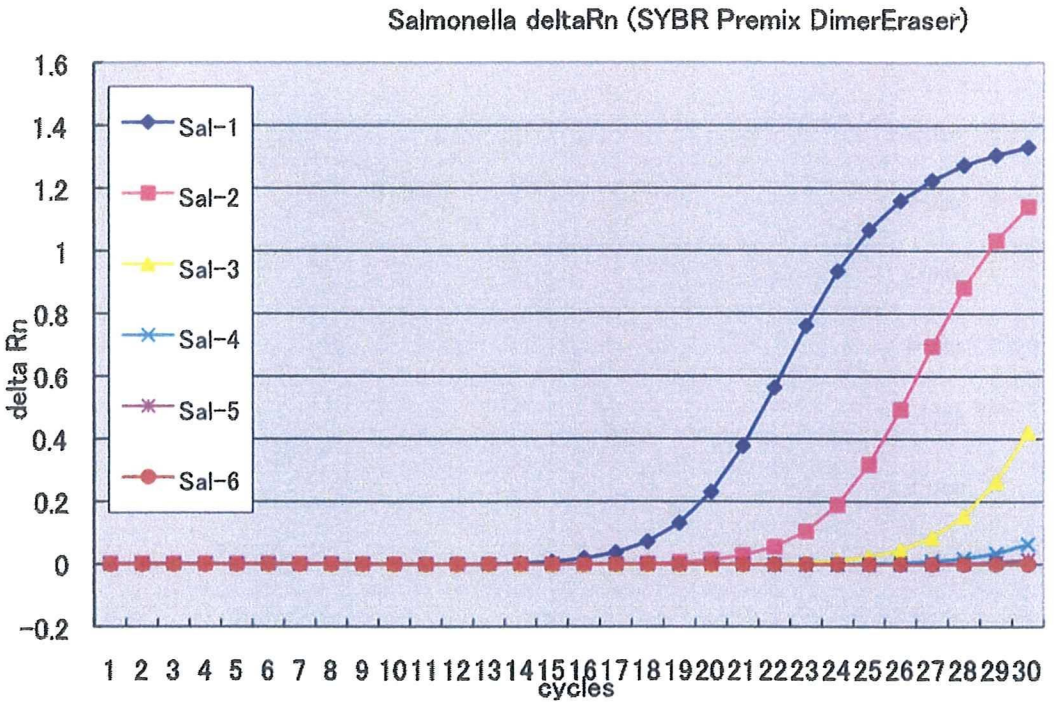


1-8 *Salmonella* spp.

施設 A



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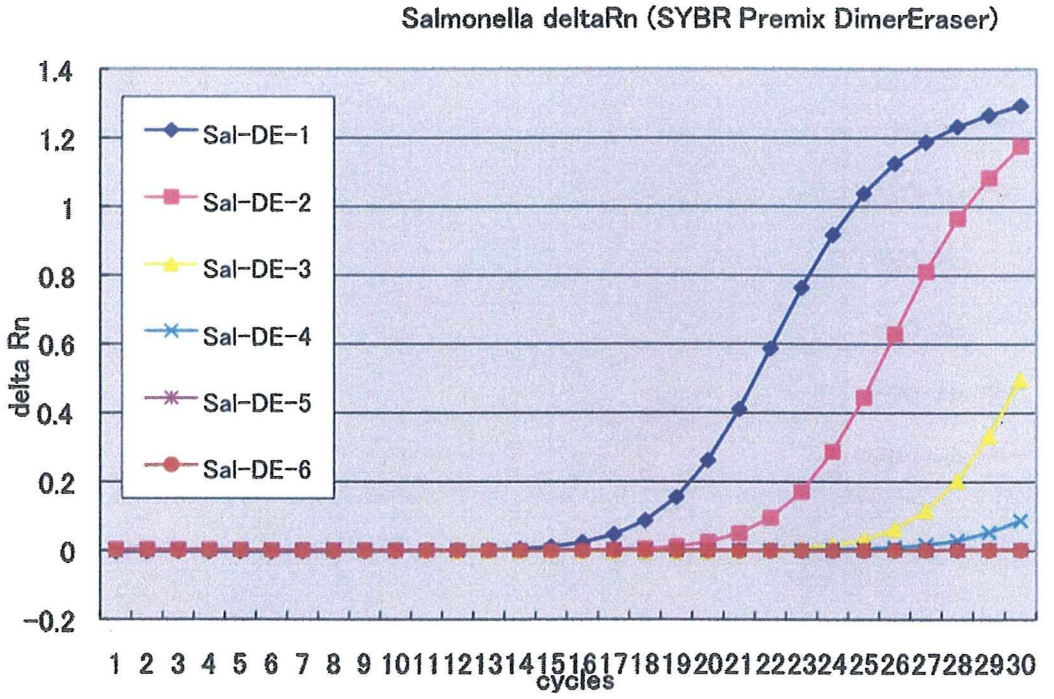
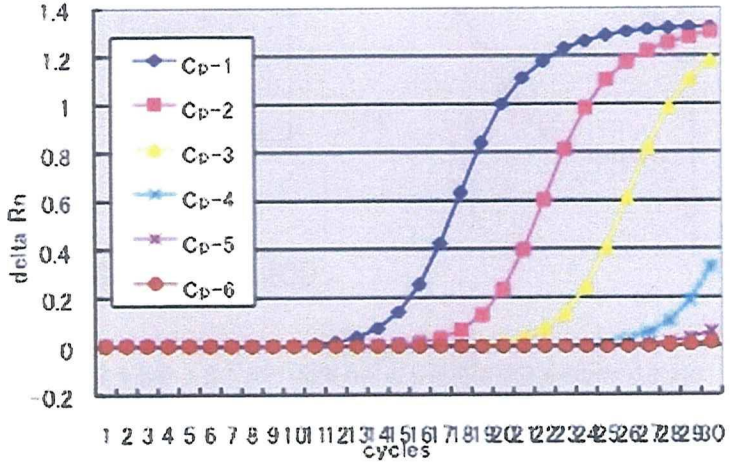


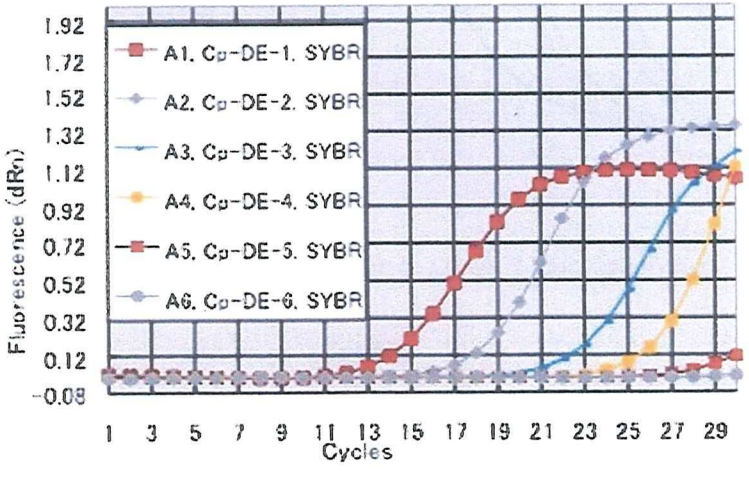
図2　菌種毎の機種によるDNA増幅曲線

2-1 *Clostridium perfringens*

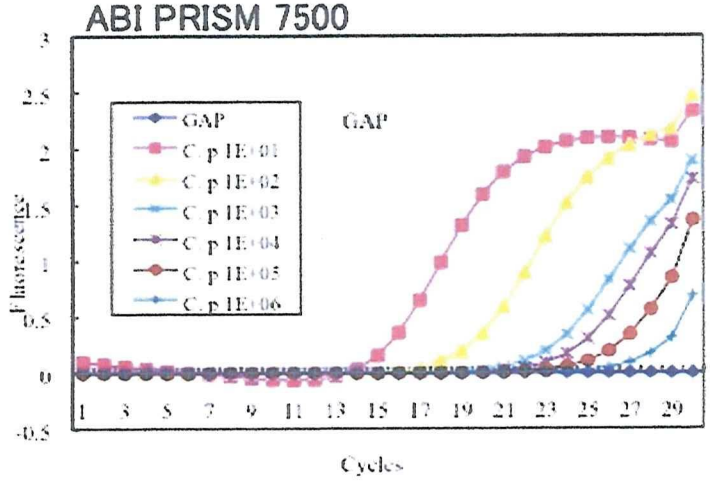
ABI PRISM 7500FAST



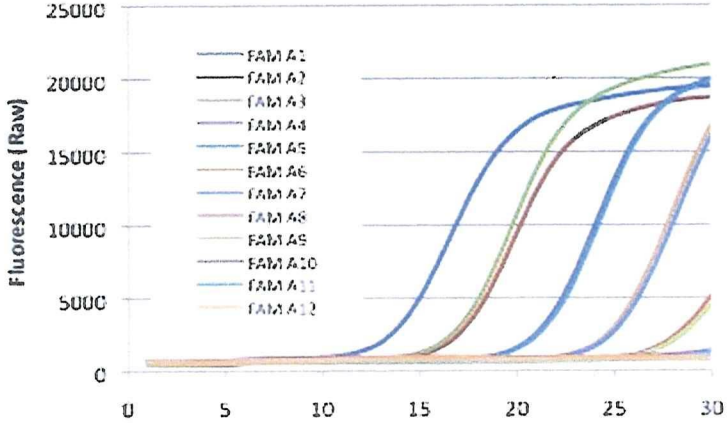
STRATAGENE Mx3000P



ABI PRISM 7500

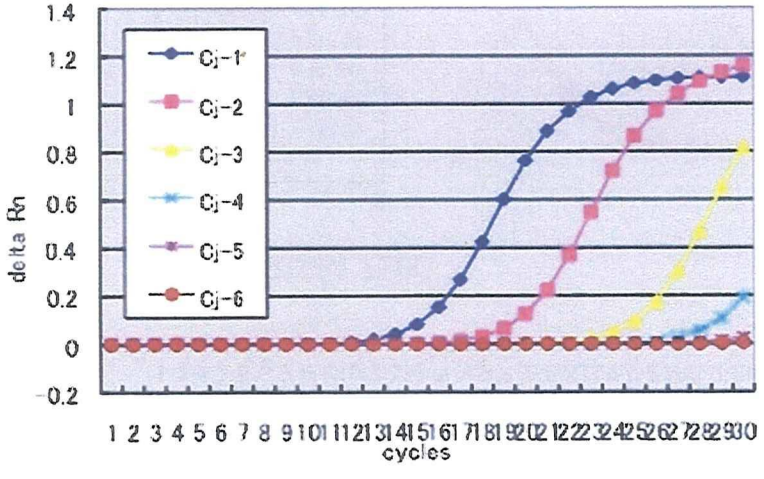


TaKaRa Thermal Cycler Dice Real Time System



2-2 *Campylobacter jejuni*

ABI PRISM 7500FAST



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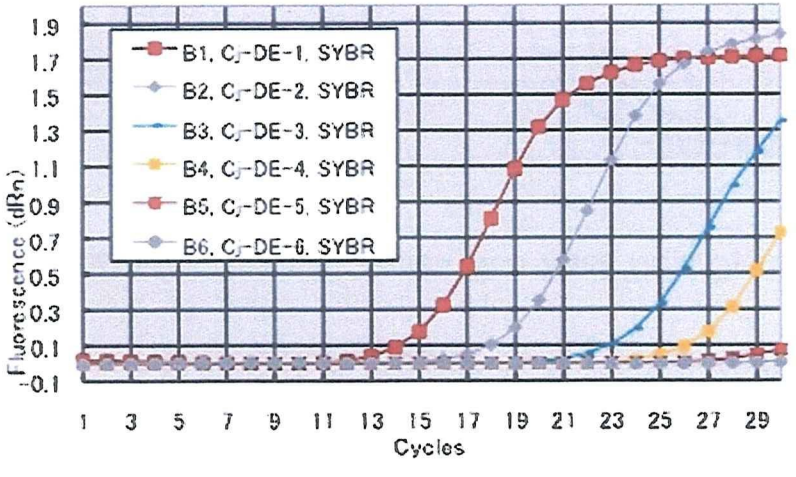
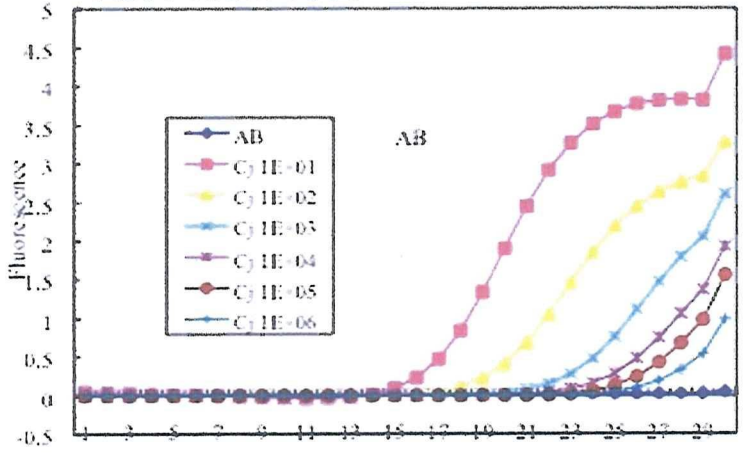
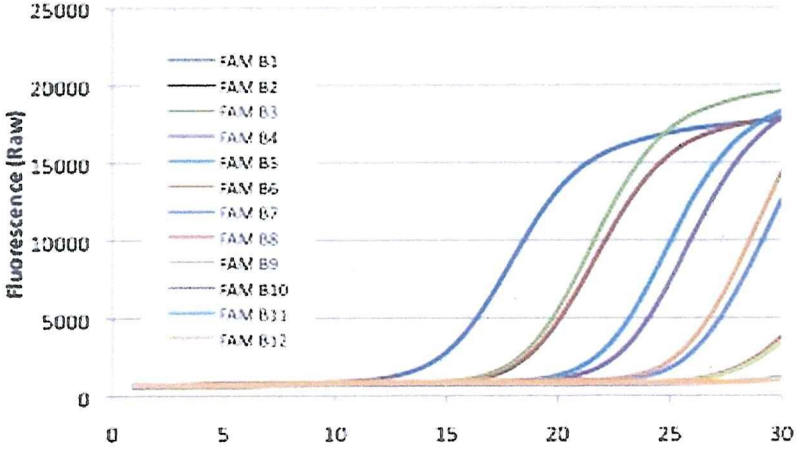


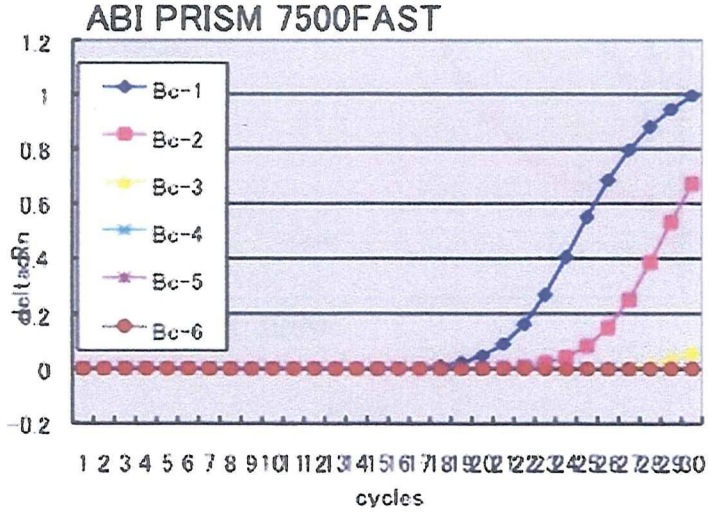
ABI PRISM 7500

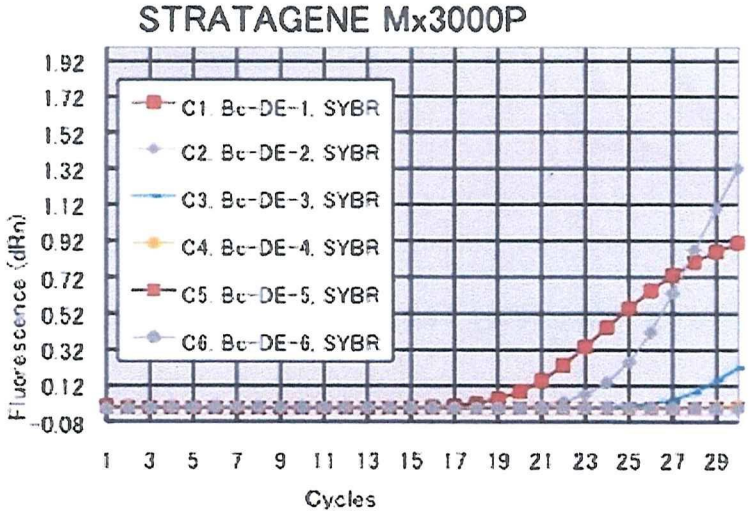


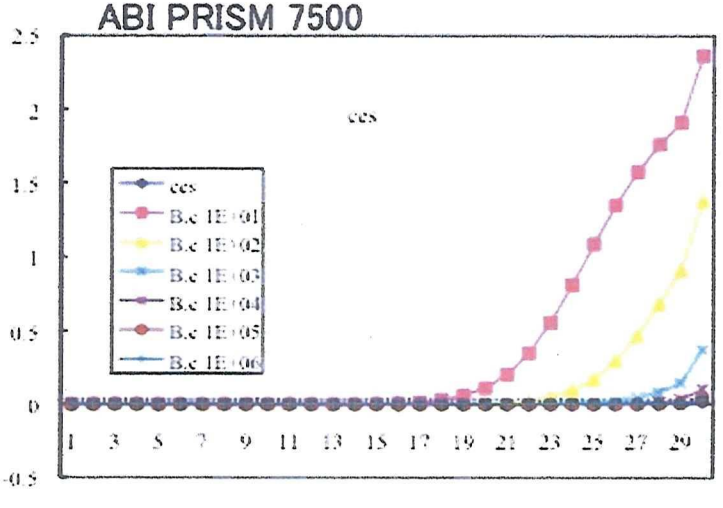
TaKaRa Thermal Cycler Dice Real Time System

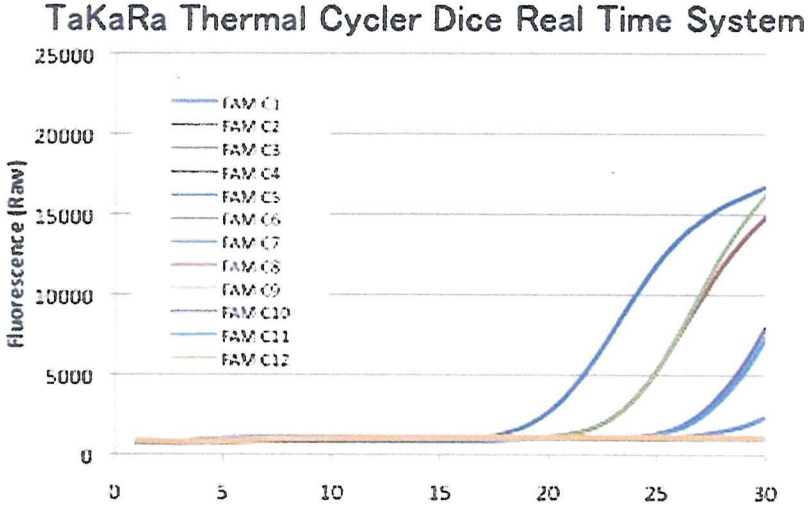


2-3 *Bacillus cereus*



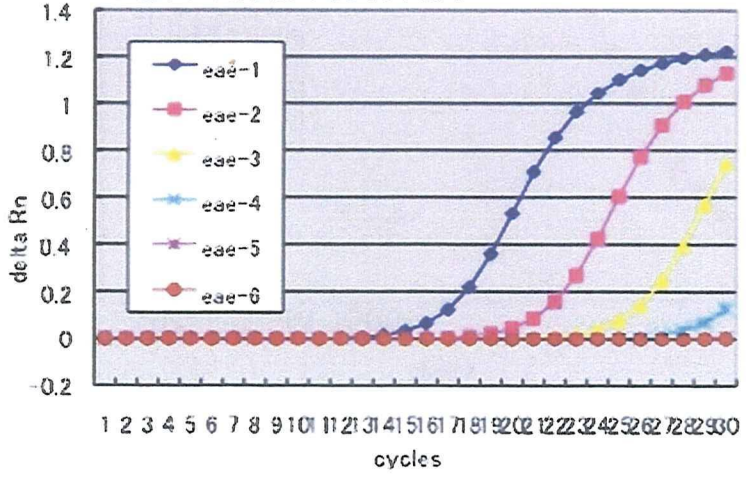




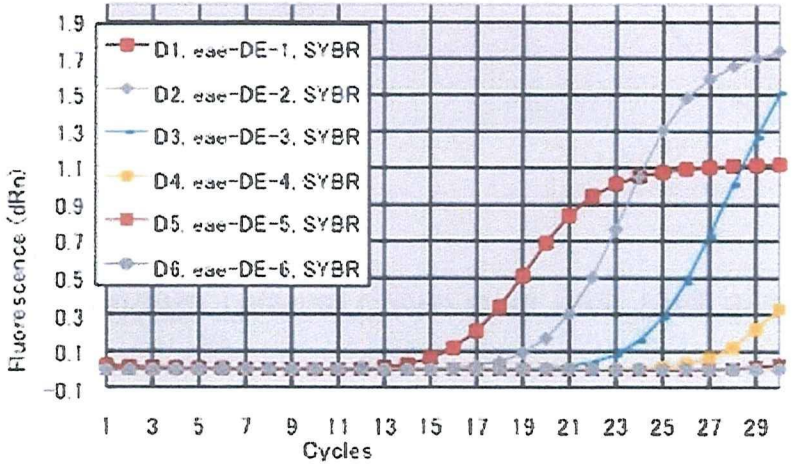


2-4 *eae* 保有*Eshericia coli*

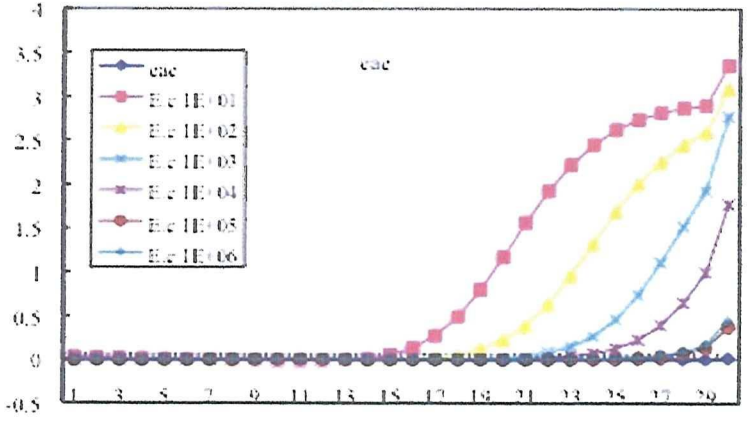
ABI PRISM 7500FAST



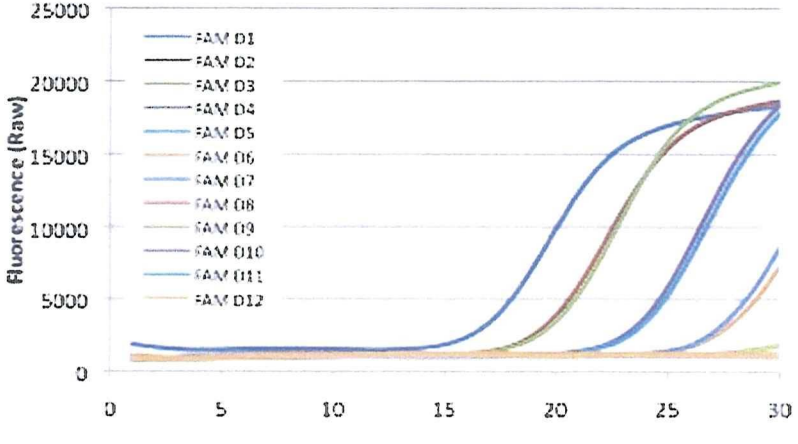
STRATAGENE Mx3000P



ABI PRISM 7500

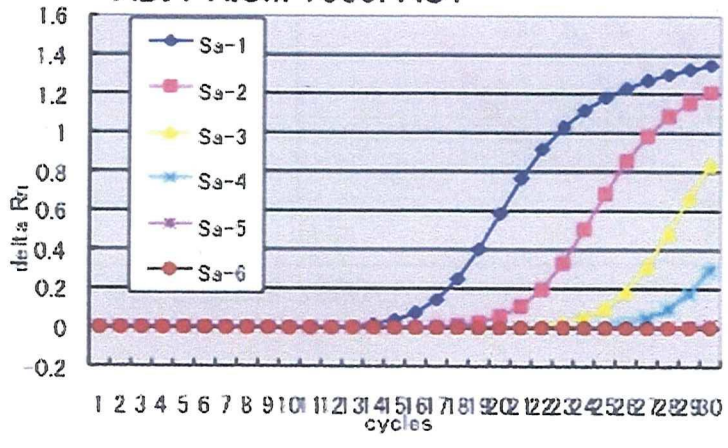


TaKaRa Thermal Cycler Dice Real Time System

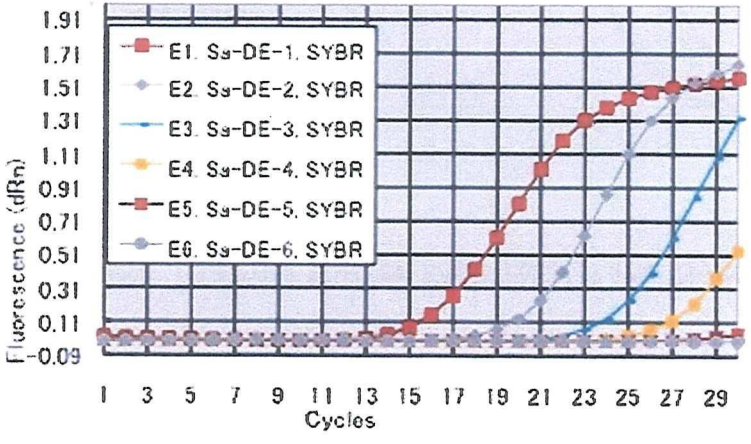


2-5 *Staphylococcus aureus*

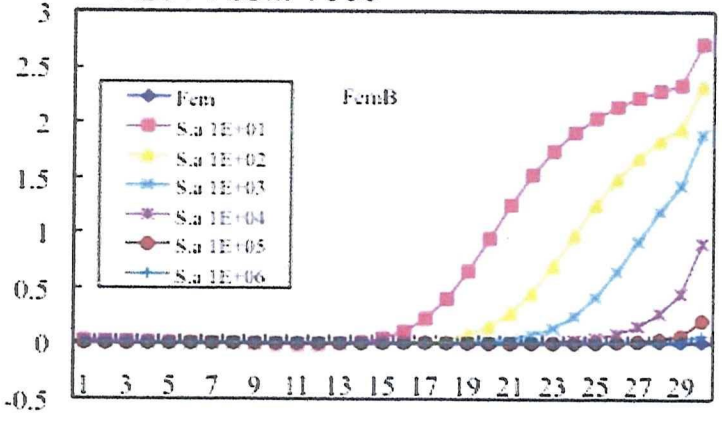
ABI PRISM 7500FAST



STRATAGENE Mx3000P



ABI PRISM 7500



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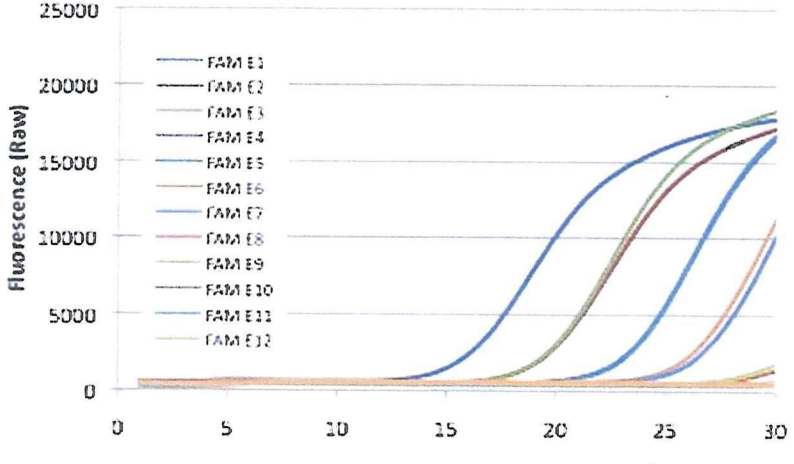
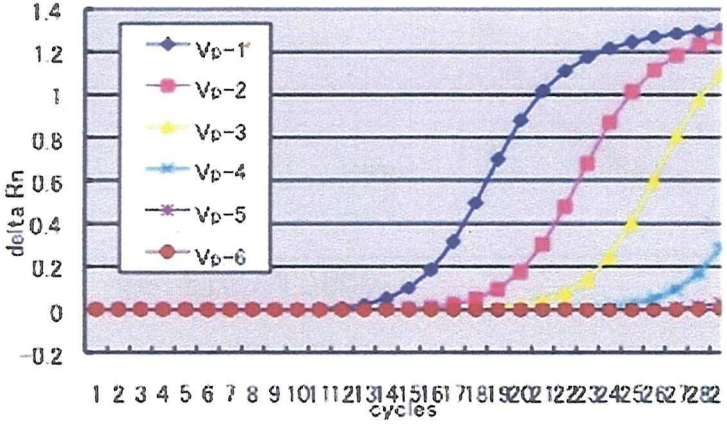
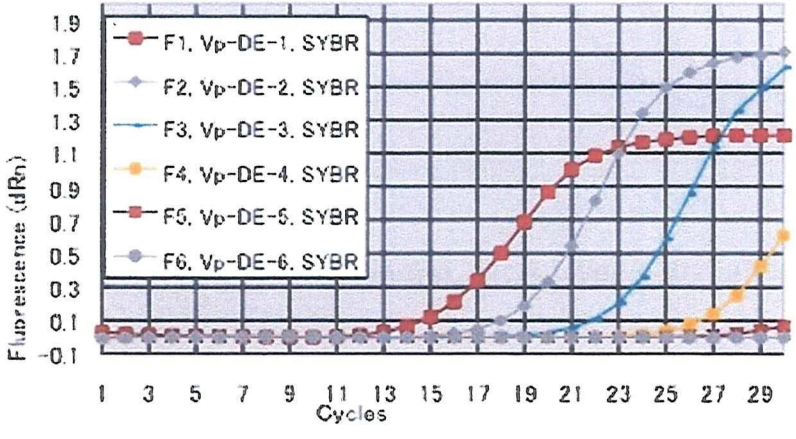


2-6 *Vibrio parahaemolyticus*

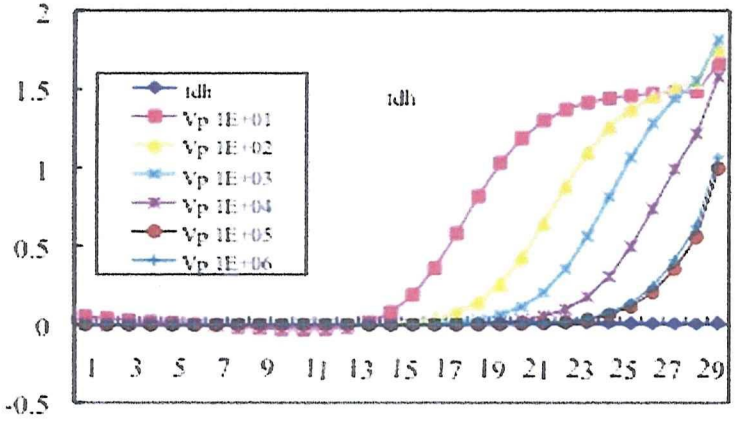
ABI PRISM 7500FAST



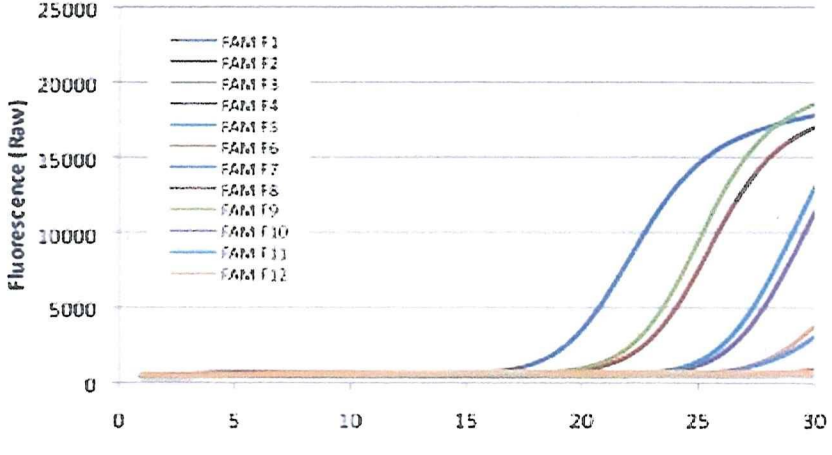
STRATAGENE Mx3000P



ABI PRISM 7500

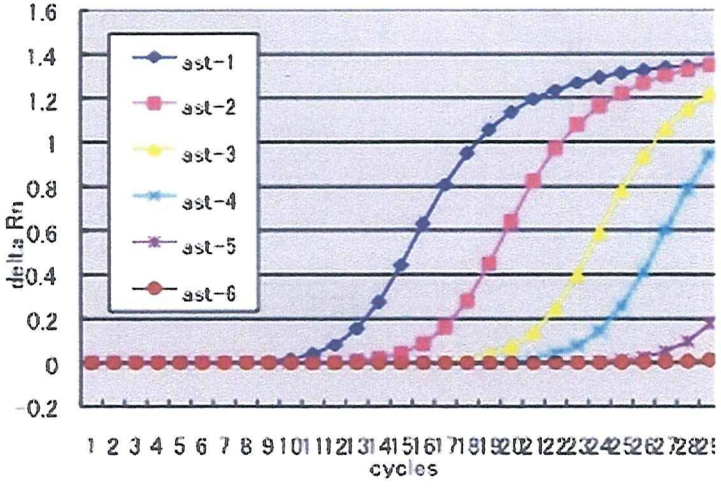


TaKaRa Thermal Cycler Dice Real Time System



2-7 *astA*保有*Escherichia coli*

ABI PRISM 7500FAST



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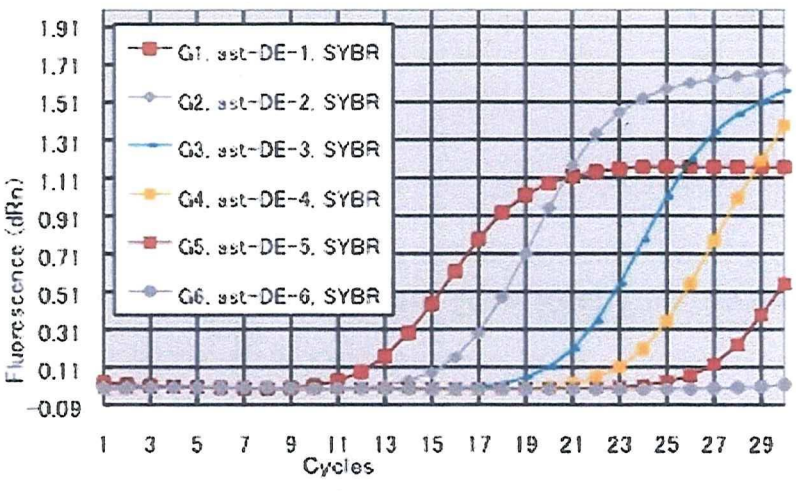
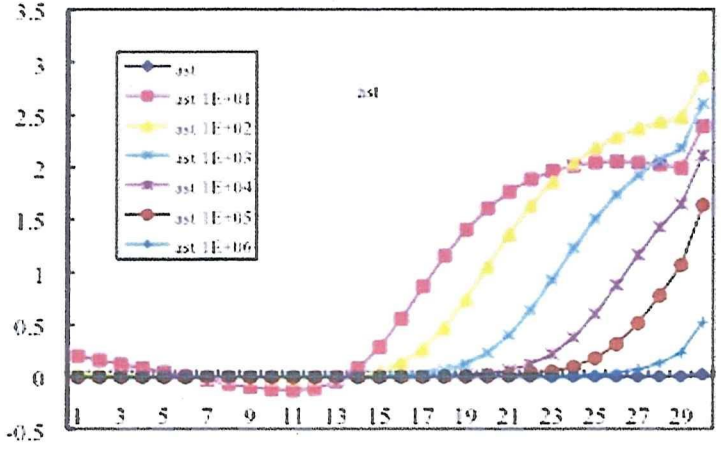


ABI PRISM 7500



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