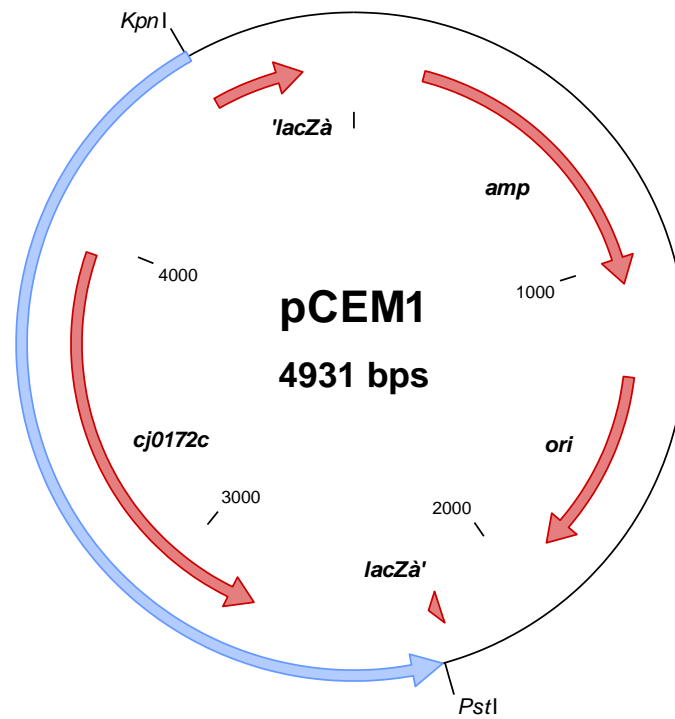
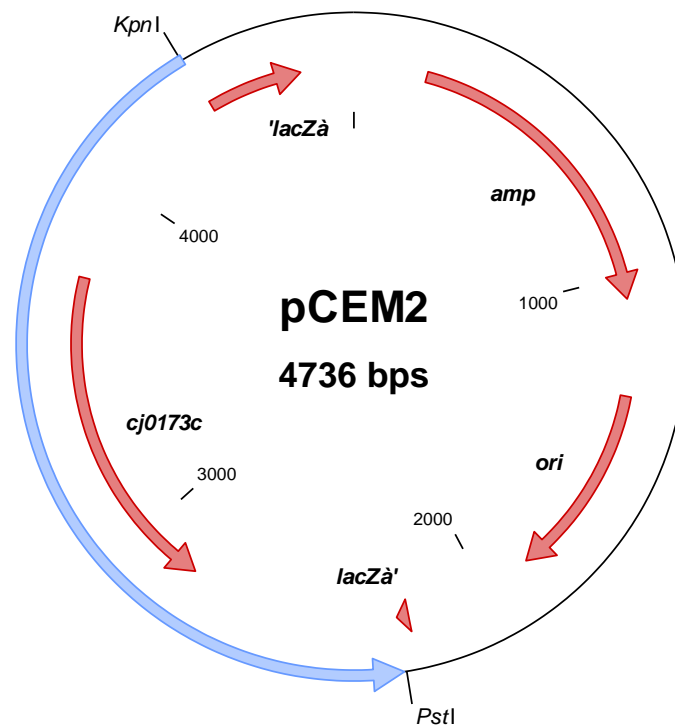


**Appendix 1.**

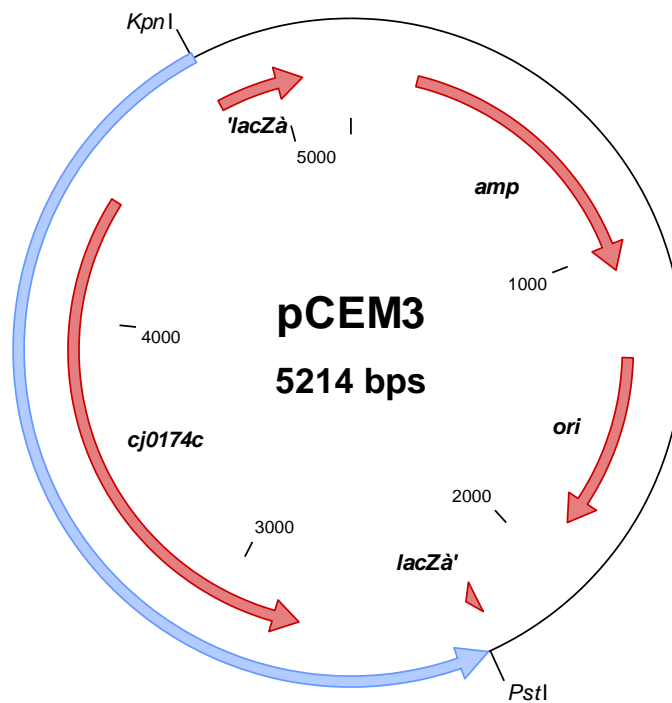
**Plasmid Construct Maps**



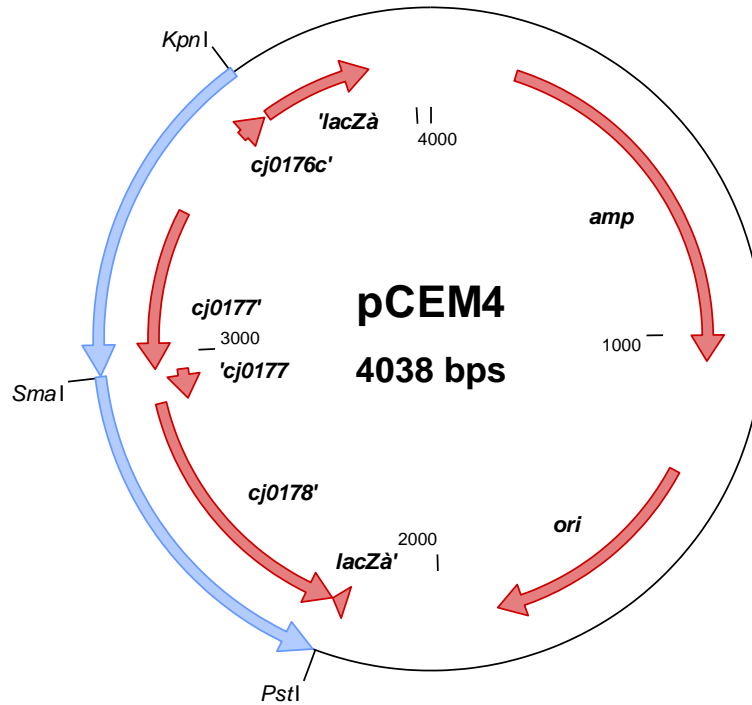
**Figure 1.** pCEM1: pUC19 containing *C. jejuni* NCTC 11168 *cj0172c* genomic fragment (bases 169571-167306) cloned between *KpnI* and *PstI* sites.



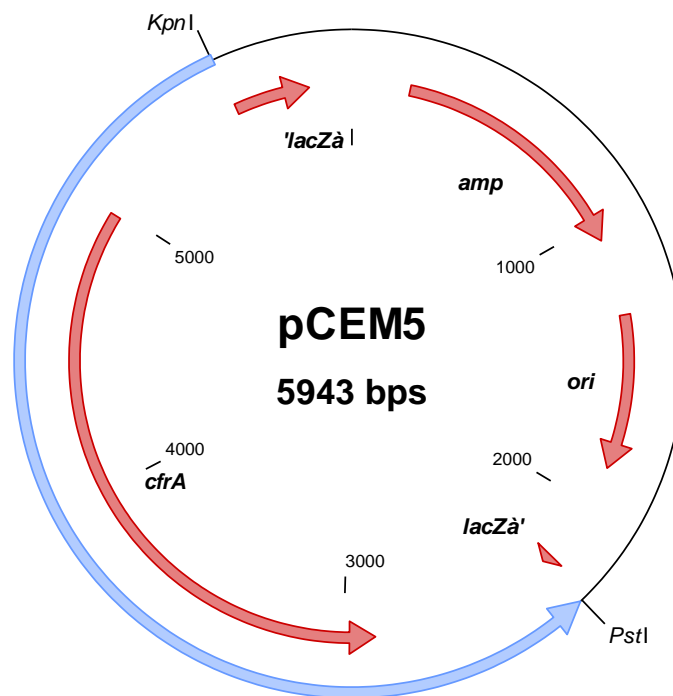
**Figure 2.** pCEM2: pUC19 containing *C. jejuni* NCTC 11168 *cj0173c* genomic fragment (bases 170551-168481) cloned between *KpnI* and *PstI* sites.



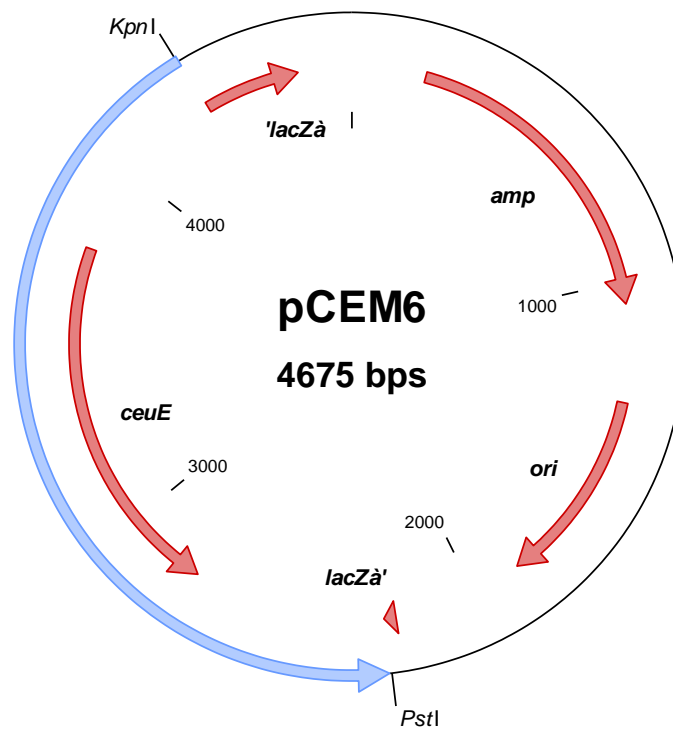
**Figure 3.** pCEM3: pUC19 containing *C. jejuni* NCTC 11168 *cj0174c* genomic fragment (bases 171984-169436) cloned between *KpnI* and *PstI* sites.



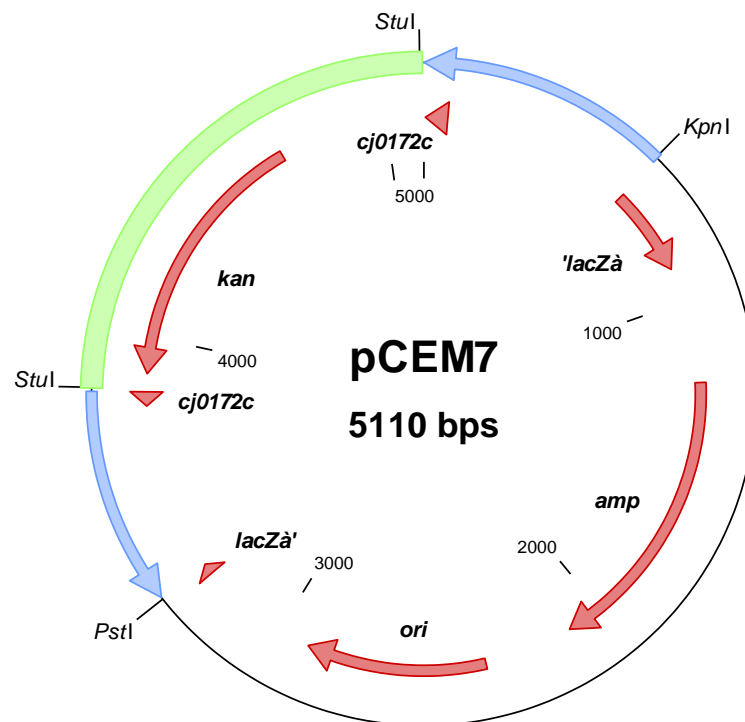
**Figure 4.** pCEM4: pUC19 containing *C. jejuni* NCTC 11168 genomic fragments (bases 172625-173291 and 173690-174389) cloned between *KpnI* and *PstI* sites with an internal *SmaI* site.



**Figure 5.** pCEM5: pUC19 containing *C. jejuni* NCTC 11168 *cfrA* genomic fragment (bases 704900-708177) cloned between *KpnI* and *PstI* sites.

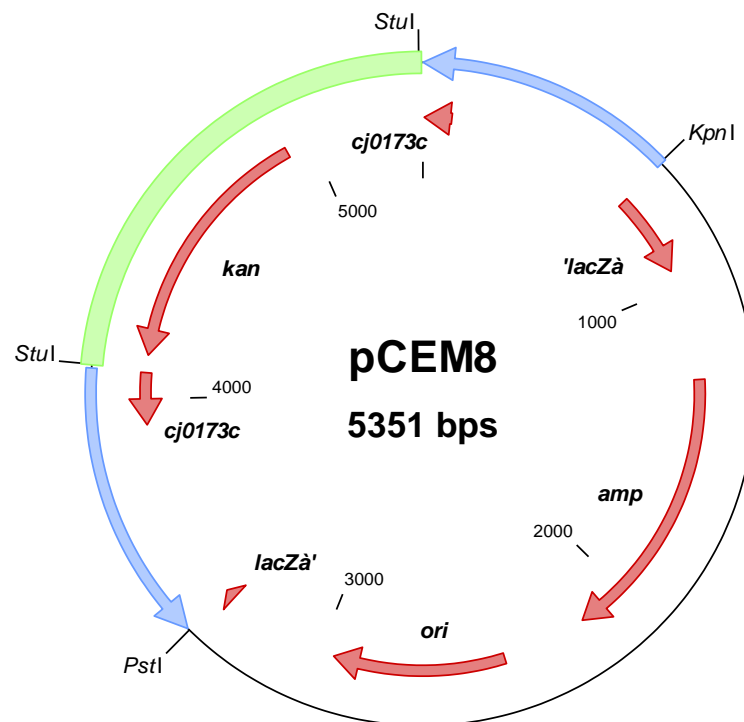


**Figure 6.** pCEM6: pUC19 containing *C. jejuni* NCTC 11168 *ceuE* genomic fragment (bases 1286177-1288186) cloned between *KpnI* and *PstI* sites.

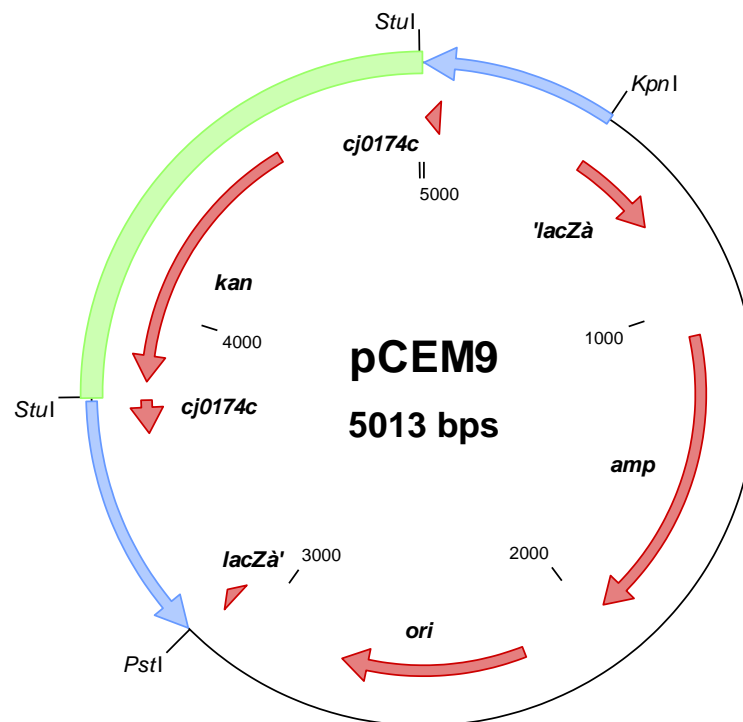


**Figure 7.** pCEM7: pCEM1,  $\Delta cj0172c::aphA-3$ . Deletion of 1097 bp of *cj0172c* ORF (bases 168946-167849) and insertion of *Km<sup>R</sup>* cassette (in forward orientation with respect to the deleted gene) into *BglIII* site created at the point of deletion.

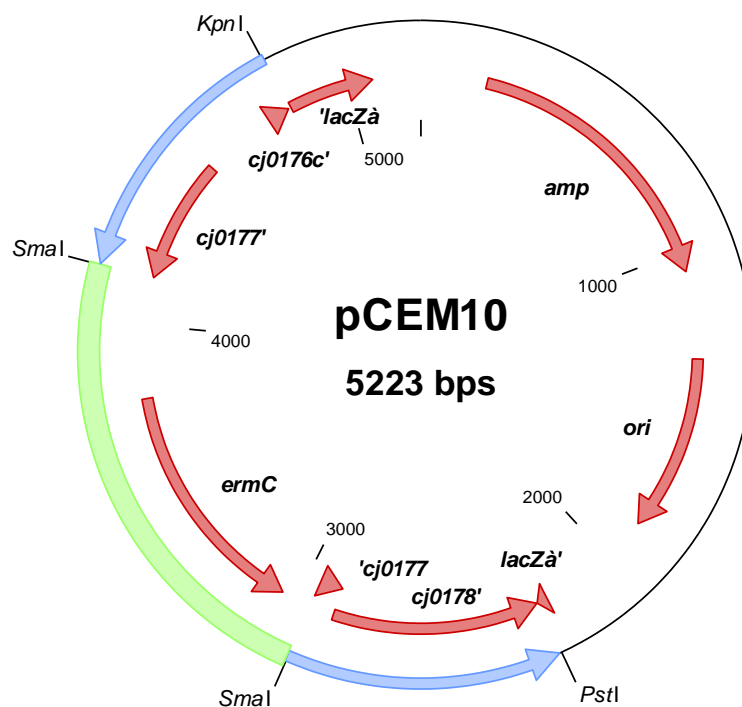




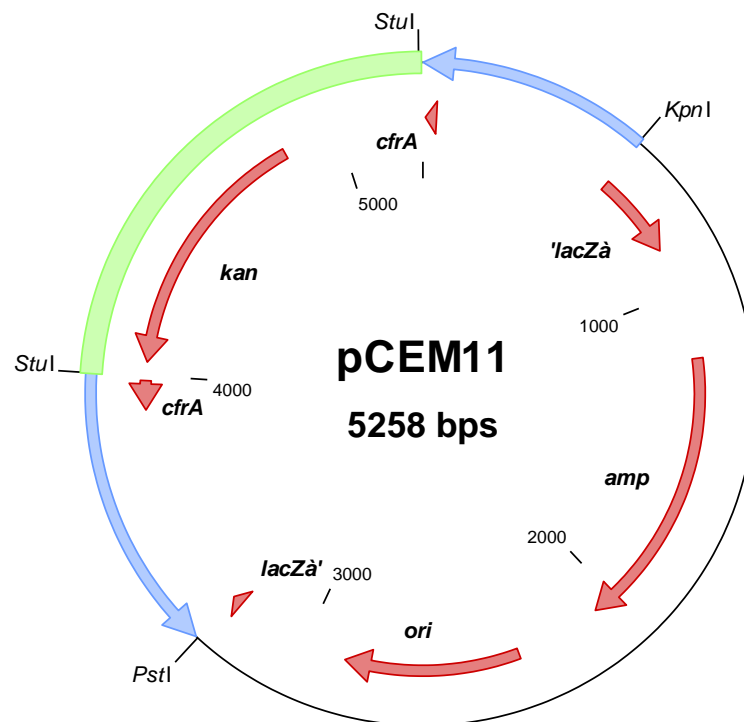
**Figure 8.** pCEM8: pCEM2,  $\Delta cj0173c::aphA-3$ . Deletion of 661 bp of *cj0173c* ORF (bases 169877-169215) and insertion of  $Km^R$  cassette (in forward orientation with respect to the deleted gene) into *Bgl*III site created at the point of deletion.



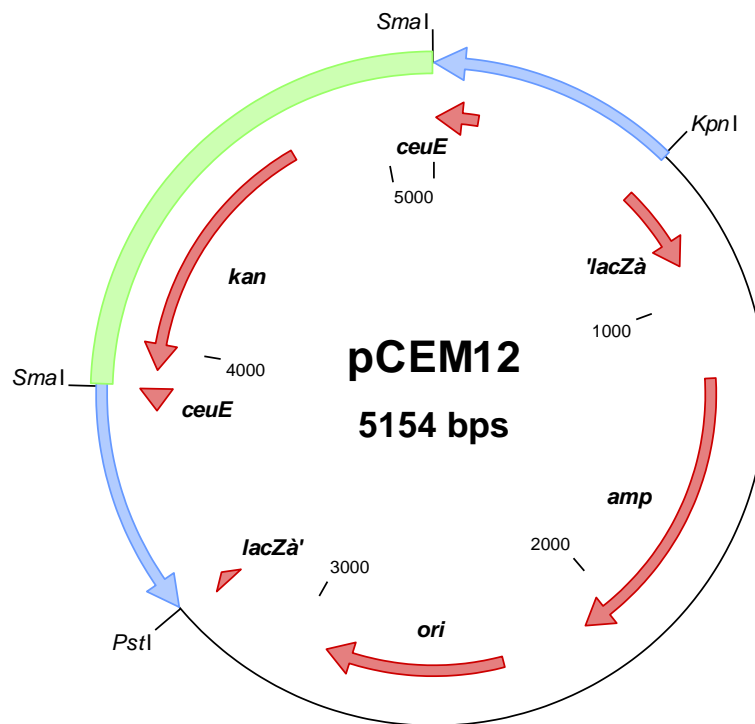
**Figure 9.** pCEM9: pCEM3,  $\Delta cj0174c::aphA-3$ . Deletion of 1477 bp of *cj0174c* ORF (bases 171520-170042) and insertion of  $Km^R$  cassette (in forward orientation with respect to the deleted gene) into *Bgl*III site created at the point of deletion.



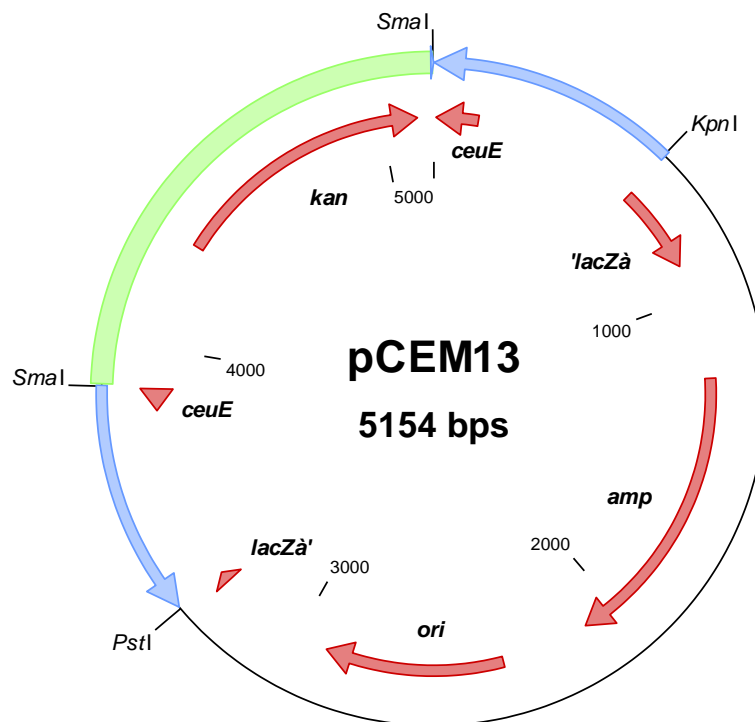
**Figure 10.** pCEM10: pCEM4,  $\Delta cj0177::ermC'$ . Insertion of  $Erm^R$  cassette (in forward orientation with respect to the deleted gene) into a *Sma*I site created between genomic fragments (bases 172625-173291 and 173690-174389).



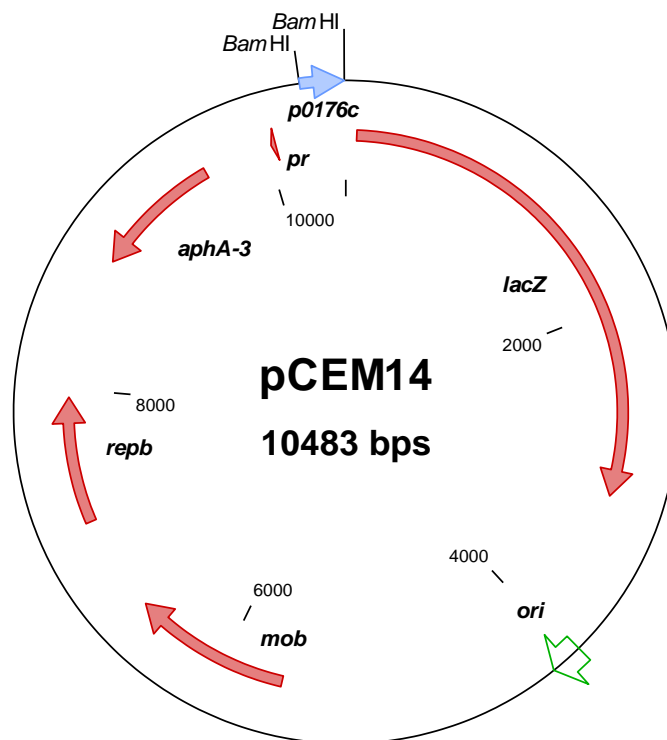
**Figure 11.** pCEM11: pCEM5,  $\Delta cfrA::aphA-3$ . Deletion of 1966 bp of *cfrA* ORF (bases 705484-707451) and insertion of  $Km^R$  cassette (in forward orientation with respect to the deleted gene) into *Bgl*III site created at the point of deletion.



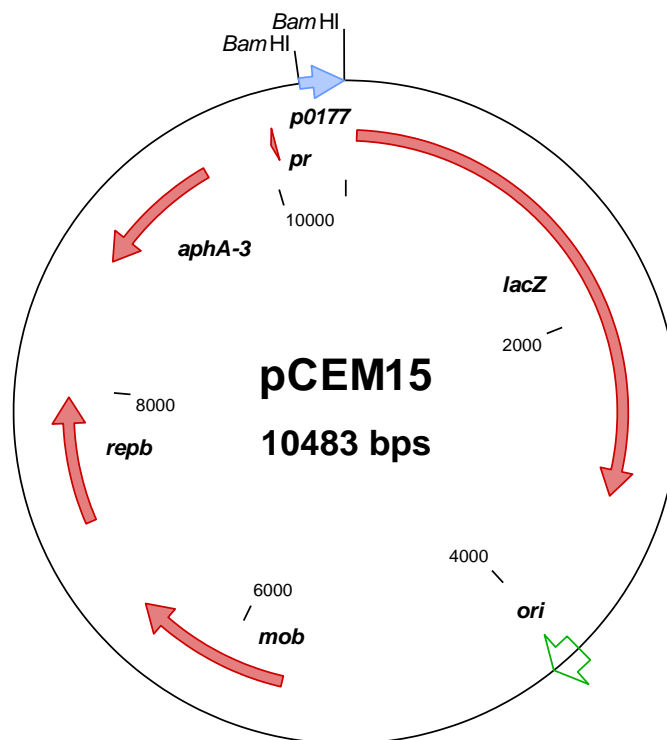
**Figure 12.** pCEM12: pCEM6,  $\Delta ceuE::aphA-3$ . Deletion of 800 bp of *ceuE* ORF (bases 1286798-1287599) and insertion of  $Km^R$  cassette (in forward orientation with respect to the deleted gene) into *Sma*I site created at the point of deletion.



**Figure 13.** pCEM13: pCEM6,  $\Delta ceuE::aphA-3$ . Deletion of 800 bp of *ceuE* ORF (bases 1286798-1287599) and insertion of  $Km^R$  cassette (in reverse orientation with respect to the deleted gene) into *Sma*I site created at the point of deletion.

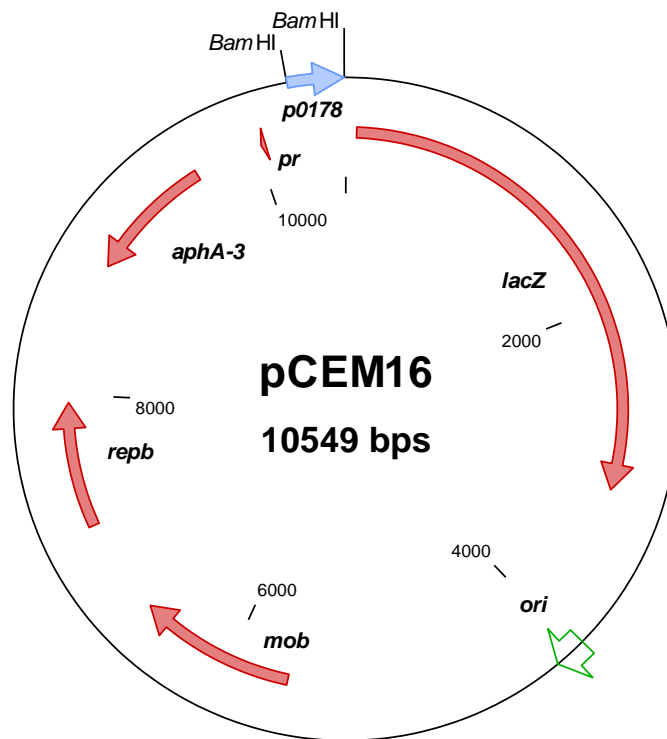


**Figure 14.** pCEM14: Reporter construct. *C. jejuni* NCTC 11168 *cj0176c-cj0177* promoter region (bases 172915-172695) inserted into *Bam*HI site of pMW10 (*cj0176c* orientation).

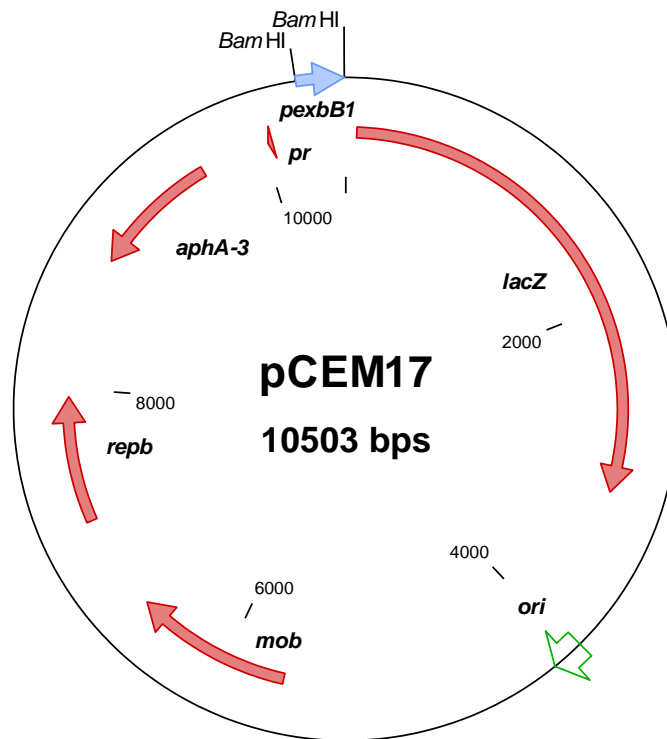


**Figure 15.** pCEM15: Reporter construct. *C. jejuni* NCTC 11168 *cj0176c-cj0177* promoter region (bases 172695-172915) inserted into *Bam*HI site of pMW10 (*cj0177* orientation).

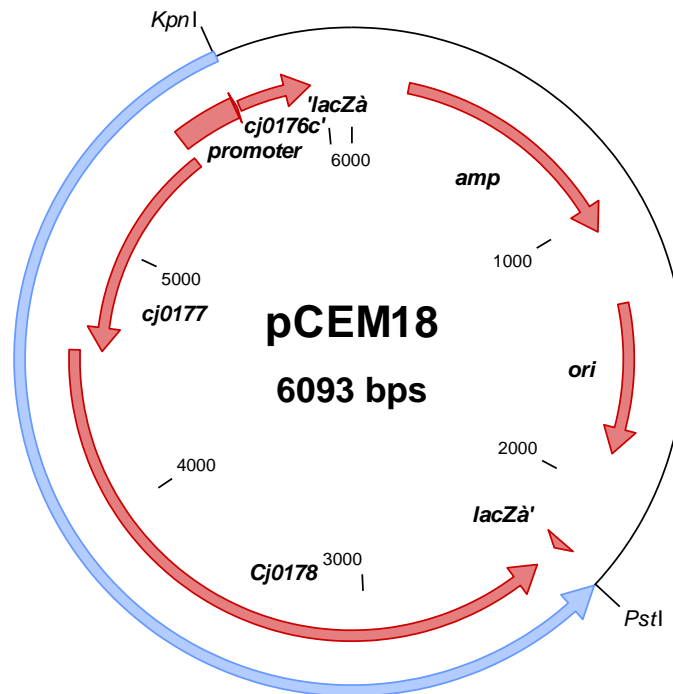




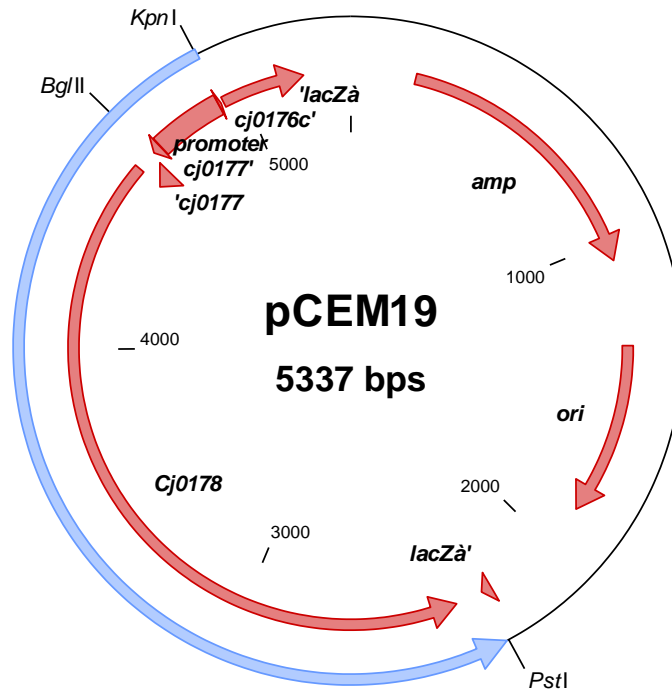
**Figure 16.** pCEM16: Reporter construct. *C. jejuni* NCTC 11168 *cj0178* putative promoter region (bases 173477-173763) inserted into *Bam*HI site of pMW10.



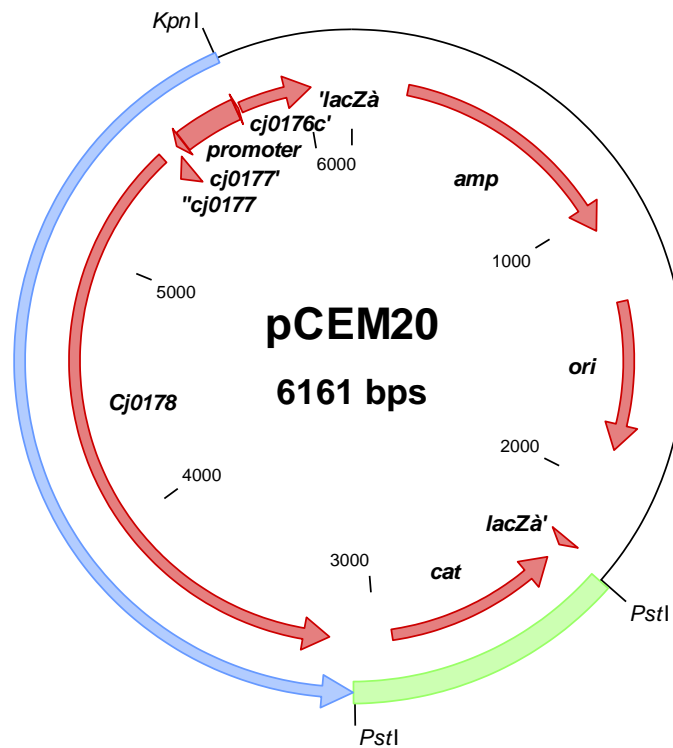
**Figure 17.** pCEM17: Reporter construct. *C. jejuni* NCTC 11168 *exbB1* putative promoter region (bases 175801-176042) inserted into *Bam*HI site of pMW10.



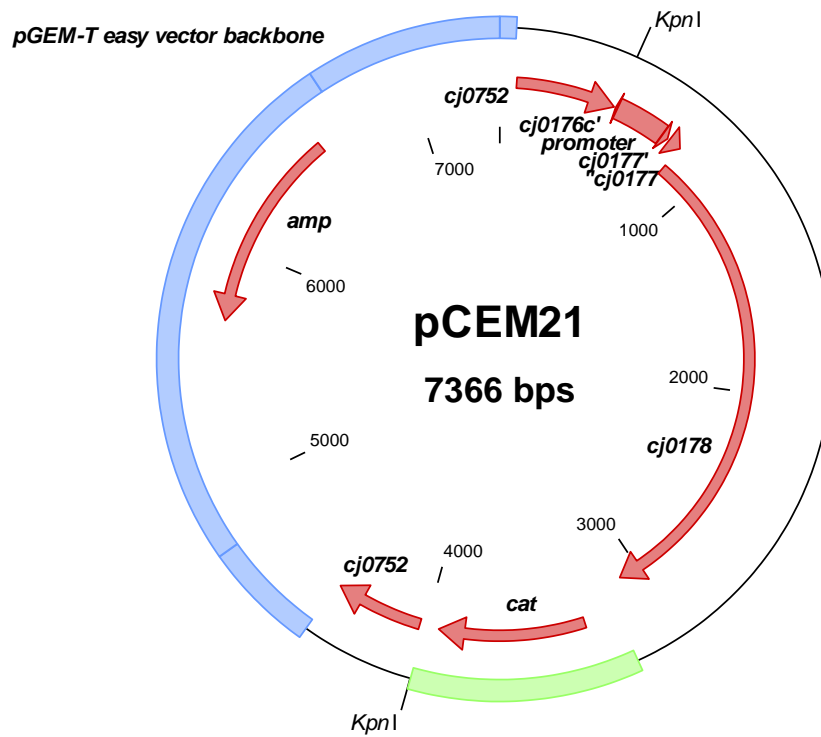
**Figure 18.** pCEM18: pUC19 containing *C. jejuni* NCTC 11168 genomic fragment (bases 172686-176113; promoter region, *cj0177*, *cj0178*) cloned between *KpnI* and *PstI* sites.



**Figure 19.** pCEM19: pCEM18,  $\Delta cj0177$ . Deletion of 764 bp of *cj0177* ORF (bases 172943-173706).



**Figure 20.** pCEM20: pCEM19 plus *cat*. Insertion of Cm<sup>R</sup> cassette at *Pst*I site downstream of *cj0178* (in forward orientation with respect to the wild-type copy of the gene).



**Figure 21.** pCEM21: Wild-type copy of *cj0178* plus promoter region and downstream Cm<sup>R</sup> cassette from pCEM20, inserted at *KpnI* site within multiple cloning region of pGEMCWH01 (in forward orientation with respect to *cj0752* flanks).

**Appendix 2.**

**Additional Primers**

**Table 1.** Additional primers used during this study.

Primer	Nucleotide sequence 5'- 3' (Restriction enzyme sites are highlighted in bold)	Product and target gene/construct
<b>Miscellaneous and screening primers</b>		
M13-F (21 mer)	GTTGTAAAACGACGGCCAGTG	pUC19
M13-R (24 mer)	GGAAACAGCTATGACCATGATTAC	pUC19
STM invkan-F	CTGGGGATCAAGCCTGATTG	Kanamycin resistance gene ( <i>aphA-3</i> )
SkanR	GGTTATTGTCCTGGGTTTCAAGCATTAG	Kanamycin resistance gene ( <i>aphA-3</i> )
CatInvF	GGAATGTCCGCAAAGCCTAATCC	Chloramphenicol resistance gene ( <i>cat</i> )
CatInvR	GCGGTCCTGAACTCTTCATGTC	Chloramphenicol resistance gene ( <i>cat</i> )
eryF	TCCCCCGGGGATCCTAGCTTTGGCTAACAC	Erythromycin resistance gene ( <i>ermC'</i> )
eryR	TCCCCCGGGCGCTCTAGAACTAGTGGATCTG	Erythromycin resistance gene ( <i>ermC'</i> )
ermCR	AACAGCTATGACCATGATTACG	Erythromycin resistance gene ( <i>ermC'</i> )
ermCIF	CATGCAGGAATTGACGATTAAAC	Erythromycin resistance gene ( <i>ermC'</i> )
ermCIR	GCCGATTTCAAAGATATTATCATGTTC	Erythromycin resistance gene ( <i>ermC'</i> )
<i>cfrA</i> for (K. A. Ridley)	ACACATTCTATGGTAAATTG	<i>C. jejuni</i> genome 705178-705197 bp
<i>cfrA</i> rev (K. A. Ridley)	GGAGCTTCTTTAATATCTTG	<i>C. jejuni</i> genome 705577-705558 bp
<i>cj0178compF2</i>	GGGGTACCGAATTATACTCGGTTTATTAAG	<i>C. jejuni</i> genome 172585-172607 bp
<i>cj0178compR2</i>	AACTGCAGCGGTTTCTATCTTAGAAGAATC	<i>C. jejuni</i> genome 176148-176127 bp
<i>cj0178InvF</i> Screen	CAAAACTAGCAAAACATAAACCTACAG	<i>C. jejuni</i> genome 173815-173789 bp
<i>cj0178InvR</i> Screen	GCTCGCGGTCGTACTTTTATAG	<i>C. jejuni</i> genome 175987-176008 bp
<i>cj0177Fprom</i> (K. A. Ridley)	TTAATTCCTTTTAGATATTTTGATAAG	<i>C. jejuni</i> genome 172694-172720 bp



## Appendix 2. Additional Primers

<i>cj0177R</i> prom (K. A. Ridley)	GCTTATAAATAGAATGCTTAAA	<i>C. jejuni</i> genome 172954-172933 bp
<i>cj0177F</i> promoter	TCCCCCGGGCATAGACATTAATTCCTTTTAG	<i>C. jejuni</i> genome 172686-172707 bp
<i>cj0177R</i> promoter	TCCCCCGGGCATAAAGCTTATAAATAGAATG	<i>C. jejuni</i> genome 172960-172939 bp
<i>cj0182R</i> Screen	CATGAATTGATCGCAACATTTAAG	<i>C. jejuni</i> genome 178116-178093 bp
<i>feoB2</i>	TTTGGATCCTTTTCATATAAGATTCACTTCTATGG	<i>C. jejuni</i> genome 1331317-1331292 bp
<i>feoB3</i>	ACCGGATCCCGGGCTTATATAGTAGCTTTTATAG	<i>C. jejuni</i> genome 1333099-1333120 bp

**Appendix 3.**

**Raw Growth Curve Data and Statistical Testing of Differences**

All growth assays were conducted using MEM $\alpha$  with cultures incubated microaerobically with agitation over 24 h. Conditions were tested in triplicate (technical replicates) in two independent assays (biological replicates).

**Growth assays of wild-type *C. jejuni* NCTC 11168 with a range of ferrous sulphate (FeSO<sub>4</sub>) concentrations.**

**Figure 3.1a.**

**0  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.01	0.01	0.03
4	0.03	0.03	0.02	0.05	0.04	0.05
8	0.04	0.06	0.06	0.07	0.05	0.07
12	0.07	0.04	0.06	0.06	0.05	0.06
24	0.08	0.05	0.05	0.04	0.06	0.07

**10  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.05	0.06	0.08	0.06	0.07	0.07
8	0.13	0.14	0.17	0.20	0.18	0.27
12	0.20	0.20	0.19	0.25	0.25	0.25
24	0.19	0.20	0.19	0.23	0.21	0.19

**20  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.01	0.03	0.03	0.02
4	0.08	0.07	0.07	0.05	0.07	0.08
8	0.19	0.18	0.17	0.15	0.17	0.19
12	0.22	0.22	0.20	0.24	0.26	0.24
24	0.21	0.21	0.22	0.22	0.22	0.20

**50  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.02	0.03	0.03	0.03
4	0.06	0.05	0.08	0.06	0.07	0.07
8	0.17	0.19	0.19	0.16	0.14	0.19
12	0.19	0.20	0.19	0.26	0.25	0.22
24	0.19	0.18	0.19	0.22	0.22	0.22

**200  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.02	0.01	0.04	0.02
4	0.07	0.07	0.06	0.06	0.07	0.05
8	0.18	0.15	0.16	0.17	0.18	0.15
12	0.25	0.20	0.25	0.24	0.25	0.23
24	0.28	0.22	0.27	0.24	0.27	0.23

**500  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.03	0.02	0.01	0.02
4	0.02	0.03	0.04	0.04	0.06	0.05
8	0.09	0.10	0.09	0.12	0.09	0.12
12	0.22	0.21	0.22	0.20	0.19	0.22
24	0.22	0.21	0.21	0.24	0.26	0.24

**Figure 3.1b.****0  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.01	0.04	0.03	0.04
4	0.05	0.04	0.05	0.03	0.03	0.03
8	0.04	0.03	0.04	0.05	0.04	0.05
12	0.06	0.06	0.04	0.06	0.06	0.05
24	0.07	0.07	0.05	0.07	0.05	0.07

**2  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.03	0.03
4	0.04	0.04	0.05	0.03	0.03	0.03
8	0.08	0.07	0.09	0.12	0.11	0.09
12	0.16	0.15	0.15	0.16	0.15	0.15
24	0.15	0.15	0.17	0.18	0.18	0.18

**4  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.04	0.05	0.05	0.05	0.03	0.03
8	0.09	0.07	0.07	0.11	0.11	0.12
12	0.16	0.16	0.16	0.16	0.14	0.15
24	0.15	0.17	0.15	0.17	0.17	0.18

**6  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.04	0.05	0.03
4	0.06	0.05	0.03	0.05	0.04	0.03
8	0.12	0.12	0.09	0.13	0.13	0.13
12	0.19	0.18	0.17	0.15	0.16	0.16
24	0.18	0.17	0.17	0.16	0.17	0.19

**8  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.04	0.03	0.04
4	0.03	0.03	0.03	0.03	0.05	0.03
8	0.10	0.09	0.09	0.11	0.14	0.13
12	0.17	0.17	0.17	0.16	0.15	0.16
24	0.15	0.18	0.17	0.17	0.15	0.17

**10  $\mu$ M ferrous sulphate**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.03	0.05
4	0.05	0.03	0.05	0.04	0.03	0.03
8	0.12	0.12	0.10	0.14	0.12	0.12
12	0.16	0.18	0.17	0.18	0.16	0.16
24	0.18	0.18	0.15	0.18	0.18	0.16

**Growth assays of wild-type *C. jejuni* NCTC 11168 with iron supplied bound to human lactoferrin (Lf), human transferrin (Tf) and ovotransferrin (ovo-Tf).**

**Figure 3.3a.**  
**NCTC 11168 with ferri-proteins.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.02	0.03	0.02
4	0.06	0.06	0.06	0.05	0.06	0.06
8	0.08	0.09	0.09	0.09	0.09	0.09
12	0.10	0.10	0.09	0.10	0.09	0.10
24	0.11	0.10	0.11	0.10	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.03	0.04	0.02
4	0.07	0.07	0.08	0.08	0.08	0.07
8	0.19	0.17	0.18	0.19	0.18	0.18
12	0.25	0.23	0.23	0.23	0.24	0.24
24	0.25	0.26	0.26	0.26	0.26	0.26

**0.27  $\mu$ M human ferri-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.02	0.02	0.02
4	0.06	0.07	0.07	0.06	0.06	0.07
8	0.16	0.16	0.17	0.16	0.16	0.16
12	0.23	0.22	0.22	0.23	0.24	0.23
24	0.26	0.26	0.27	0.27	0.26	0.27

**0.27  $\mu$ M human ferri-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.02	0.03	0.02	0.03
4	0.08	0.08	0.07	0.07	0.08	0.07
8	0.17	0.16	0.18	0.17	0.17	0.18
12	0.27	0.26	0.26	0.27	0.27	0.26
24	0.27	0.27	0.27	0.28	0.29	0.28

**0.27  $\mu$ M ferri-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.02	0.03	0.03
4	0.07	0.07	0.07	0.06	0.07	0.07
8	0.18	0.18	0.18	0.17	0.17	0.18
12	0.27	0.27	0.27	0.26	0.27	0.27
24	0.28	0.28	0.29	0.28	0.30	0.30

**Mean of 0.27  $\mu$ M human ferri-Lf optical density values at 24 h from experimental run 1 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0005
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 47.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.1567
95% confidence interval	0.1423 to 0.1710
R squared	0.9991

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf optical density values at 24 h from experimental run 2 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0004
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 50.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.1667
95% confidence interval	0.1523 to 0.1810
R squared	0.9992

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.1835
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.006667
95% confidence interval	-0.007677 to 0.02101
R squared	0.6667

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.1835
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.006667
95% confidence interval	-0.007677 to 0.02101
R squared	0.6667

**Mean of 0.27  $\mu$ M human ferri-Tf optical density values at 24 h from experimental run 1 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0004
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 49.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.1633
95% confidence interval	0.1490 to 0.1777
R squared	0.9992

**Mean of 0.27  $\mu$ M human ferri-Tf optical density values at 24 h from experimental run 2 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0003
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 55.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.1833
95% confidence interval	0.1690 to 0.1977
R squared	0.9993

**Mean of 0.27  $\mu$ M human ferri-Tf optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0572
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	t = 4.000, df = 2
Number of pairs	3



How big is the difference?

Mean of differences	0.01333
95% confidence interval	-0.001010 to 0.02768
R squared	0.8889

**Mean of 0.27  $\mu$ M human ferri-Tf optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0198
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 7.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.02333
95% confidence interval	0.008990 to 0.03768
R squared	0.9608

**Mean of 0.27  $\mu$ M ferri-ovo-Tf optical density values at 24 h from experimental run 1 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0004
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 53.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.1767
95% confidence interval	0.1623 to 0.1910
R squared	0.9993

**Mean of 0.27  $\mu$ M ferri-ovo-Tf optical density values at 24 h from experimental run 2 vs. mean of negative (unsupplemented MEM $\alpha$ ) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0012
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 29.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.1933
95% confidence interval	0.1646 to 0.2220
R squared	0.9976

**Mean of 0.27  $\mu$ M ferri-ovo-Tf optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>) optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0153
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 8.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.02667
95% confidence interval	0.01232 to 0.04101
R squared	0.9697

**Mean of 0.27  $\mu$ M ferri-ovo-Tf optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>) optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0377
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 5.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.03333
95% confidence interval	0.004647 to 0.06202
R squared	0.9259

**Figure 3.3b.**  
**NCTC 11168 with apo-proteins.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.02	0.03	0.02
4	0.06	0.06	0.06	0.05	0.06	0.06
8	0.08	0.09	0.09	0.09	0.09	0.09
12	0.10	0.10	0.09	0.10	0.09	0.10
24	0.11	0.10	0.11	0.10	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.03	0.04	0.02
4	0.07	0.07	0.08	0.08	0.08	0.07
8	0.19	0.17	0.18	0.19	0.18	0.18
12	0.25	0.23	0.23	0.23	0.24	0.24
24	0.25	0.26	0.26	0.26	0.26	0.26

**0.27  $\mu$ M human apo-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.02	0.02	0.03
4	0.06	0.07	0.06	0.07	0.07	0.07
8	0.12	0.13	0.11	0.12	0.12	0.11
12	0.15	0.14	0.15	0.15	0.15	0.13
24	0.19	0.19	0.18	0.17	0.17	0.17

**0.27  $\mu$ M human apo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.02	0.03
4	0.05	0.04	0.05	0.04	0.04	0.05
8	0.05	0.05	0.05	0.06	0.06	0.05
12	0.05	0.04	0.06	0.05	0.06	0.06
24	0.05	0.03	0.03	0.05	0.04	0.05

**0.27  $\mu$ M apo-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.06	0.05	0.05	0.05	0.05	0.04
8	0.05	0.05	0.05	0.05	0.04	0.06
12	0.05	0.05	0.05	0.05	0.05	0.05
24	0.03	0.03	0.04	0.03	0.03	0.05

**Mean of 0.27  $\mu$ M human ferri-Lf optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human apo-Lf optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0021
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.07333
95% confidence interval	0.05899 to 0.08768
R squared	0.9959

**Mean of 0.27  $\mu$ M human ferri-Lf optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human apo-Lf optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0012
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 29.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.09667
95% confidence interval	0.08232 to 0.1110
R squared	0.9976

**Growth assay of wild-type *C. jejuni* 81-176 with iron supplied bound to human Lf, human Tf and ovo-Tf.**

**Figure 3.4.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.02	0.02	0.03
4	0.01	0.02	0.01	0.03	0.02	0.02
8	0.09	0.08	0.09	0.07	0.07	0.09
12	0.10	0.11	0.10	0.09	0.09	0.10
24	0.10	0.11	0.10	0.09	0.11	0.11

**Positive (10  $\mu$ M FeSO<sub>4</sub>)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.02	0.02	0.02
4	0.04	0.03	0.04	0.04	0.04	0.04
8	0.19	0.19	0.20	0.18	0.18	0.19
12	0.29	0.28	0.28	0.29	0.29	0.30
24	0.31	0.29	0.28	0.29	0.30	0.30

**0.27  $\mu$ M human ferri-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.02
4	0.02	0.03	0.02	0.03	0.04	0.04
8	0.22	0.21	0.20	0.20	0.20	0.20
12	0.33	0.31	0.32	0.32	0.32	0.31
24	0.32	0.33	0.34	0.33	0.33	0.34

**0.27  $\mu$ M human ferri-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.03	0.02
4	0.02	0.02	0.02	0.03	0.03	0.04
8	0.19	0.20	0.22	0.21	0.20	0.21
12	0.30	0.30	0.30	0.31	0.31	0.32
24	0.35	0.32	0.33	0.34	0.33	0.32

**0.27  $\mu$ M ferri-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.03	0.02
4	0.03	0.01	0.02	0.04	0.04	0.02
8	0.23	0.21	0.22	0.22	0.21	0.23
12	0.31	0.31	0.34	0.33	0.34	0.34
24	0.33	0.34	0.35	0.33	0.35	0.35

**Growth assays of wild-type *C. jejuni* NCTC 11168 mixed with or partitioned from iron supplied bound to human Lf, human Tf or ovo-Tf.**

**Figure 3.5a.**

**NCTC 11168 with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.02	0.03
4	0.04	0.04	0.05	0.04	0.04	0.04
8	0.09	0.10	0.10	0.08	0.09	0.09
12	0.08	0.09	0.08	0.10	0.10	0.09
24	0.09	0.08	0.10	0.09	0.09	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.06	0.07	0.08	0.06	0.06	0.08
8	0.20	0.21	0.23	0.24	0.21	0.22
12	0.25	0.25	0.27	0.25	0.26	0.27
24	0.29	0.27	0.29	0.28	0.29	0.29

**Positive (10  $\mu$ M FeSO<sub>4</sub>), partitioned**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.03	0.02	0.03	0.03
4	0.07	0.07	0.07	0.07	0.08	0.06
8	0.22	0.24	0.23	0.22	0.22	0.21
12	0.25	0.26	0.27	0.26	0.26	0.26
24	0.27	0.30	0.29	0.26	0.28	0.28

**250  $\mu$ g human ferri-Lf, mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.03	0.03	0.02
4	0.06	0.06	0.07	0.06	0.06	0.06
8	0.22	0.21	0.21	0.21	0.21	0.21
12	0.28	0.28	0.29	0.29	0.29	0.29
24	0.30	0.29	0.31	0.28	0.28	0.30

**250  $\mu$ g human ferri-Lf, partitioned**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.03
4	0.06	0.05	0.05	0.05	0.05	0.05
8	0.12	0.14	0.14	0.12	0.12	0.13
12	0.13	0.16	0.16	0.15	0.15	0.16
24	0.17	0.17	0.17	0.16	0.16	0.16

**Mean of 250  $\mu$ g human ferri-Lf, mixed optical density values at 24 h from experimental run 1 vs. mean of 250  $\mu$ g human ferri-Lf, partitioned optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0020
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.52$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1300
95% confidence interval	-0.1548 to -0.1052
R squared	0.9961

**Mean of 250 µg human ferri-Lf, mixed optical density values at 24 h from experimental run 2 vs. mean of 250 µg human ferri-Lf, partitioned optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0028
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 19.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1267
95% confidence interval	-0.1554 to -0.09798
R squared	0.9945

**Mean of positive (10 µM FeSO<sub>4</sub>), mixed optical density values at 24 h from experimental run 1 vs. mean of positive (10 µM FeSO<sub>4</sub>), partitioned optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.8399
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 0.2294, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.003333
95% confidence interval	-0.05919 to 0.06585
R squared	0.02564

**Mean of positive (10 µM FeSO<sub>4</sub>), mixed optical density values at 24 h from experimental run 2 vs. mean of positive (10 µM FeSO<sub>4</sub>), partitioned optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0572
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 4.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.01333
95% confidence interval	-0.02768 to 0.001010
R squared	0.8889

**Figure 3.5b.**  
**NCTC 11168 with human ferri-Tf.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.02	0.03
4	0.04	0.04	0.05	0.04	0.04	0.04
8	0.09	0.10	0.10	0.08	0.09	0.09
12	0.08	0.09	0.08	0.10	0.10	0.09
24	0.09	0.08	0.10	0.09	0.09	0.10

**Positive (10  $\mu$ M FeSO $_4$ ), mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.06	0.07	0.08	0.06	0.06	0.08
8	0.20	0.21	0.23	0.24	0.21	0.22
12	0.25	0.25	0.27	0.25	0.26	0.27
24	0.29	0.27	0.29	0.28	0.29	0.29

**Positive (10  $\mu$ M FeSO $_4$ ), partitioned**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.03	0.02	0.03	0.03
4	0.07	0.07	0.07	0.07	0.08	0.06
8	0.22	0.24	0.23	0.22	0.22	0.21
12	0.25	0.26	0.27	0.26	0.26	0.26
24	0.27	0.30	0.29	0.26	0.28	0.28

**250  $\mu$ g human ferri-Tf, mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.02	0.02	0.04
4	0.07	0.07	0.06	0.07	0.07	0.07
8	0.20	0.21	0.21	0.21	0.21	0.22
12	0.28	0.28	0.28	0.29	0.28	0.28
24	0.29	0.30	0.31	0.30	0.27	0.29



**250 µg human ferri-Tf, partitioned**

<b>Hours at 37°C</b>	<b>Experimental Run 1 (optical density at 600 nm)</b>			<b>Experimental Run 2 (optical density at 600 nm)</b>		
0	0.03	0.02	0.03	0.04	0.03	0.02
4	0.05	0.05	0.05	0.05	0.05	0.06
8	0.13	0.15	0.14	0.13	0.14	0.14
12	0.16	0.15	0.16	0.14	0.15	0.15
24	0.17	0.16	0.16	0.16	0.17	0.17

**Mean of 250 µg human ferri-Tf, mixed optical density values at 24 h from experimental run 1 vs. mean of 250 µg human ferri-Tf, partitioned optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0041
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 15.50$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1367
95% confidence interval	-0.1746 to -0.09872
R squared	0.9917

**Mean of 250 µg human ferri-Tf, mixed optical density values at 24 h from experimental run 2 vs. mean of 250 µg human ferri-Tf, partitioned optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0051
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 13.98$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1233
95% confidence interval	-0.1613 to -0.08538
R squared	0.9899

**Figure 3.5c.**  
**NCTC 11168 with ferri-ovo-Tf.**

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.02	0.03
4	0.04	0.04	0.05	0.04	0.04	0.04
8	0.09	0.10	0.10	0.08	0.09	0.09
12	0.08	0.09	0.08	0.10	0.10	0.09
24	0.09	0.08	0.10	0.09	0.09	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.06	0.07	0.08	0.06	0.06	0.08
8	0.20	0.21	0.23	0.24	0.21	0.22
12	0.25	0.25	0.27	0.25	0.26	0.27
24	0.29	0.27	0.29	0.28	0.29	0.29

**Positive (10  $\mu$ M FeSO<sub>4</sub>), partitioned**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.03	0.02	0.03	0.03
4	0.07	0.07	0.07	0.07	0.08	0.06
8	0.22	0.24	0.23	0.22	0.22	0.21
12	0.25	0.26	0.27	0.26	0.26	0.26
24	0.27	0.30	0.29	0.26	0.28	0.28

**250  $\mu$ g ferri-ovo-Tf, mixed**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.03	0.03
4	0.07	0.07	0.08	0.07	0.07	0.06
8	0.21	0.21	0.22	0.20	0.22	0.21
12	0.27	0.30	0.29	0.28	0.28	0.27
24	0.28	0.29	0.30	0.30	0.31	0.31

**250  $\mu$ g ferri-ovo-Tf, partitioned**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.03	0.03	0.03	0.03
4	0.05	0.05	0.05	0.05	0.05	0.05
8	0.14	0.15	0.13	0.14	0.14	0.12
12	0.16	0.17	0.17	0.14	0.14	0.16
24	0.15	0.17	0.16	0.15	0.16	0.17

**Mean of 250 µg ferri-ovo-Tf, mixed optical density values at 24 h from experimental run 1 vs. mean of 250 µg ferri-ovo-Tf, partitioned optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0020
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.52$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1300
95% confidence interval	-0.1548 to -0.1052
R squared	0.9961

**Mean of 250 µg ferri-ovo-Tf, mixed optical density values at 24 h from experimental run 2 vs. mean of 250 µg ferri-ovo-Tf, partitioned optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0005
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 44.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1467
95% confidence interval	-0.1610 to -0.1323
R squared	0.9990

### Competition growth assays of wild-type *C. jejuni* NCTC 11168

#### Figure 3.6a.

**NCTC 11168 with competing ferri-protein and bovine serum albumin (BSA).**

#### Negative (unsupplemented MEM $\alpha$ )

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.02	0.03	0.02	0.03
4	0.04	0.04	0.04	0.04	0.04	0.05
8	0.09	0.08	0.10	0.08	0.08	0.09
12	0.09	0.10	0.10	0.09	0.10	0.10
24	0.09	0.08	0.08	0.09	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.02
4	0.06	0.06	0.07	0.06	0.07	0.06
8	0.18	0.16	0.19	0.17	0.17	0.19
12	0.25	0.25	0.24	0.26	0.26	0.25
24	0.26	0.26	0.27	0.28	0.28	0.28

**0.27  $\mu$ M human ferri-Lf vs. 0.81  $\mu$ M BSA**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.03	0.03	0.02
4	0.05	0.06	0.06	0.06	0.06	0.06
8	0.17	0.17	0.16	0.17	0.18	0.17
12	0.22	0.23	0.23	0.23	0.23	0.24
24	0.26	0.26	0.27	0.26	0.27	0.27

**0.27  $\mu$ M human ferri-Tf vs. 0.81  $\mu$ M BSA**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.02	0.02
4	0.06	0.05	0.06	0.06	0.05	0.05
8	0.16	0.18	0.19	0.17	0.17	0.19
12	0.27	0.28	0.27	0.28	0.27	0.26
24	0.28	0.26	0.26	0.28	0.29	0.29

**0.27  $\mu$ M ferri-ovo-Tf vs. 0.81  $\mu$ M BSA**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.03	0.02	0.02
4	0.06	0.05	0.06	0.06	0.06	0.07
8	0.19	0.18	0.19	0.19	0.19	0.18
12	0.28	0.27	0.28	0.29	0.27	0.27
24	0.26	0.28	0.28	0.28	0.28	0.26

**Figure 3.6b.**

NCTC 11168 with competing cognate ferri- and apo-proteins.

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.02	0.03	0.02	0.03
4	0.04	0.04	0.04	0.04	0.04	0.05
8	0.09	0.08	0.10	0.08	0.08	0.09
12	0.09	0.10	0.10	0.09	0.10	0.10
24	0.09	0.08	0.08	0.09	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.02
4	0.06	0.06	0.07	0.06	0.07	0.06
8	0.18	0.16	0.19	0.17	0.17	0.19
12	0.25	0.25	0.24	0.26	0.26	0.25
24	0.26	0.26	0.27	0.28	0.28	0.28

**0.27  $\mu$ M human ferri-Lf vs. 0.81  $\mu$ M human apo-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.01	0.02	0.02	0.03
4	0.02	0.03	0.03	0.02	0.02	0.03
8	0.11	0.13	0.12	0.11	0.11	0.12
12	0.18	0.19	0.18	0.18	0.18	0.17
24	0.23	0.23	0.23	0.19	0.20	0.21

**0.27  $\mu$ M human ferri-Tf vs. 0.81  $\mu$ M human apo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.03	0.02
4	0.02	0.01	0.03	0.02	0.02	0.01
8	0.02	0.01	0.02	0.02	0.02	0.02
12	0.01	0.02	0.01	0.02	0.03	0.02
24	0.05	0.04	0.05	0.05	0.05	0.05

**0.27  $\mu$ M ferri-ovo-Tf vs. 0.81  $\mu$ M apo-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.03	0.02	0.03
4	0.03	0.02	0.02	0.02	0.02	0.02
8	0.05	0.03	0.03	0.04	0.04	0.05
12	0.06	0.07	0.07	0.07	0.06	0.07
24	0.12	0.12	0.12	0.11	0.12	0.11

**Figure 3.6c.**

NCTC 11168 with competing opposite ferri- and apo-proteins.

**Negative (unsupplemented MEM $\alpha$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.02	0.03	0.02	0.03
4	0.04	0.04	0.04	0.04	0.04	0.05
8	0.09	0.08	0.10	0.08	0.08	0.09
12	0.09	0.10	0.10	0.09	0.10	0.10
24	0.09	0.08	0.08	0.09	0.10	0.10

**Positive (10  $\mu\text{M}$   $\text{FeSO}_4$ )**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.02
4	0.06	0.06	0.07	0.06	0.07	0.06
8	0.18	0.16	0.19	0.17	0.17	0.19
12	0.25	0.25	0.24	0.26	0.26	0.25
24	0.26	0.26	0.27	0.28	0.28	0.28

**0.27  $\mu\text{M}$  human ferri-Lf vs. 0.81  $\mu\text{M}$  human apo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.02	0.03	0.02
4	0.04	0.05	0.03	0.04	0.04	0.05
8	0.07	0.07	0.07	0.06	0.07	0.07
12	0.09	0.10	0.11	0.10	0.10	0.09
24	0.15	0.16	0.15	0.16	0.15	0.15

**0.27  $\mu\text{M}$  human ferri-Lf vs. 0.81  $\mu\text{M}$  apo-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.03	0.02	0.02	0.02
4	0.04	0.04	0.05	0.04	0.05	0.05
8	0.07	0.06	0.08	0.06	0.06	0.07
12	0.07	0.08	0.07	0.08	0.08	0.07
24	0.13	0.14	0.14	0.13	0.13	0.14

**0.27  $\mu\text{M}$  human ferri-Tf vs. 0.81  $\mu\text{M}$  human apo-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.01	0.02	0.02	0.02
4	0.04	0.04	0.05	0.05	0.05	0.04
8	0.10	0.08	0.09	0.09	0.09	0.10
12	0.13	0.12	0.14	0.13	0.13	0.14
24	0.20	0.21	0.20	0.20	0.19	0.19

**0.27  $\mu\text{M}$  human ferri-Tf vs. 0.81  $\mu\text{M}$  apo-ovo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.01	0.02	0.02	0.03
4	0.03	0.02	0.02	0.03	0.03	0.03
8	0.01	0.02	0.02	0.01	0.01	0.02
12	0.04	0.02	0.02	0.03	0.03	0.05
24	0.03	0.02	0.03	0.03	0.04	0.03

**0.27  $\mu$ M ferri-ovo-Tf vs. 0.81  $\mu$ M human apo-Lf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.02	0.03
4	0.04	0.04	0.05	0.05	0.04	0.04
8	0.11	0.12	0.12	0.10	0.11	0.12
12	0.15	0.16	0.16	0.16	0.16	0.16
24	0.19	0.20	0.21	0.19	0.20	0.20

**0.27  $\mu$ M ferri-ovo-Tf vs. 0.81  $\mu$ M human apo-Tf**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.02	0.02
4	0.02	0.03	0.02	0.02	0.03	0.02
8	0.03	0.04	0.04	0.03	0.03	0.05
12	0.06	0.06	0.08	0.07	0.07	0.08
24	0.09	0.12	0.11	0.10	0.11	0.10

**Growth of wild-type and mutant *C. jejuni* strains in rich medium (Mueller-Hinton broth, MHB).**

**Figure 4.10a.**

**JDR5 ( $\Delta chuA::cat$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.03	0.03	0.02
4	0.07	0.06	0.07	0.07	0.08	0.07
8	0.26	0.26	0.28	0.27	0.27	0.29
12	0.36	0.37	0.38	0.38	0.39	0.37
24	0.64	0.64	0.62	0.60	0.58	0.62

**CEM5 ( $\Delta cfrA::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.02	0.02	0.02
4	0.07	0.09	0.09	0.08	0.08	0.08
8	0.28	0.28	0.30	0.29	0.27	0.28
12	0.40	0.40	0.41	0.39	0.39	0.38
24	0.62	0.63	0.63	0.59	0.59	0.60

**CEM3 ( $\Delta cj0174c::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.01	0.01	0.03
4	0.08	0.07	0.08	0.07	0.07	0.09
8	0.25	0.26	0.26	0.29	0.27	0.27
12	0.39	0.36	0.37	0.38	0.38	0.36
24	0.63	0.61	0.62	0.59	0.62	0.60

**81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.03	0.01
4	0.08	0.09	0.09	0.09	0.08	0.08
8	0.28	0.30	0.30	0.29	0.29	0.29
12	0.37	0.39	0.37	0.39	0.40	0.38
24	0.57	0.58	0.57	0.60	0.60	0.61

**NCTC 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.02	0.02	0.01
4	0.07	0.07	0.07	0.06	0.07	0.08
8	0.27	0.27	0.25	0.25	0.26	0.26
12	0.38	0.35	0.35	0.36	0.36	0.38
24	0.57	0.55	0.55	0.59	0.58	0.60

**KAR3 ( $p19::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.03	0.03	0.02
4	0.08	0.07	0.08	0.08	0.07	0.07
8	0.27	0.27	0.26	0.26	0.28	0.28
12	0.39	0.37	0.38	0.36	0.36	0.39
24	0.58	0.59	0.55	0.57	0.58	0.56

**KAR2 ( $cj0178::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.03	0.02	0.03	0.01
4	0.08	0.07	0.08	0.08	0.08	0.06
8	0.28	0.25	0.25	0.28	0.26	0.26
12	0.39	0.35	0.36	0.37	0.36	0.38
24	0.52	0.53	0.52	0.55	0.56	0.54



**Figure 4.10b.****JDR21 ( $\Delta cj0178::cat$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.03
4	0.06	0.05	0.07	0.07
8	0.26	0.26	0.26	0.25
12	0.42	0.42	0.41	0.39
24	0.72	0.73	0.71	0.70

**CEM10 (CEM8,  $\Delta cj0177::ermC'$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.03
4	0.04	0.03	0.05	0.05
8	0.19	0.19	0.22	0.22
12	0.36	0.36	0.35	0.35
24	0.73	0.74	0.70	0.68

 **$\Delta feoB::ermC'$  11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.03
4	0.04	0.06	0.05	0.07
8	0.25	0.27	0.26	0.24
12	0.38	0.42	0.40	0.39
24	0.63	0.63	0.62	0.60

**NCTC 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.04	0.04	0.03
4	0.03	0.04	0.05	0.06
8	0.20	0.21	0.25	0.25
12	0.37	0.36	0.35	0.37
24	0.64	0.64	0.59	0.60

**JDR20 ( $\Delta tonB1::cat$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.04	0.04
4	0.04	0.04	0.06	0.06
8	0.21	0.20	0.22	0.21
12	0.36	0.34	0.35	0.37
24	0.59	0.60	0.60	0.58

**CEM9 (KAR2,  $\Delta cj0177::ermC'$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.05	0.03	0.03
4	0.02	0.03	0.04	0.04
8	0.15	0.20	0.19	0.21
12	0.30	0.32	0.33	0.35
24	0.51	0.52	0.55	0.55

**CEM4 ( $\Delta cj0177::ermC'$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.03	0.03	0.02
4	0.04	0.04	0.05	0.06
8	0.19	0.16	0.20	0.21
12	0.34	0.33	0.35	0.35
24	0.54	0.51	0.55	0.53

**Figure 4.10c.****CEM11 (KAR2,  $\Delta cfrA::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.02
4	0.04	0.05	0.07	0.07
8	0.24	0.23	0.25	0.26
12	0.42	0.38	0.37	0.39
24	0.71	0.67	0.65	0.68

**CEM12 (KAR2,  $\Delta feoB::ermC'$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.03	0.02	0.02
4	0.06	0.05	0.05	0.07
8	0.27	0.25	0.26	0.27
12	0.42	0.39	0.38	0.37
24	0.72	0.67	0.65	0.64

**NCTC 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.04	0.04	0.03
4	0.03	0.04	0.05	0.06
8	0.20	0.21	0.25	0.25
12	0.37	0.36	0.35	0.37
24	0.64	0.64	0.59	0.60

**CEM6 ( $\Delta ceuE::aphA-3$ ) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.05	0.04	0.03
4	0.03	0.03	0.04	0.04
8	0.14	0.12	0.16	0.15
12	0.32	0.30	0.34	0.33
24	0.60	0.60	0.58	0.57

**CEM8 (complemented KAR2 strain) 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.03	0.02
4	0.03	0.04	0.05	0.05
8	0.19	0.21	0.22	0.24
12	0.33	0.35	0.36	0.35
24	0.54	0.55	0.56	0.58

 **$\Delta cj0444::aphA-3$  81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.03
4	0.01	0.01	0.02	0.02
8	0.03	0.05	0.06	0.06
12	0.07	0.08	0.09	0.10
24	0.51	0.52	0.50	0.53

Growth assays of *C. jejuni* strains with mutated iron outer membrane receptor proteins supplied with iron bound to human-Lf.

**Figure 4.11a.**

NCTC 11168 and JDR5 ( $\Delta chuA::cat$ ) with human ferri-Lf.

**Negative (unsupplemented MEMa), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.03	0.03	0.01	0.03
4	0.02	0.02	0.03	0.02	0.03	0.03
8	0.03	0.05	0.06	0.04	0.03	0.05
12	0.07	0.07	0.05	0.06	0.07	0.06
24	0.07	0.04	0.06	0.06	0.05	0.06

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.01	0.03	0.03
4	0.06	0.05	0.05	0.05	0.04	0.05
8	0.18	0.19	0.18	0.16	0.16	0.16
12	0.26	0.25	0.26	0.22	0.22	0.22
24	0.30	0.32	0.29	0.26	0.27	0.29

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.02	0.01	0.02	0.02
4	0.05	0.06	0.04	0.05	0.03	0.05
8	0.15	0.16	0.19	0.18	0.15	0.14
12	0.23	0.26	0.25	0.24	0.25	0.22
24	0.27	0.25	0.26	0.26	0.26	0.26

**Negative (unsupplemented MEM $\alpha$ ), JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.01	0.02	0.05	0.04
4	0.02	0.03	0.02	0.02	0.01	0.01
8	0.06	0.06	0.07	0.08	0.05	0.08
12	0.06	0.08	0.07	0.07	0.07	0.07
24	0.05	0.06	0.08	0.07	0.06	0.06

**Positive (10  $\mu$ M FeSO<sub>4</sub>), JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.03	0.04	0.04
4	0.05	0.05	0.07	0.06	0.05	0.04
8	0.18	0.19	0.19	0.20	0.18	0.16
12	0.23	0.24	0.25	0.25	0.22	0.25
24	0.29	0.28	0.27	0.26	0.28	0.27

**0.27  $\mu$ M human ferri-Lf, JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.01	0.02	0.03	0.02
4	0.06	0.06	0.04	0.06	0.05	0.03
8	0.17	0.19	0.18	0.18	0.16	0.16
12	0.27	0.27	0.28	0.26	0.28	0.26
24	0.28	0.28	0.29	0.27	0.27	0.29

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, JDR5 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0728
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 3.500$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.02333
95% confidence interval	-0.005353 to 0.05202
R squared	0.8596

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, JDR5 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.1296
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.500$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.01667
95% confidence interval	-0.01202 to 0.04535
R squared	0.7576

**Figure 4.11b.**

**NCTC 11168 and KAR3 (*p19::aphA-3*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.02	0.03	0.01
4	0.03	0.03	0.03	0.03	0.02	0.02
8	0.05	0.04	0.05	0.05	0.06	0.08
12	0.06	0.05	0.06	0.05	0.05	0.07
24	0.06	0.08	0.07	0.05	0.05	0.07

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.02	0.03	0.02
4	0.06	0.05	0.05	0.05	0.04	0.04
8	0.14	0.16	0.13	0.13	0.11	0.11
12	0.20	0.21	0.19	0.19	0.17	0.17
24	0.24	0.23	0.25	0.21	0.21	0.23

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.03	0.01	0.01	0.02
4	0.03	0.04	0.04	0.03	0.03	0.04
8	0.14	0.14	0.14	0.13	0.12	0.13
12	0.21	0.20	0.20	0.20	0.19	0.19
24	0.23	0.23	0.24	0.22	0.23	0.23

**Negative (unsupplemented MEM $\alpha$ ), KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.02	0.03	0.01
4	0.02	0.03	0.02	0.02	0.01	0.01
8	0.04	0.03	0.03	0.02	0.03	0.03
12	0.04	0.03	0.04	0.03	0.03	0.03
24	0.06	0.07	0.04	0.05	0.05	0.05

**Positive (10  $\mu$ M FeSO<sub>4</sub>), KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.03	0.02	0.04
4	0.05	0.06	0.04	0.04	0.04	0.04
8	0.12	0.13	0.13	0.11	0.10	0.11
12	0.18	0.17	0.17	0.16	0.16	0.17
24	0.23	0.24	0.22	0.21	0.22	0.21

**0.27  $\mu$ M human ferri-Lf, KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.02	0.03	0.03
4	0.04	0.04	0.04	0.04	0.03	0.04
8	0.11	0.11	0.12	0.10	0.11	0.10
12	0.18	0.19	0.19	0.19	0.19	0.20
24	0.23	0.23	0.24	0.24	0.25	0.24

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, KAR3 optical density values at 24 h from experimental run 1.**

Each row has the same difference. Cannot calculate a paired t test.

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, KAR3 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.1835
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.01333
95% confidence interval	-0.01535 to 0.04202
R squared	0.6667

**Figure 4.11c.**

**NCTC 11168 and CEM5 ( $\Delta$ *frA::aphA-3*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.02	0.02	0.01	0.02
4	0.04	0.04	0.04	0.04	0.05	0.05
8	0.07	0.06	0.07	0.08	0.08	0.09
12	0.08	0.08	0.07	0.08	0.08	0.08
24	0.10	0.09	0.09	0.08	0.09	0.08

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.01	0.03
4	0.06	0.06	0.06	0.05	0.07	0.08
8	0.16	0.16	0.16	0.15	0.15	0.14
12	0.21	0.20	0.22	0.21	0.20	0.19
24	0.24	0.25	0.25	0.26	0.25	0.25

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.02	0.03	0.04
4	0.05	0.05	0.05	0.06	0.06	0.06
8	0.15	0.16	0.16	0.17	0.16	0.16
12	0.23	0.22	0.22	0.21	0.21	0.21
24	0.25	0.25	0.24	0.29	0.28	0.27

**Negative (unsupplemented MEM $\alpha$ ), CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.02	0.03	0.03	0.04	0.05	0.06
8	0.07	0.07	0.07	0.07	0.08	0.08
12	0.08	0.09	0.09	0.09	0.10	0.09
24	0.09	0.10	0.09	0.10	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.05	0.05	0.04
4	0.06	0.06	0.06	0.08	0.08	0.09
8	0.14	0.14	0.15	0.16	0.17	0.15
12	0.20	0.23	0.23	0.22	0.21	0.23
24	0.25	0.25	0.26	0.25	0.25	0.26

**0.27  $\mu$ M human ferri-Lf, CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.04	0.03	0.04
4	0.04	0.03	0.04	0.04	0.04	0.04
8	0.07	0.08	0.08	0.09	0.09	0.09
12	0.10	0.12	0.10	0.13	0.13	0.12
24	0.18	0.18	0.16	0.20	0.19	0.20

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM5 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0021
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 22.00, df = 2
Number of pairs	3



How big is the difference?

Mean of differences	-0.07333
95% confidence interval	-0.08768 to -0.05899
R squared	0.9959

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM5 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0063
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 12.50, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.08333
95% confidence interval	-0.1120 to -0.05465
R squared	0.9874

**Figure 4.11d.**

**NCTC 11168 and KAR2 (*cj0178::aphA-3*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.01	0.01
4	0.02	0.03	0.02	0.01	0.05	0.03
8	0.05	0.04	0.04	0.04	0.05	0.07
12	0.08	0.05	0.06	0.05	0.07	0.06
24	0.09	0.07	0.05	0.05	0.08	0.09

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.03	0.03	0.02	0.03
4	0.07	0.07	0.03	0.05	0.05	0.04
8	0.17	0.18	0.14	0.15	0.16	0.19
12	0.25	0.28	0.24	0.24	0.26	0.26
24	0.30	0.29	0.28	0.27	0.29	0.28

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.02	0.04	0.02	0.03	0.02
4	0.08	0.08	0.04	0.02	0.03	0.03
8	0.15	0.15	0.15	0.15	0.14	0.13
12	0.20	0.19	0.19	0.18	0.20	0.21
24	0.23	0.25	0.21	0.20	0.22	0.22

**Negative (unsupplemented MEM $\alpha$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.03	0.01	0.02	0.02
4	0.02	0.01	0.01	0.02	0.02	0.02
8	0.06	0.04	0.02	0.04	0.02	0.03
12	0.04	0.03	0.03	0.01	0.05	0.03
24	0.08	0.05	0.06	0.05	0.08	0.07

**Positive (10  $\mu$ M FeSO<sub>4</sub>), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.03	0.03	0.03	0.03
4	0.04	0.05	0.01	0.01	0.03	0.02
8	0.16	0.18	0.15	0.14	0.13	0.14
12	0.31	0.28	0.24	0.26	0.23	0.25
24	0.33	0.32	0.29	0.30	0.30	0.32

**0.27  $\mu$ M human ferri-Lf, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.02	0.02	0.04
4	0.03	0.04	0.03	0.03	0.02	0.02
8	0.03	0.02	0.01	0.02	0.05	0.03
12	0.05	0.03	0.09	0.06	0.08	0.08
24	0.08	0.09	0.11	0.10	0.10	0.08

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, KAR2 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0179
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 7.364, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1367
95% confidence interval	-0.2165 to -0.05681
R squared	0.9644

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, KAR2 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0091
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 10.39, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1200
95% confidence interval	-0.1697 to -0.07031
R squared	0.9818

**Growth assays of *C. jejuni* strain KAR2 (*cj0178::aphA-3*) with iron supplied bound to human Tf and ovo-Tf.**

**Figure 4.12a.**

**NCTC 11168 and KAR2 with human ferri-Tf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.01	0.02	0.04	0.02
4	0.04	0.04	0.03	0.02	0.06	0.06
8	0.07	0.08	0.06	0.06	0.10	0.10
12	0.09	0.07	0.07	0.07	0.08	0.10
24	0.08	0.07	0.09	0.08	0.10	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.03	0.03	0.04
4	0.07	0.05	0.06	0.06	0.09	0.10
8	0.11	0.12	0.18	0.19	0.22	0.25
12	0.16	0.21	0.29	0.29	0.26	0.30
24	0.23	0.24	0.29	0.26	0.28	0.29

**0.27  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.03	0.02	0.03	0.03
4	0.06	0.06	0.05	0.06	0.11	0.10
8	0.15	0.15	0.22	0.23	0.25	0.27
12	0.30	0.30	0.30	0.29	0.30	0.30
24	0.32	0.30	0.29	0.29	0.28	0.28

**Negative (unsupplemented MEM $\alpha$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.03	0.03	0.03
4	0.04	0.03	0.04	0.04	0.05	0.07
8	0.08	0.08	0.07	0.08	0.08	0.09
12	0.10	0.10	0.08	0.08	0.05	0.07
24	0.10	0.07	0.07	0.08	0.05	0.05

**Positive (10  $\mu$ M FeSO<sub>4</sub>), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.03	0.04	0.02
4	0.02	0.02	0.06	0.07	0.09	0.08
8	0.13	0.13	0.22	0.21	0.21	0.21
12	0.16	0.17	0.29	0.27	0.28	0.30
24	0.25	0.23	0.28	0.26	0.30	0.30

**0.27  $\mu$ M human ferri-Tf, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.04	0.04	0.04	0.03
4	0.06	0.06	0.06	0.05	0.08	0.11
8	0.11	0.11	0.20	0.19	0.23	0.25
12	0.22	0.20	0.22	0.20	0.27	0.27
24	0.19	0.17	0.19	0.19	0.25	0.27

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Tf, KAR2 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0069
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 12.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1200
95% confidence interval	-0.1630 to -0.07697
R squared	0.9863

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Tf, KAR2 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.2293
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 1.710$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04667
95% confidence interval	-0.1641 to 0.07074
R squared	0.5939

**Figure 4.12b.**

**NCTC 11168 and KAR2 with ferri-ovo-Tf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.04	0.01
4	0.04	0.04	0.05	0.03	0.05	0.05
8	0.10	0.10	0.08	0.09	0.09	0.08
12	0.08	0.09	0.10	0.09	0.09	0.11
24	0.08	0.09	0.08	0.10	0.10	0.09

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.03	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06	0.07
8	0.20	0.18	0.18	0.22	0.17	0.17
12	0.24	0.23	0.23	0.25	0.23	0.24
24	0.27	0.29	0.28	0.28	0.27	0.28

**0.27  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.03	0.03	0.03
4	0.08	0.08	0.07	0.06	0.07	0.06
8	0.20	0.21	0.20	0.19	0.18	0.20
12	0.25	0.26	0.25	0.24	0.24	0.25
24	0.30	0.30	0.30	0.31	0.27	0.29

**Negative (unsupplemented MEM $\alpha$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.03	0.03	0.04
4	0.03	0.03	0.04	0.04	0.04	0.04
8	0.06	0.06	0.07	0.08	0.08	0.07
12	0.08	0.08	0.09	0.09	0.11	0.10
24	0.09	0.10	0.11	0.10	0.11	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.03	0.01	0.02
4	0.04	0.05	0.05	0.06	0.05	0.05
8	0.20	0.20	0.19	0.19	0.18	0.18
12	0.21	0.23	0.24	0.24	0.20	0.21
24	0.26	0.27	0.29	0.29	0.29	0.30

**0.27  $\mu$ M ferri-ovo-Tf, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.02	0.02
4	0.04	0.05	0.05	0.05	0.05	0.05
8	0.09	0.09	0.10	0.10	0.10	0.11
12	0.13	0.14	0.13	0.12	0.13	0.11
24	0.15	0.16	0.15	0.14	0.15	0.15

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf, KAR2 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0005
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 44.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1467
95% confidence interval	-0.1610 to -0.1323
R squared	0.9990

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf, KAR2 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0101
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 9.865$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1433
95% confidence interval	-0.2059 to -0.08081
R squared	0.9799

**Growth assays of *C. jejuni* strain  $\Delta cj0444::aphA-3$  81-176 with iron supplied bound to human Lf, human Tf or ovo-Tf.**

**Figure 4.13a.**

**NCTC 11168, 81-176 and  $\Delta cj0444::aphA-3$  81-176 with human ferri-Lf.**

**Negative (unsupplemented MEMa), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.01
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.13	0.13
12	0.16	0.16	0.14	0.14
24	0.15	0.16	0.14	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.02
4	0.05	0.07	0.06	0.06
8	0.16	0.15	0.16	0.16
12	0.25	0.29	0.27	0.27
24	0.25	0.25	0.29	0.30

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.02	0.03
4	0.07	0.06	0.07	0.06
8	0.18	0.19	0.17	0.18
12	0.29	0.27	0.29	0.30
24	0.28	0.28	0.29	0.29

**Negative (unsupplemented MEM $\alpha$ ), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.02	0.02	0.03
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.12	0.14
12	0.15	0.16	0.15	0.15
24	0.22	0.24	0.22	0.21

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.01
4	0.04	0.06	0.05	0.06
8	0.15	0.16	0.16	0.16
12	0.20	0.19	0.21	0.23
24	0.40	0.38	0.41	0.38

**0.27  $\mu$ M human ferri-Lf, 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.01	0.03
4	0.04	0.05	0.05	0.05
8	0.13	0.12	0.12	0.13
12	0.29	0.29	0.29	0.31
24	0.42	0.41	0.43	0.44

**Negative (unsupplemented MEM $\alpha$ ),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.03	0.02
4	0.07	0.09	0.08	0.07
8	0.19	0.18	0.17	0.17
12	0.23	0.23	0.21	0.22
24	0.27	0.26	0.27	0.28



**Positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.02	0.02
4	0.09	0.06	0.08	0.09
8	0.20	0.21	0.19	0.22
12	0.37	0.36	0.35	0.35
24	0.40	0.41	0.42	0.42

**0.27  $\mu$ M human ferri-Lf,  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.02
4	0.06	0.07	0.08	0.08	0.08	0.09
8	0.22	0.25	0.25	0.23	0.24	0.25
12	0.38	0.39	0.41	0.40	0.38	0.37
24	0.46	0.42	0.41	0.47	0.45	0.44

**Figure 4.13b.**

NCTC 11168, 81-176 and  $\Delta$ *cj0444::aphA-3* 81-176 with human ferri-Tf.

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.01
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.13	0.13
12	0.16	0.16	0.14	0.14
24	0.15	0.16	0.14	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.02
4	0.05	0.07	0.06	0.06
8	0.16	0.15	0.16	0.16
12	0.25	0.29	0.27	0.27
24	0.25	0.25	0.29	0.30

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.02	0.03
4	0.07	0.06	0.07	0.06
8	0.18	0.19	0.17	0.18
12	0.29	0.27	0.29	0.30
24	0.28	0.28	0.29	0.29

**Negative (unsupplemented MEM $\alpha$ ), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.02	0.02	0.03
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.12	0.14
12	0.15	0.16	0.15	0.15
24	0.22	0.24	0.22	0.21

**Positive (10  $\mu$ M FeSO $_4$ ), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.01
4	0.04	0.06	0.05	0.06
8	0.15	0.16	0.16	0.16
12	0.20	0.19	0.21	0.23
24	0.40	0.38	0.41	0.38

**0.27  $\mu$ M human ferri-Tf, 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.04	0.02	0.02
4	0.05	0.06	0.06	0.05
8	0.14	0.12	0.13	0.12
12	0.31	0.27	0.28	0.27
24	0.44	0.46	0.41	0.42

**Negative (unsupplemented MEM $\alpha$ ),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.03	0.02
4	0.07	0.09	0.08	0.07
8	0.19	0.18	0.17	0.17
12	0.23	0.23	0.21	0.22
24	0.27	0.26	0.27	0.28

**Positive (10  $\mu$ M FeSO $_4$ ),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.02	0.02
4	0.09	0.06	0.08	0.09
8	0.20	0.21	0.19	0.22
12	0.37	0.36	0.35	0.35
24	0.40	0.41	0.42	0.42

**0.27  $\mu$ M human ferri-Tf,  $\Delta cj0444::aphA-3$  81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.02	0.03
4	0.10	0.09	0.08	0.09	0.08	0.09
8	0.24	0.22	0.23	0.24	0.25	0.22
12	0.40	0.41	0.38	0.38	0.38	0.39
24	0.42	0.45	0.46	0.42	0.43	0.45

**Figure 4.13c.**

NCTC 11168, 81-176 and  $\Delta cj0444::aphA-3$  81-176 with ferri-ovo-Tf.

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.01
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.13	0.13
12	0.16	0.16	0.14	0.14
24	0.15	0.16	0.14	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.02
4	0.05	0.07	0.06	0.06
8	0.16	0.15	0.16	0.16
12	0.25	0.29	0.27	0.27
24	0.25	0.25	0.29	0.30

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.02	0.03
4	0.07	0.06	0.07	0.06
8	0.18	0.19	0.17	0.18
12	0.29	0.27	0.29	0.30
24	0.28	0.28	0.29	0.29

**Negative (unsupplemented MEM $\alpha$ ), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.02	0.02	0.03
4	0.05	0.06	0.04	0.05
8	0.12	0.12	0.12	0.14
12	0.15	0.16	0.15	0.15
24	0.22	0.24	0.22	0.21

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.02	0.01
4	0.04	0.06	0.05	0.06
8	0.15	0.16	0.16	0.16
12	0.20	0.19	0.21	0.23
24	0.40	0.38	0.41	0.38

**0.27  $\mu$ M ferri-ovo-Tf, 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.04	0.03	0.03
4	0.06	0.06	0.06	0.05
8	0.12	0.13	0.14	0.14
12	0.29	0.29	0.28	0.29
24	0.39	0.43	0.44	0.41

**Negative (unsupplemented MEM $\alpha$ ),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.04	0.03	0.02
4	0.07	0.09	0.08	0.07
8	0.19	0.18	0.17	0.17
12	0.23	0.23	0.21	0.22
24	0.27	0.26	0.27	0.28

**Positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)		Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.02	0.02
4	0.09	0.06	0.08	0.09
8	0.20	0.21	0.19	0.22
12	0.37	0.36	0.35	0.35
24	0.40	0.41	0.42	0.42

**0.27  $\mu$ M ferri-ovo-Tf,  $\Delta$ *cj0444::aphA-3* 81-176**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.02	0.02
4	0.08	0.07	0.08	0.10	0.09	0.10
8	0.23	0.22	0.24	0.25	0.25	0.26
12	0.38	0.37	0.40	0.39	0.40	0.41
24	0.43	0.45	0.42	0.44	0.44	0.42

**Growth assays of *C. jejuni* strains with mutations in components of the *cj0173c-cj0178* system supplied with iron bound to human Lf.**

**Figure 4.15a.**

**NCTC 11168 and CEM3 ( $\Delta cj0174c::aphA-3$ ) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.02	0.02	0.03	0.03
4	0.01	0.02	0.03	0.06	0.06	0.06
8	0.06	0.05	0.06	0.08	0.07	0.08
12	0.05	0.08	0.07	0.08	0.09	0.08
24	0.07	0.07	0.08	0.08	0.08	0.07

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.01	0.03	0.02	0.03
4	0.05	0.05	0.04	0.09	0.09	0.09
8	0.17	0.17	0.17	0.17	0.15	0.16
12	0.25	0.26	0.25	0.23	0.21	0.22
24	0.27	0.27	0.25	0.24	0.23	0.23

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.04	0.03	0.03
4	0.04	0.05	0.06	0.10	0.09	0.09
8	0.16	0.15	0.17	0.15	0.17	0.14
12	0.23	0.23	0.24	0.23	0.23	0.25
24	0.26	0.26	0.26	0.25	0.25	0.26

**Negative (unsupplemented MEM $\alpha$ ), CEM3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.02	0.02	0.02	0.04
4	0.03	0.02	0.02	0.06	0.06	0.06
8	0.06	0.06	0.06	0.07	0.08	0.08
12	0.08	0.08	0.07	0.09	0.09	0.09
24	0.09	0.08	0.08	0.08	0.08	0.09

**Positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), CEM3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.03	0.02
4	0.03	0.04	0.04	0.10	0.09	0.09
8	0.16	0.16	0.16	0.15	0.15	0.14
12	0.22	0.23	0.23	0.25	0.23	0.22
24	0.27	0.27	0.25	0.23	0.23	0.23

**0.27  $\mu\text{M}$  human ferri-Lf, CEM3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.04	0.02	0.04
4	0.04	0.03	0.03	0.08	0.07	0.06
8	0.11	0.12	0.13	0.13	0.12	0.12
12	0.19	0.19	0.18	0.19	0.19	0.19
24	0.24	0.24	0.25	0.22	0.20	0.21

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, CEM3 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0377
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 5.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.01667
95% confidence interval	-0.03101 to -0.002323
R squared	0.9259

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, CEM3 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0229
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 6.500$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04333
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95% confidence interval	-0.07202 to -0.01465
R squared	0.9548

**Figure 4.15b.**  
NCTC 11168 and CEM4 ( $\Delta cj0177::ermC'$ ) with human ferri-Lf.

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.03	0.03	0.03
4	0.05	0.04	0.05	0.05	0.05	0.05
8	0.12	0.11	0.12	0.11	0.13	0.10
12	0.13	0.13	0.13	0.14	0.14	0.14
24	0.13	0.13	0.14	0.14	0.13	0.14

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.04	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06	0.06
8	0.16	0.16	0.16	0.15	0.17	0.16
12	0.25	0.23	0.22	0.24	0.24	0.23
24	0.27	0.28	0.28	0.28	0.28	0.28

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.03	0.03	0.03
4	0.05	0.06	0.06	0.06	0.06	0.05
8	0.16	0.17	0.18	0.18	0.18	0.17
12	0.32	0.32	0.31	0.32	0.32	0.32
24	0.31	0.30	0.30	0.30	0.30	0.30

### Negative (unsupplemented MEM $\alpha$ ), CEM4

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.02
4	0.04	0.04	0.05	0.04	0.04	0.04
8	0.10	0.08	0.09	0.10	0.08	0.08
12	0.11	0.11	0.11	0.11	0.10	0.11
24	0.12	0.11	0.12	0.11	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM4**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.03	0.03	0.03
4	0.05	0.05	0.06	0.05	0.05	0.05
8	0.15	0.15	0.15	0.14	0.14	0.14
12	0.18	0.19	0.20	0.19	0.20	0.20
24	0.25	0.27	0.27	0.24	0.24	0.25

**0.27  $\mu$ M human ferri-Lf, CEM4**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.04	0.04	0.04
8	0.08	0.08	0.10	0.08	0.09	0.09
12	0.14	0.15	0.15	0.16	0.16	0.16
24	0.24	0.25	0.24	0.24	0.24	0.24

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 12 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM4 optical density values at 12 h from experimental run 1.**

Paired t test

P value	0.0012
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 29.44, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1700
95% confidence interval	-0.1948 to -0.1452
R squared	0.9977

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 12 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM4 optical density values at 12 h from experimental run 2.**

Each row has the same difference. Cannot calculate a paired t test.

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM4 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0091
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes



### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

One- or two-tailed P value?	Two-tailed
t, df	t = 10.39, df = 2
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.06000
95% confidence interval	-0.08484 to -0.03516
R squared	0.9818

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, CEM4 optical density values at 24 h from experimental run 2.**

Each row has the same different. Cannot calculate a paired t test.

**Mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), 11168 optical density values at 12 h from experimental run 1 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), CEM4 optical density values at 12 h from experimental run 1.**

<u>Paired t test</u>	
P value	0.0964
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	t = 2.982, df = 2
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.04333
95% confidence interval	-0.1059 to -0.01919
R squared	0.8164

**Mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), 11168 optical density values at 12 h from experimental run 2 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), CEM4 optical density values at 12 h from experimental run 2.**

<u>Paired t test</u>	
P value	0.0202
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 6.928, df = 2
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.0400
95% confidence interval	-0.06484 to -0.01516
R squared	0.9600

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>), CEM4 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0572
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 4.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.01333
95% confidence interval	-0.02768 to -0.001010
R squared	0.8889

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>), CEM4 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0082
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 11.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.03667
95% confidence interval	-0.05101 to -0.02232
R squared	0.9837

**Growth assays of *C. jejuni* strain  $\Delta$ feoB::ermC' 11168 with iron supplied bound to human Lf, human Tf or ovo-Tf.**

**Figure 4.16a.**

**NCTC 11168 and  $\Delta$ feoB::ermC' 11168 with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.05	0.05
8	0.12	0.12	0.10	0.10	0.11	0.11
12	0.13	0.13	0.13	0.11	0.11	0.11
24	0.14	0.13	0.12	0.12	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.03	0.03
4	0.08	0.07	0.08	0.08	0.07	0.07
8	0.18	0.19	0.21	0.19	0.18	0.18
12	0.31	0.31	0.31	0.25	0.25	0.25
24	0.30	0.32	0.32	0.28	0.29	0.29

**Positive (25  $\mu$ M haem), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.08	0.08	0.08	0.09	0.09	0.09
8	0.22	0.24	0.25	0.19	0.19	0.19
12	0.29	0.29	0.28	0.35	0.37	0.36
24	0.32	0.31	0.33	0.28	0.28	0.28

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.08	0.08	0.08	0.08	0.08	0.08
8	0.23	0.24	0.25	0.19	0.18	0.19
12	0.32	0.33	0.34	0.30	0.31	0.31
24	0.32	0.33	0.33	0.30	0.30	0.30

**Negative (unsupplemented MEMa),  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.01	0.02
4	0.06	0.05	0.05	0.06	0.06	0.06
8	0.10	0.10	0.09	0.09	0.09	0.09
12	0.12	0.12	0.12	0.09	0.10	0.10
24	0.12	0.10	0.10	0.10	0.11	0.11

**Positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.07	0.07	0.07	0.06	0.06	0.07
8	0.15	0.14	0.15	0.10	0.10	0.10
12	0.16	0.17	0.17	0.19	0.20	0.20
24	0.18	0.19	0.19	0.24	0.23	0.24

**Positive (25  $\mu$ M haem),  $\Delta$ feoB::*ermC'* 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.08	0.08	0.08	0.07	0.07	0.07
8	0.29	0.28	0.28	0.15	0.15	0.15
12	0.32	0.33	0.32	0.25	0.26	0.26
24	0.36	0.36	0.36	0.29	0.29	0.29

**0.27  $\mu$ M human ferri-Lf,  $\Delta$ feoB::*ermC'* 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.04	0.04	0.05	0.06	0.06	0.06
8	0.11	0.11	0.12	0.10	0.10	0.10
12	0.21	0.21	0.21	0.22	0.19	0.19
24	0.22	0.21	0.21	0.25	0.25	0.25

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ feoB::*ermC'* 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0007
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 38.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1267
95% confidence interval	-0.1410 to -0.1123
R squared	0.9986

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ feoB::*ermC'* 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0131
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 8.660, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.0500
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95% confidence interval	-0.07484 to -0.02516
R squared	0.9740

Paired t test	
P value	0.0034
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 17.00, df = 2
Number of pairs	3

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf,  $\Delta$ *feoB::ermC* 11168 optical density values at 24 h from experimental run 2.**

**Figure 4.16b.**  
NCTC 11168 and  $\Delta feoB::ermC'$  11168 with human ferri-Tf.

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.05	0.05
8	0.12	0.12	0.10	0.10	0.11	0.11
12	0.13	0.13	0.13	0.11	0.11	0.11
24	0.14	0.13	0.12	0.12	0.12	0.12

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.03	0.03
4	0.08	0.07	0.08	0.08	0.07	0.07
8	0.18	0.19	0.21	0.19	0.18	0.18
12	0.31	0.31	0.31	0.25	0.25	0.25
24	0.30	0.32	0.32	0.28	0.29	0.29

**Positive (25  $\mu$ M haem), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.08	0.08	0.08	0.09	0.09	0.09
8	0.22	0.24	0.25	0.19	0.19	0.19
12	0.29	0.29	0.28	0.35	0.37	0.36
24	0.32	0.31	0.33	0.28	0.28	0.28

**0.27  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.01	0.03
4	0.08	0.07	0.07	0.07	0.07	0.07
8	0.23	0.23	0.21	0.18	0.18	0.18
12	0.34	0.34	0.34	0.31	0.31	0.31
24	0.31	0.32	0.32	0.30	0.29	0.29

**Negative (unsupplemented MEM $\alpha$ ),  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.01	0.02
4	0.06	0.05	0.05	0.06	0.06	0.06
8	0.10	0.10	0.09	0.09	0.09	0.09
12	0.12	0.12	0.12	0.09	0.10	0.10
24	0.12	0.10	0.10	0.10	0.11	0.11

**Positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.07	0.07	0.07	0.06	0.06	0.07
8	0.15	0.14	0.15	0.10	0.10	0.10
12	0.16	0.17	0.17	0.19	0.20	0.20
24	0.18	0.19	0.19	0.24	0.23	0.24

**Positive (25  $\mu$ M haem),  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.08	0.08	0.08	0.07	0.07	0.07
8	0.29	0.28	0.28	0.15	0.15	0.15
12	0.32	0.33	0.32	0.25	0.26	0.26
24	0.36	0.36	0.36	0.29	0.29	0.29

**0.27  $\mu$ M human ferri-Tf,  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.02	0.02	0.02
4	0.05	0.05	0.05	0.06	0.06	0.06
8	0.12	0.14	0.13	0.10	0.09	0.09
12	0.21	0.21	0.21	0.22	0.21	0.21
24	0.21	0.21	0.21	0.25	0.25	0.25

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Tf,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0010
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 32.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1067
95% confidence interval	-0.1210 to -0.09232
R squared	0.9981

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Tf,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0059
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 13.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.05768 to -0.02899
R squared	0.9883

**Figure 4.16c.**  
**NCTC 11168 and  $\Delta feoB::ermC'$  11168 with ferri-ovo-Tf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.05	0.05
8	0.12	0.12	0.10	0.10	0.11	0.11
12	0.13	0.13	0.13	0.11	0.11	0.11
24	0.14	0.13	0.12	0.12	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.03	0.03
4	0.08	0.07	0.08	0.08	0.07	0.07
8	0.18	0.19	0.21	0.19	0.18	0.18
12	0.31	0.31	0.31	0.25	0.25	0.25
24	0.30	0.32	0.32	0.28	0.29	0.29

**Positive (25  $\mu$ M haem), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.08	0.08	0.08	0.09	0.09	0.09
8	0.22	0.24	0.25	0.19	0.19	0.19
12	0.29	0.29	0.28	0.35	0.37	0.36
24	0.32	0.31	0.33	0.28	0.28	0.28

**0.27  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.03	0.01	0.03
4	0.07	0.08	0.08	0.07	0.08	0.08
8	0.21	0.22	0.22	0.19	0.19	0.21
12	0.33	0.34	0.34	0.29	0.30	0.31
24	0.33	0.34	0.34	0.29	0.30	0.30

**Negative (unsupplemented MEM $\alpha$ ),  $\Delta feoB::ermC'$  11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.01	0.02
4	0.06	0.05	0.05	0.06	0.06	0.06
8	0.10	0.10	0.09	0.09	0.09	0.09
12	0.12	0.12	0.12	0.09	0.10	0.10
24	0.12	0.10	0.10	0.10	0.11	0.11



**Positive (10  $\mu$ M FeSO<sub>4</sub>),  $\Delta$ feoB::*ermC'* 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.07	0.07	0.07	0.06	0.06	0.07
8	0.15	0.14	0.15	0.10	0.10	0.10
12	0.16	0.17	0.17	0.19	0.20	0.20
24	0.18	0.19	0.19	0.24	0.23	0.24

**Positive (25  $\mu$ M haem),  $\Delta$ feoB::*ermC'* 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.02	0.02	0.02
4	0.08	0.08	0.08	0.07	0.07	0.07
8	0.29	0.28	0.28	0.15	0.15	0.15
12	0.32	0.33	0.32	0.25	0.26	0.26
24	0.36	0.36	0.36	0.29	0.29	0.29

**0.27  $\mu$ M ferri-ovo-Tf,  $\Delta$ feoB::*ermC'* 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.02	0.02	0.02
4	0.06	0.06	0.06	0.06	0.06	0.06
8	0.13	0.12	0.12	0.10	0.10	0.11
12	0.22	0.21	0.21	0.22	0.23	0.23
24	0.23	0.22	0.21	0.24	0.25	0.25

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf,  $\Delta$ feoB::*ermC'* 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0057
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 13.23, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.1167
95% confidence interval	-0.1546 to -0.07872
R squared	0.9887

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf,  $\Delta$ feoB::*ermC'* 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 9913000, df = 2
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.05000
95% confidence interval	-0.05000 to -0.05000
R squared	1.000

**Growth assays of *C. jejuni* strain JDR20 ( $\Delta$ tonB1::cat) supplied with iron bound to human Lf, human Tf and ovo-Tf.**

**Figure 4.17a.**

**NCTC 11168 and JDR20 with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.02	0.02	0.03	0.03
4	0.06	0.06	0.05	0.04	0.05	0.04
8	0.13	0.12	0.10	0.12	0.13	0.12
12	0.17	0.16	0.17	0.14	0.15	0.14
24	0.16	0.16	0.15	0.15	0.15	0.15

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.04	0.02
4	0.07	0.08	0.06	0.05	0.06	0.06
8	0.14	0.14	0.14	0.15	0.15	0.15
12	0.24	0.25	0.23	0.25	0.24	0.23
24	0.26	0.26	0.26	0.29	0.29	0.29

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.07	0.07	0.07	0.07	0.06	0.06
8	0.18	0.16	0.16	0.16	0.17	0.16
12	0.32	0.28	0.28	0.28	0.29	0.29
24	0.29	0.30	0.29	0.30	0.31	0.29

**Negative (unsupplemented MEM $\alpha$ ), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.02	0.02	0.02	0.02
4	0.05	0.04	0.05	0.04	0.03	0.04
8	0.10	0.10	0.10	0.09	0.10	0.10
12	0.15	0.16	0.17	0.12	0.13	0.13
24	0.15	0.16	0.15	0.15	0.14	0.15

**Positive (10  $\mu$ M FeSO $_4$ ), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.04	0.03	0.02	0.03
4	0.06	0.06	0.06	0.05	0.05	0.05
8	0.12	0.12	0.12	0.13	0.12	0.13
12	0.21	0.19	0.19	0.21	0.19	0.19
24	0.25	0.24	0.24	0.26	0.25	0.26

**0.27  $\mu$ M human ferri-Lf, JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.02	0.02	0.02	0.01
4	0.06	0.06	0.05	0.05	0.05	0.05
8	0.10	0.12	0.13	0.11	0.11	0.12
12	0.23	0.24	0.26	0.24	0.25	0.25
24	0.24	0.23	0.23	0.26	0.26	0.26

**Mean of positive (10  $\mu$ M FeSO $_4$ ), 11168 optical density values at 12 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO $_4$ ), JDR20 optical density values at 12 h from experimental run 1.**

Paired t test

P value	0.0390
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 4.914, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.08128 to -0.005384
R squared	0.9235

**Mean of positive (10  $\mu$ M FeSO $_4$ ), 11168 optical density values at 12 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO $_4$ ), JDR20 optical density values at 12 h from experimental run 2.**

Paired t test

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

P value	0.0059
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 13.00$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.05768 to -0.02899
R squared	0.9883

**Mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), JDR20 optical density values at 24 h from experimental run 1.**

#### Paired t test

P value	0.0377
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 5.000$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.01667
95% confidence interval	-0.03101 to -0.002323
R squared	0.9259

**Mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), JDR20 optical density values at 24 h from experimental run 2.**

#### Paired t test

P value	0.0099
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 10.00$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.03333
95% confidence interval	-0.04768 to -0.01899
R squared	0.9804

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, JDR20 optical density values at 24 h from experimental run 1.**

#### Paired t test

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

P value	0.0091
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 10.39$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.0600
95% confidence interval	-0.08484 to -0.03516
R squared	0.9818

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, JDR20 optical density values at 24 h from experimental run 2.**

<u>Paired t test</u>	
P value	0.0202
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 6.928$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.04000
95% confidence interval	-0.06484 to -0.01516
R squared	0.9600

**Figure 4.17b.**  
**NCTC 11168 and JDR20 with human ferri-Tf.**

#### **Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.02	0.02	0.03	0.03
4	0.06	0.06	0.05	0.04	0.05	0.04
8	0.13	0.12	0.10	0.12	0.13	0.12
12	0.17	0.16	0.17	0.14	0.15	0.14
24	0.16	0.16	0.15	0.15	0.15	0.15

#### **Positive (10 $\mu\text{M}$ FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.04	0.02
4	0.07	0.08	0.06	0.05	0.06	0.06
8	0.14	0.14	0.14	0.15	0.15	0.15
12	0.24	0.25	0.23	0.25	0.24	0.23
24	0.26	0.26	0.26	0.29	0.29	0.29

**0.27  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.02	0.03	0.01
4	0.06	0.06	0.06	0.06	0.05	0.06
8	0.15	0.15	0.16	0.15	0.15	0.16
12	0.28	0.27	0.28	0.29	0.28	0.28
24	0.27	0.29	0.28	0.30	0.28	0.28

**Negative (unsupplemented MEM $\alpha$ ), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.02	0.02	0.02	0.02
4	0.05	0.04	0.05	0.04	0.03	0.04
8	0.10	0.10	0.10	0.09	0.10	0.10
12	0.15	0.16	0.17	0.12	0.13	0.13
24	0.15	0.16	0.15	0.15	0.14	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.04	0.03	0.02	0.03
4	0.06	0.06	0.06	0.05	0.05	0.05
8	0.12	0.12	0.12	0.13	0.12	0.13
12	0.21	0.19	0.19	0.21	0.19	0.19
24	0.25	0.24	0.24	0.26	0.25	0.26

**0.27  $\mu$ M human ferri-Tf, JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.04	0.03	0.02	0.02
4	0.05	0.05	0.05	0.05	0.04	0.05
8	0.12	0.10	0.10	0.10	0.10	0.10
12	0.26	0.23	0.24	0.25	0.25	0.23
24	0.27	0.27	0.26	0.27	0.26	0.27

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Tf, JDR20 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.1835
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 2.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.01333
95% confidence interval	-0.04202 to 0.01535
R squared	0.6667

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Tf, JDR20 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0742
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 3.464$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0200
95% confidence interval	-0.04484 to 0.004843
R squared	0.8571

**Figure 4.17c.**

**NCTC 11168 and JDR20 with ferri-ovo-Tf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.02	0.02	0.03	0.03
4	0.06	0.06	0.05	0.04	0.05	0.04
8	0.13	0.12	0.10	0.12	0.13	0.12
12	0.17	0.16	0.17	0.14	0.15	0.14
24	0.16	0.16	0.15	0.15	0.15	0.15

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.04	0.02
4	0.07	0.08	0.06	0.05	0.06	0.06
8	0.14	0.14	0.14	0.15	0.15	0.15
12	0.24	0.25	0.23	0.25	0.24	0.23
24	0.26	0.26	0.26	0.29	0.29	0.29

**0.27  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.01	0.01	0.03
4	0.06	0.06	0.06	0.06	0.07	0.06
8	0.16	0.15	0.15	0.17	0.17	0.17
12	0.29	0.28	0.28	0.29	0.29	0.30
24	0.28	0.32	0.30	0.31	0.32	0.31

**Negative (unsupplemented MEM $\alpha$ ), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.02	0.02	0.02	0.02
4	0.05	0.04	0.05	0.04	0.03	0.04
8	0.10	0.10	0.10	0.09	0.10	0.10
12	0.15	0.16	0.17	0.12	0.13	0.13
24	0.15	0.16	0.15	0.15	0.14	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.04	0.03	0.02	0.03
4	0.06	0.06	0.06	0.05	0.05	0.05
8	0.12	0.12	0.12	0.13	0.12	0.13
12	0.21	0.19	0.19	0.21	0.19	0.19
24	0.25	0.24	0.24	0.26	0.25	0.26

**0.27  $\mu$ M ferri-ovo-Tf, JDR20**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.02	0.01	0.01
4	0.04	0.05	0.05	0.04	0.04	0.05
8	0.11	0.12	0.14	0.11	0.12	0.13
12	0.25	0.26	0.27	0.26	0.25	0.26
24	0.26	0.27	0.26	0.27	0.27	0.27

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf, JDR20 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0059
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 13.00, df = 2
Number of pairs	3



How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.05768 to -0.02899
R squared	0.9883

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf, JDR20 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0059
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 13.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.05768 to -0.02899
R squared	0.9883

**Growth assays of *C. jejuni* strains CEM11 (KAR2,  $\Delta cfrA::aphA-3$ ) and CEM12 (KAR2,  $\Delta feoB::ermC'$ ) supplied with iron bound to human Lf.**

**Figure 4.18a.**

**NCTC 11168 and CEM11 with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.02	0.02	0.02
4	0.06	0.04	0.05	0.04	0.04	0.04
8	0.13	0.13	0.13	0.12	0.14	0.11
12	0.14	0.14	0.14	0.14	0.14	0.14
24	0.13	0.13	0.13	0.12	0.14	0.14

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.02	0.03	0.03
4	0.06	0.06	0.06	0.05	0.05	0.05
8	0.18	0.18	0.20	0.16	0.16	0.15
12	0.28	0.28	0.30	0.25	0.26	0.26
24	0.29	0.30	0.31	0.32	0.31	0.31

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.03	0.03
4	0.05	0.05	0.05	0.05	0.06	0.04
8	0.19	0.19	0.22	0.18	0.18	0.17
12	0.34	0.34	0.35	0.33	0.32	0.31
24	0.34	0.32	0.32	0.30	0.30	0.31

**Negative (unsupplemented MEM $\alpha$ ), CEM11**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.06	0.04	0.05	0.06	0.06	0.07
8	0.12	0.12	0.13	0.14	0.15	0.15
12	0.19	0.19	0.19	0.22	0.23	0.22
24	0.27	0.18	0.17	0.25	0.22	0.24

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM11**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.06	0.05	0.06	0.06	0.06	0.07
8	0.17	0.17	0.20	0.19	0.19	0.20
12	0.28	0.26	0.26	0.29	0.28	0.28
24	0.25	0.25	0.25	0.24	0.23	0.23

**0.27  $\mu$ M human ferri-Lf, CEM11**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.02	0.03	0.02
4	0.05	0.05	0.05	0.05	0.06	0.05
8	0.18	0.17	0.15	0.16	0.17	0.17
12	0.29	0.30	0.31	0.31	0.30	0.28
24	0.27	0.26	0.28	0.29	0.25	0.25

**Mean of negative (unsupplemented MEM $\alpha$ ), 11168 optical density values at 24 h from experimental run 1 vs. mean of negative (unsupplemented MEM $\alpha$ ), CEM11 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.1374
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 2.411, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.07667
95% confidence interval	-0.06016 to 0.2135
R squared	0.7440

**Mean of negative (unsupplemented MEM $\alpha$ ), 11168 optical density values at 24 h from experimental run 2 vs. mean of negative (unsupplemented MEM $\alpha$ ), CEM11 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0192
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 7.112$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.1033
95% confidence interval	0.04081 to 0.1659
R squared	0.9620

**Mean of positive (10  $\mu\text{M}$  FeSO $_4$ ), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu\text{M}$  FeSO $_4$ ), CEM11 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0131
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 8.660$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0500
95% confidence interval	-0.07484 to -0.02516
R squared	0.9740

**Mean of positive (10  $\mu\text{M}$  FeSO $_4$ ), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu\text{M}$  FeSO $_4$ ), CEM11 optical density values at 24 h from experimental run 2.**

Each row has the same difference. Cannot calculate a paired t test.

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, CEM11 optical density values at 24 h from experimental run 1.**

Paired t test

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

P value	0.0234
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 6.425$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.05667
95% confidence interval	-0.09462 to -0.01872
R squared	0.9538

**Mean of 0.27  $\mu\text{M}$  human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu\text{M}$  human ferri-Lf, CEM11 optical density values at 24 h from experimental run 2.**

<u>Paired t test</u>	
P value	0.1201
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.619$ , $df = 2$
Number of pairs	3

<u>How big is the difference?</u>	
Mean of differences	-0.04000
95% confidence interval	-0.1057 to 0.02573
R squared	0.7742

**Figure 4.18b.**  
**NCTC 11168 and CEM12 with human ferri-Lf.**

#### **Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.02	0.02	0.02
4	0.06	0.04	0.05	0.04	0.04	0.04
8	0.13	0.13	0.13	0.12	0.14	0.11
12	0.14	0.14	0.14	0.14	0.14	0.14
24	0.13	0.13	0.13	0.12	0.14	0.14

#### **Positive (10 $\mu\text{M}$ FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.02	0.03	0.03
4	0.06	0.06	0.06	0.05	0.05	0.05
8	0.18	0.18	0.20	0.16	0.16	0.15
12	0.28	0.28	0.30	0.25	0.26	0.26
24	0.29	0.30	0.31	0.32	0.31	0.31

**Positive (25  $\mu$ M haem), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.02	0.03	0.03	0.02
4	0.11	0.08	0.10	0.10	0.09	0.09
8	0.25	0.24	0.24	0.22	0.23	0.22
12	0.40	0.40	0.41	0.37	0.36	0.36
24	0.37	0.37	0.38	0.38	0.39	0.38

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.03	0.03
4	0.05	0.05	0.05	0.05	0.06	0.04
8	0.19	0.19	0.22	0.18	0.18	0.17
12	0.34	0.34	0.35	0.33	0.32	0.31
24	0.34	0.32	0.32	0.30	0.30	0.31

**Negative (unsupplemented MEM $\alpha$ ), CEM12**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.02	0.02	0.03	0.02
4	0.04	0.04	0.03	0.03	0.03	0.04
8	0.10	0.09	0.10	0.11	0.09	0.09
12	0.13	0.14	0.12	0.12	0.12	0.14
24	0.13	0.13	0.12	0.13	0.13	0.13

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM12**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.03	0.02
4	0.04	0.04	0.04	0.04	0.04	0.04
8	0.08	0.08	0.09	0.07	0.08	0.08
12	0.17	0.18	0.18	0.18	0.19	0.16
24	0.24	0.24	0.25	0.23	0.25	0.25

**Positive (25  $\mu$ M haem), CEM12**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.02	0.02	0.02
4	0.08	0.08	0.10	0.09	0.09	0.08
8	0.19	0.19	0.19	0.20	0.18	0.17
12	0.33	0.31	0.38	0.36	0.35	0.36
24	0.35	0.35	0.34	0.35	0.35	0.36

**0.27  $\mu$ M human ferri-Lf, CEM12**

<b>Hours at 37°C</b>	<b>Experimental Run 1 (optical density at 600 nm)</b>			<b>Experimental Run 2 (optical density at 600 nm)</b>		
0	0.01	0.02	0.01	0.02	0.02	0.02
4	0.05	0.05	0.05	0.04	0.04	0.04
8	0.08	0.08	0.09	0.10	0.09	0.10
12	0.17	0.16	0.18	0.18	0.19	0.19
24	0.27	0.24	0.25	0.26	0.26	0.27

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>), CEM12 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0034
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 17.00, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.05667
95% confidence interval	0.04232 to 0.07101
R squared	0.9931

**Mean of positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (10  $\mu$ M FeSO<sub>4</sub>), CEM12 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0198
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 7.000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.0700
95% confidence interval	0.02697 to 0.1130
R squared	0.9608

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM12 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0021
P value summary	**

Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.00$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	0.07333
95% confidence interval	0.05899 to 0.08768
R squared	0.9959

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf, CEM12 optical density values at 24 h from experimental run 2.**

<u>Paired t test</u>	
P value	$<0.0001$
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 4011000$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	0.04000
95% confidence interval	0.04000 to 0.04000
R squared	1.000

**Growth assays of *C. jejuni* strain CEM8 (strain KAR2: *cj0178::aphA-3*, complemented with a wild-type copy of *cj0178* inserted into the pseudogene *cj0752*) with iron supplied bound to human Lf, human Tf and ovo-Tf.**

**Figure 5.4a.**  
**NCTC 11168 and CEM8 with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.06
8	0.12	0.12	0.12	0.12	0.13
12	0.12	0.12	0.12	0.13	0.13
24	0.12	0.13	0.13	0.13	0.13

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.04	0.04
4	0.08	0.08	0.08	0.10	0.09
8	0.25	0.23	0.24	0.26	0.26
12	0.30	0.30	0.30	0.29	0.30
24	0.29	0.28	0.28	0.31	0.31

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.03	0.03
4	0.08	0.10	0.08	0.10	0.11
8	0.26	0.29	0.28	0.30	0.31
12	0.32	0.31	0.31	0.33	0.32
24	0.32	0.31	0.31	0.32	0.32

**Negative (unsupplemented MEM $\alpha$ ), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06
8	0.08	0.09	0.08	0.09	0.09
12	0.13	0.11	0.11	0.12	0.13
24	0.12	0.13	0.12	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.08	0.08	0.08	0.08	0.08
8	0.21	0.21	0.21	0.19	0.19
12	0.28	0.28	0.28	0.28	0.26
24	0.25	0.26	0.25	0.24	0.26

**0.27  $\mu$ M human ferri-Lf, CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.04	0.03	0.03	0.04	0.04
4	0.07	0.07	0.07	0.07	0.07
8	0.11	0.11	0.11	0.11	0.13
12	0.28	0.28	0.29	0.26	0.27
24	0.25	0.24	0.24	0.25	0.26



**Figure 5.4b.**  
**NCTC 11168 and CEM8 with human ferri-Tf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.06
8	0.12	0.12	0.12	0.12	0.13
12	0.12	0.12	0.12	0.13	0.13
24	0.12	0.13	0.13	0.13	0.13

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.04	0.04
4	0.08	0.08	0.08	0.10	0.09
8	0.25	0.23	0.24	0.26	0.26
12	0.30	0.30	0.30	0.29	0.30
24	0.29	0.28	0.28	0.31	0.31

**0.27  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.02	0.02	0.03	0.03
4	0.09	0.09	0.09	0.10	0.10
8	0.28	0.26	0.27	0.30	0.28
12	0.31	0.33	0.32	0.33	0.33
24	0.30	0.31	0.30	0.32	0.31

**Negative (unsupplemented MEM $\alpha$ ), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06
8	0.08	0.09	0.08	0.09	0.09
12	0.13	0.11	0.11	0.12	0.13
24	0.12	0.13	0.12	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.08	0.08	0.08	0.08	0.08
8	0.21	0.21	0.21	0.19	0.19
12	0.28	0.28	0.28	0.28	0.26
24	0.25	0.26	0.25	0.24	0.26

**0.27  $\mu$ M human ferri-Tf, CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.07	0.07	0.07	0.07	0.07
8	0.12	0.12	0.12	0.11	0.11
12	0.29	0.29	0.29	0.27	0.27
24	0.24	0.25	0.25	0.26	0.25

**Figure 5.4c.****NCTC 11168 and CEM8 with ferri-ovo-Tf.****Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.03	0.03
4	0.06	0.06	0.06	0.06	0.06
8	0.12	0.12	0.12	0.12	0.13
12	0.12	0.12	0.12	0.13	0.13
24	0.12	0.13	0.13	0.13	0.13

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.04	0.04
4	0.08	0.08	0.08	0.10	0.09
8	0.25	0.23	0.24	0.26	0.26
12	0.30	0.30	0.30	0.29	0.30
24	0.29	0.28	0.28	0.31	0.31

**0.27  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.02	0.02	0.02	0.03	0.03
4	0.09	0.09	0.09	0.10	0.10
8	0.25	0.24	0.24	0.29	0.29
12	0.33	0.32	0.32	0.33	0.34
24	0.31	0.31	0.31	0.33	0.34

**Negative (unsupplemented MEM $\alpha$ ), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06
8	0.08	0.09	0.08	0.09	0.09
12	0.13	0.11	0.11	0.12	0.13
24	0.12	0.13	0.12	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.04	0.04
4	0.08	0.08	0.08	0.08	0.08
8	0.21	0.21	0.21	0.19	0.19
12	0.28	0.28	0.28	0.28	0.26
24	0.25	0.26	0.25	0.24	0.26

**0.27  $\mu$ M ferri-ovo-Tf, CEM8**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)	
0	0.03	0.03	0.03	0.03	0.04
4	0.07	0.07	0.07	0.07	0.07
8	0.10	0.13	0.13	0.12	0.12
12	0.29	0.27	0.28	0.26	0.27
24	0.25	0.25	0.25	0.25	0.25

Growth assays of *C. jejuni* strains KAR2 (*cj0178::aphA-3*) and CEM4 ( *$\Delta$ cj0177::ermC'*) with iron supplied solely in the form of haem.

**Figure 5.5a.**

NCTC 11168 and KAR2 with porcine haem.

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.04	0.01	0.01	0.01
4	0.06	0.06	0.06	0.02	0.02	0.02
8	0.11	0.10	0.11	0.12	0.09	0.10
12	0.12	0.11	0.12	0.12	0.10	0.10
24	0.12	0.11	0.11	0.12	0.12	0.10

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.01	0.01	0.01
4	0.08	0.08	0.08	0.04	0.04	0.05
8	0.21	0.25	0.24	0.23	0.22	0.22
12	0.29	0.28	0.29	0.30	0.30	0.29
24	0.28	0.27	0.28	0.29	0.30	0.30

**25  $\mu$ M porcine haem, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.01	0.01	0.01
4	0.13	0.11	0.11	0.05	0.04	0.04
8	0.26	0.22	0.22	0.28	0.26	0.27
12	0.33	0.33	0.33	0.35	0.36	0.37
24	0.30	0.32	0.31	0.32	0.33	0.34

**Negative (unsupplemented MEM $\alpha$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.01	0.01	0.01
4	0.05	0.06	0.05	0.01	0.02	0.01
8	0.11	0.10	0.12	0.09	0.10	0.11
12	0.12	0.12	0.12	0.12	0.10	0.12
24	0.12	0.12	0.13	0.11	0.11	0.11

**Positive (10  $\mu$ M FeSO<sub>4</sub>), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.01	0.01	0.01
4	0.07	0.07	0.06	0.05	0.04	0.05
8	0.20	0.20	0.22	0.20	0.22	0.25
12	0.29	0.28	0.30	0.28	0.28	0.28
24	0.29	0.27	0.28	0.26	0.28	0.27

**25  $\mu$ M porcine haem, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.01	0.01	0.01
4	0.11	0.12	0.10	0.03	0.03	0.03
8	0.23	0.19	0.19	0.24	0.24	0.25
12	0.34	0.34	0.34	0.35	0.34	0.36
24	0.32	0.31	0.30	0.29	0.30	0.32

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 24 h from experimental run 1 vs. mean of 25  $\mu$ M porcine haem, KAR2 optical density values at 24 h from experimental run 1.**

Paired t test

P value	1.0000
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 0.0000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	0.0000
95% confidence interval	-0.04303 to 0.04303
R squared	0.0000

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 24 h from experimental run 2 vs. mean of 25  $\mu$ M porcine haem, KAR2 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.1835
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0200
95% confidence interval	-0.06303 to 0.02303
R squared	0.6667

**Figure 5.5b.**

**NCTC 11168 and CEM4 with porcine haem.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.03	0.03	0.03
4	0.05	0.04	0.05	0.05	0.05	0.05
8	0.12	0.11	0.12	0.11	0.13	0.10
12	0.13	0.13	0.13	0.14	0.14	0.14
24	0.13	0.13	0.14	0.14	0.13	0.14

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.04	0.04	0.04
4	0.06	0.06	0.06	0.06	0.06	0.06
8	0.16	0.16	0.16	0.15	0.17	0.16
12	0.25	0.23	0.22	0.24	0.24	0.23
24	0.27	0.28	0.28	0.28	0.28	0.28

**25  $\mu$ M porcine haem, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.10	0.09	0.11	0.09	0.09	0.10
8	0.22	0.19	0.21	0.19	0.20	0.20
12	0.37	0.36	0.37	0.35	0.35	0.36
24	0.34	0.31	0.35	0.32	0.32	0.35

**Negative (unsupplemented MEM $\alpha$ ), CEM4**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.02
4	0.04	0.04	0.05	0.04	0.04	0.04
8	0.10	0.08	0.09	0.10	0.08	0.08
12	0.11	0.11	0.11	0.11	0.10	0.11
24	0.12	0.11	0.12	0.11	0.12	0.12

**Positive (10  $\mu$ M FeSO<sub>4</sub>), CEM4**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.03	0.03	0.03
4	0.05	0.05	0.06	0.05	0.05	0.05
8	0.15	0.15	0.15	0.14	0.14	0.14
12	0.18	0.19	0.20	0.19	0.20	0.20
24	0.25	0.27	0.27	0.24	0.24	0.25

**25  $\mu$ M porcine haem, CEM4**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.04	0.03	0.03	0.03
4	0.10	0.09	0.10	0.10	0.09	0.09
8	0.15	0.15	0.15	0.14	0.14	0.14
12	0.25	0.23	0.22	0.24	0.24	0.24
24	0.32	0.34	0.30	0.30	0.30	0.30

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 12 h from experimental run 1 vs. mean of 25  $\mu$ M porcine haem, CEM4 optical density values at 12 h from experimental run 1.**

Paired t test

P value	0.0043
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 15.12$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1333
95% confidence interval	-0.1713 to -0.09538
R squared	0.9913

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 12 h from experimental run 2 vs. mean of 25  $\mu$ M porcine haem, CEM4 optical density values at 12 h from experimental run 2.**

Paired t test

P value	0.0009
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 34.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1133
95% confidence interval	-0.1277 to 0.09899
R squared	0.9983

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 24 h from experimental run 1 vs. mean of 25  $\mu$ M porcine haem, CEM4 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.6254
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.5714$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.01333
95% confidence interval	-0.1137 to 0.08707
R squared	0.1404

**Mean of 25  $\mu$ M porcine haem, 11168 optical density values at 24 h from experimental run 2 vs. mean of 25  $\mu$ M porcine haem, CEM4 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0955
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 3.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.03000
95% confidence interval	-0.07303 to 0.01303
R squared	0.8182

**Growth assay of *C. jejuni* NCTC 11168 with and without noradrenaline (NA) in the presence and absence of iron.**

**Figure 6.3.**

**Negative (unsupplemented MEM $\alpha$ ), 11168 (inoculated to initial OD<sub>600</sub> of 0.0125)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.01	0.01	0.01	0.02
4	0.04	0.03	0.03	0.07	0.05	0.05
8	0.09	0.08	0.08	0.11	0.11	0.10
12	0.09	0.09	0.09	0.13	0.12	0.11
24	0.10	0.11	0.10	0.12	0.11	0.12

**Negative (unsupplemented MEM $\alpha$ ), 11168 (inoculated to initial OD<sub>600</sub> of 0.025)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.02	0.02	0.02
4	0.06	0.06	0.06	0.06	0.06	0.06
8	0.11	0.10	0.10	0.09	0.08	0.09
12	0.11	0.11	0.11	0.11	0.10	0.11
24	0.11	0.10	0.10	0.13	0.13	0.13

**Negative (unsupplemented MEM $\alpha$ ) with 100  $\mu$ M NA, 11168 (inoculated to initial OD<sub>600</sub> of 0.0125)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.02	0.01	0.01
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.12	0.11	0.12	0.13	0.13	0.14
12	0.12	0.12	0.13	0.15	0.16	0.16
24	0.13	0.13	0.13	0.15	0.14	0.15

**Negative (unsupplemented MEM $\alpha$ ) with 100  $\mu$ M NA, 11168 (inoculated to initial OD<sub>600</sub> of 0.025)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.03	0.02	0.03
4	0.08	0.08	0.08	0.08	0.08	0.10
8	0.13	0.14	0.14	0.15	0.16	0.15
12	0.16	0.15	0.15	0.20	0.20	0.19
24	0.14	0.13	0.13	0.16	0.17	0.17



**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 (inoculated to initial OD<sub>600</sub> of 0.0125)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.02	0.02	0.01
4	0.06	0.05	0.05	0.07	0.08	0.07
8	0.18	0.17	0.16	0.18	0.15	0.16
12	0.26	0.26	0.27	0.28	0.29	0.29
24	0.27	0.27	0.27	0.26	0.27	0.26

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168 (inoculated to initial OD<sub>600</sub> of 0.025)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.04	0.03
4	0.09	0.08	0.08	0.10	0.10	0.09
8	0.25	0.24	0.28	0.23	0.23	0.22
12	0.29	0.29	0.28	0.30	0.29	0.30
24	0.26	0.27	0.27	0.26	0.26	0.27

**Positive (10  $\mu$ M FeSO<sub>4</sub>) with 100  $\mu$ M NA, 11168 (inoculated to initial OD<sub>600</sub> of 0.0125)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.01	0.01	0.01
4	0.03	0.04	0.04	0.08	0.09	0.10
8	0.15	0.16	0.18	0.17	0.18	0.20
12	0.27	0.27	0.28	0.30	0.30	0.30
24	0.26	0.25	0.26	0.29	0.30	0.29

**Positive (10  $\mu$ M FeSO<sub>4</sub>) with 100  $\mu$ M NA, 11168 (inoculated to initial OD<sub>600</sub> of 0.025)**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.02	0.02	0.02
4	0.07	0.07	0.08	0.11	0.11	0.12
8	0.25	0.28	0.29	0.23	0.25	0.26
12	0.30	0.29	0.30	0.32	0.32	0.32
24	0.28	0.28	0.30	0.29	0.30	0.29

**Mean of negative (lower inoculum), 11168 optical density values at 24 h from experimental run 1 vs. mean of negative (lower inoculum) with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0153
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 8.000, df = 2

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.02667
95% confidence interval	-0.04101 to -0.01232
R squared	0.9697

**Mean of negative (lower inoculum), 11168 optical density values at 24 h from experimental run 2 vs. mean of negative (lower inoculum), with NA, 11168 optical density values at 24 h from experimental run 2.**

<u>Paired t test</u>	
P value	<0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 11900000$ , $df = 2$
Number of pairs	3

<u>How big is the difference?</u>	
Mean of differences	-0.03000
95% confidence interval	-0.03000 to -0.03000
R squared	1.000

**Mean of negative (higher inoculum), 11168 optical density values at 24 h from experimental run 1 vs. mean of negative (higher inoculum) with NA, 11168 optical density values at 24 h from experimental run 1.**

<u>Paired t test</u>	
P value	<0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 11900000$ , $df = 2$
Number of pairs	3

<u>How big is the difference?</u>	
Mean of differences	-0.0300
95% confidence interval	-0.03000 to -0.03000
R squared	1.000

**Mean of negative (higher inoculum), 11168 optical density values at 24 h from experimental run 2 vs. mean of negative (higher inoculum), with NA, 11168 optical density values at 24 h from experimental run 2.**

<u>Paired t test</u>	
P value	0.0082
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

t, df	t = 11.00, df = 2
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.03667
95% confidence interval	-0.05101 to -0.02232
R squared	0.9837

**Mean of positive (lower inoculum), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (lower inoculum) with NA, 11168 optical density values at 24 h from experimental run 1.**

#### Paired t test

P value	0.0572
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t = 4.000, df = 2
Number of pairs	3

#### How big is the difference?

Mean of differences	0.01333
95% confidence interval	-0.001010 to 0.02768
R squared	0.8889

**Mean of positive (lower inoculum), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (lower inoculum) with NA, 11168 optical density values at 24 h from experimental run 2.**

Each row has the same difference. Cannot calculate a paired t test.

**Mean of positive (higher inoculum), 11168 optical density values at 24 h from experimental run 1 vs. mean of positive (higher inoculum) with NA, 11168 optical density values at 24 h from experimental run 1.**

#### Paired t test

P value	0.0153
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 8.000, df = 2
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.02667
95% confidence interval	-0.04101 to 0.01232
R squared	0.9697

**Mean of positive (higher inoculum), 11168 optical density values at 24 h from experimental run 2 vs. mean of positive (higher inoculum), with NA, 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0351
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 5.196$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0300
95% confidence interval	-0.05484 to -0.005157
R squared	0.9310

**Growth assay of *C. jejuni* NCTC 11168 with and without NA in the presence of human ferri-Lf, human ferri-Tf and ferri-ovo-Tf.**

**Figure 6.4a.**

**NCTC 11168 with 0.111  $\mu$ M human ferri-Lf, human ferri-Tf or ferri-ovo-Tf in the presence and absence of NA.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.02	0.01	0.02
4	0.03	0.03	0.03	0.04	0.05	0.04
8	0.06	0.06	0.07	0.10	0.10	0.10
12	0.09	0.10	0.11	0.10	0.11	0.11
24	0.12	0.11	0.11	0.11	0.12	0.14

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.03	0.03	0.03
4	0.04	0.04	0.05	0.06	0.06	0.06
8	0.09	0.09	0.10	0.14	0.14	0.14
12	0.22	0.21	0.22	0.28	0.28	0.28
24	0.28	0.29	0.29	0.31	0.29	0.28

**0.111  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.04	0.03
4	0.03	0.03	0.03	0.07	0.06	0.06
8	0.09	0.09	0.09	0.16	0.17	0.16
12	0.20	0.19	0.19	0.28	0.27	0.27
24	0.24	0.23	0.22	0.30	0.29	0.30

**0.111  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.04	0.04
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.09	0.09	0.08	0.13	0.15	0.14
12	0.21	0.21	0.21	0.29	0.28	0.28
24	0.24	0.21	0.21	0.30	0.29	0.29

**0.111  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.08	0.08	0.09	0.14	0.12	0.12
12	0.19	0.18	0.19	0.27	0.27	0.27
24	0.21	0.22	0.22	0.27	0.28	0.28

**0.111  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.03	0.03	0.03	0.06	0.06	0.06
8	0.09	0.09	0.08	0.21	0.21	0.21
12	0.22	0.23	0.22	0.30	0.32	0.32
24	0.32	0.32	0.32	0.34	0.35	0.34

**0.111  $\mu$ M human ferri-Tf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.03	0.03	0.03	0.04	0.03
4	0.04	0.04	0.04	0.06	0.07	0.06
8	0.10	0.10	0.08	0.19	0.20	0.19
12	0.24	0.24	0.25	0.32	0.33	0.32
24	0.33	0.33	0.31	0.32	0.32	0.32

**0.111  $\mu$ M ferri-ovo-Tf with 100  $\mu$ M NA, 11168**

<b>Hours at 37°C</b>	<b>Experimental Run 1 (optical density at 600 nm)</b>			<b>Experimental Run 2 (optical density at 600 nm)</b>		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.10	0.11	0.11	0.16	0.18	0.17
12	0.23	0.25	0.24	0.30	0.31	0.30
24	0.30	0.32	0.32	0.31	0.31	0.31

**Mean of 0.111  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.111  $\mu$ M human ferri-Lf with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0041
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 15.59$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0900
95% confidence interval	-0.1148 to -0.06516
R squared	0.9918

**Mean of 0.111  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.111  $\mu$ M human ferri-Lf with NA, 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0198
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 7.000$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04667
95% confidence interval	-0.07535 to -0.01798
R squared	0.9608

**Mean of 0.111  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.111  $\mu$ M human ferri-Tf with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0072
P value summary	**

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 11.72$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.1033
95% confidence interval	-0.1413 to -0.06538
R squared	0.9856

**Mean of 0.111  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.111  $\mu$ M human ferri-Tf with NA, 11168 optical density values at 24 h from experimental run 2.**

#### Paired t test

P value	0.0153
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 8.000$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.02667
95% confidence interval	-0.04101 to -0.01232
R squared	0.9697

**Mean of 0.111  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.111  $\mu$ M ferri-ovo-Tf with NA, 11168 optical density values at 24 h from experimental run 1.**

#### Paired t test

P value	0.0012
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 29.00$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.09667
95% confidence interval	-0.1110 to -0.08232
R squared	0.9976

**Mean of 0.111  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.111  $\mu$ M ferri-ovo-Tf with NA, 11168 optical density values at 24 h from experimental run 2.**

#### Paired t test

P value	0.0099
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P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 10.00$ , $df = 2$
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.03333
95% confidence interval	-0.04768 to -0.01899
R squared	0.9804

**Figure 6.4b.**

NCTC 11168 with 0.27  $\mu\text{M}$  human ferri-Lf, human ferri-Tf or ferri-ovo-Tf in the presence and absence of NA.

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.02	0.01	0.02
4	0.03	0.03	0.03	0.04	0.05	0.04
8	0.06	0.06	0.07	0.10	0.10	0.10
12	0.09	0.10	0.11	0.10	0.11	0.11
24	0.12	0.11	0.11	0.11	0.12	0.14

**Positive (10  $\mu\text{M}$  FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.03	0.03	0.03
4	0.04	0.04	0.05	0.06	0.06	0.06
8	0.09	0.09	0.10	0.14	0.14	0.14
12	0.22	0.21	0.22	0.28	0.28	0.28
24	0.28	0.29	0.29	0.31	0.29	0.28

**0.27  $\mu\text{M}$  human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.09	0.09	0.09	0.15	0.15	0.17
12	0.23	0.22	0.23	0.29	0.28	0.28
24	0.28	0.29	0.28	0.32	0.30	0.30



**0.27  $\mu$ M human ferri-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.09	0.08	0.08	0.15	0.15	0.15
12	0.21	0.21	0.21	0.30	0.29	0.29
24	0.27	0.26	0.27	0.31	0.31	0.31

**0.27  $\mu$ M ferri-ovo-Tf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.09	0.09	0.10	0.16	0.15	0.15
12	0.23	0.23	0.22	0.26	0.27	0.27
24	0.30	0.32	0.31	0.30	0.30	0.30

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.10	0.08	0.08	0.19	0.21	0.20
12	0.25	0.25	0.26	0.30	0.33	0.31
24	0.35	0.36	0.36	0.33	0.35	0.34

**0.27  $\mu$ M human ferri-Tf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.07	0.07
8	0.09	0.09	0.08	0.19	0.19	0.19
12	0.25	0.26	0.26	0.33	0.33	0.33
24	0.34	0.37	0.35	0.34	0.34	0.35

**0.27  $\mu$ M ferri-ovo-Tf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.04	0.04	0.04	0.06	0.06	0.06
8	0.10	0.11	0.11	0.19	0.20	0.20
12	0.27	0.27	0.28	0.33	0.33	0.34
24	0.35	0.36	0.37	0.35	0.36	0.36

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0067
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 12.12$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.07000
95% confidence interval	-0.09484 to -0.04516
R squared	0.9866

**Mean of 0.27  $\mu$ M human ferri-Lf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0202
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 6.928$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04000
95% confidence interval	-0.06484 to -0.01516
R squared	0.9600

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Tf with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0187
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 7.211$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.08667
95% confidence interval	-0.1384 to -0.03495
R squared	0.9630

**Mean of 0.27  $\mu$ M human ferri-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Tf with NA, 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0099
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 10.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.03333
95% confidence interval	-0.04768 to -0.01899
R squared	0.9804

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf with NA, 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0131
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 8.660$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.0500
95% confidence interval	-0.07484 to -0.02516
R squared	0.9740

**Mean of 0.27  $\mu$ M ferri-ovo-Tf, 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M ferri-ovo-Tf with NA, 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0034
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 17.00$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.05667
95% confidence interval	-0.07101 to -0.04232
R squared	0.9931

**Growth assays of *C. jejuni* mutant strains with iron supplied bound to human Lf in the presence and absence of NA.**

**Figure 6.5a.**

**NCTC 11168 and JDR5 (*AchuA::cat*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.02	0.03
4	0.05	0.04	0.05	0.04	0.04	0.05
8	0.09	0.09	0.09	0.10	0.10	0.10
12	0.14	0.15	0.14	0.13	0.14	0.14
24	0.16	0.15	0.15	0.14	0.14	0.15

**Negative (unsupplemented MEM $\alpha$ ), JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.02	0.02	0.02	0.03
4	0.04	0.04	0.05	0.04	0.05	0.04
8	0.11	0.11	0.11	0.11	0.12	0.10
12	0.14	0.13	0.14	0.14	0.14	0.14
24	0.16	0.17	0.17	0.15	0.15	0.15

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.01	0.02	0.02
4	0.05	0.05	0.06	0.06	0.05	0.05
8	0.12	0.12	0.12	0.15	0.15	0.16
12	0.27	0.29	0.30	0.30	0.29	0.28
24	0.29	0.31	0.30	0.32	0.30	0.30

**Positive (10  $\mu$ M FeSO<sub>4</sub>), JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.01	0.01	0.02	0.02	0.01
4	0.05	0.06	0.05	0.05	0.05	0.06
8	0.13	0.12	0.15	0.16	0.16	0.17
12	0.29	0.31	0.31	0.28	0.28	0.29
24	0.25	0.26	0.26	0.29	0.32	0.30

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.02	0.01	0.02
4	0.05	0.05	0.05	0.05	0.05	0.05
8	0.15	0.15	0.15	0.16	0.17	0.18
12	0.33	0.31	0.31	0.30	0.30	0.30
24	0.31	0.33	0.33	0.29	0.31	0.29

**0.27  $\mu$ M human ferri-Lf, JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.01	0.01	0.02	0.01
4	0.06	0.06	0.06	0.05	0.06	0.06
8	0.18	0.17	0.17	0.18	0.18	0.16
12	0.28	0.30	0.32	0.28	0.29	0.30
24	0.29	0.28	0.27	0.29	0.27	0.27

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.01
4	0.05	0.06	0.05	0.05	0.06	0.06
8	0.16	0.16	0.16	0.18	0.18	0.19
12	0.28	0.28	0.34	0.33	0.33	0.33
24	0.31	0.30	0.30	0.31	0.32	0.32

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, JDR5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.02	0.02	0.03	0.03
4	0.06	0.06	0.07	0.07	0.06	0.07
8	0.15	0.15	0.16	0.15	0.15	0.17
12	0.35	0.32	0.32	0.34	0.32	0.32
24	0.28	0.27	0.27	0.30	0.32	0.31

**Mean of 0.27  $\mu$ M human ferri-Lf, JDR5 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, JDR5 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0315
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 5.500$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.03667
95% confidence interval	-0.06535 to -0.007980
R squared	0.9380

**Mean of 0.27  $\mu$ M human ferri-Lf, JDR5 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, JDR5 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0229
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 6.500$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.04333
95% confidence interval	-0.07202 to -0.01465
R squared	0.9548

**Figure 6.5b.**

**NCTC 11168 and KAR3 (*p19::aphA-3*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.03	0.02	0.01	0.02
4	0.04	0.05	0.04	0.04	0.04	0.05
8	0.09	0.09	0.10	0.10	0.10	0.11
12	0.15	0.15	0.13	0.14	0.14	0.14
24	0.15	0.14	0.14	0.14	0.15	0.14

**Negative (unsupplemented MEM $\alpha$ ), KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.02	0.03	0.01
4	0.05	0.05	0.05	0.05	0.05	0.04
8	0.10	0.10	0.09	0.10	0.09	0.12
12	0.15	0.14	0.13	0.14	0.13	0.13
24	0.15	0.15	0.14	0.15	0.14	0.15

**Positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.01	0.01	0.02
4	0.05	0.05	0.06	0.05	0.06	0.06
8	0.11	0.14	0.13	0.15	0.16	0.15
12	0.20	0.20	0.22	0.21	0.20	0.22
24	0.24	0.28	0.29	0.29	0.29	0.30

**Positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.02	0.02	0.02	0.03
4	0.06	0.05	0.06	0.06	0.06	0.05
8	0.13	0.13	0.14	0.15	0.15	0.17
12	0.21	0.23	0.25	0.23	0.20	0.21
24	0.28	0.25	0.25	0.28	0.29	0.29

**0.27  $\mu\text{M}$  human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.02	0.01
4	0.04	0.05	0.05	0.05	0.05	0.05
8	0.12	0.12	0.12	0.14	0.14	0.14
12	0.26	0.26	0.24	0.27	0.25	0.26
24	0.32	0.32	0.34	0.32	0.32	0.32

**0.27  $\mu\text{M}$  human ferri-Lf, KAR3**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.01	0.03	0.03	0.03	0.01
4	0.04	0.06	0.06	0.04	0.05	0.06
8	0.15	0.13	0.12	0.13	0.13	0.15
12	0.26	0.26	0.23	0.26	0.24	0.24
24	0.27	0.29	0.31	0.28	0.30	0.31

**0.27  $\mu\text{M}$  human ferri-Lf with 100  $\mu\text{M}$  NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.02	0.01	0.03
4	0.05	0.06	0.05	0.06	0.06	0.05
8	0.12	0.13	0.15	0.13	0.15	0.15
12	0.21	0.23	0.26	0.22	0.22	0.27
24	0.35	0.32	0.36	0.35	0.34	0.35

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, KAR3**

<b>Hours at 37°C</b>	<b>Experimental Run 1 (optical density at 600 nm)</b>			<b>Experimental Run 2 (optical density at 600 nm)</b>		
0	0.02	0.02	0.02	0.02	0.02	0.01
4	0.06	0.07	0.06	0.07	0.07	0.06
8	0.15	0.16	0.17	0.16	0.16	0.17
12	0.25	0.27	0.26	0.26	0.26	0.27
24	0.32	0.32	0.32	0.33	0.33	0.33

**Mean of 0.27  $\mu$ M human ferri-Lf, KAR3 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, KAR3 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0202
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 6.928, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.0400
95% confidence interval	-0.06484 to -0.01516
R squared	0.9600

**Mean of 0.27  $\mu$ M human ferri-Lf, KAR3 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, KAR3 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0202
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 6.928, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.0400
95% confidence interval	-0.06484 to -0.01516
R squared	0.9600



**Figure 6.5c.**  
**NCTC 11168 and KAR2 (*cj0178::aphA-3*) with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.02	0.02	0.02
4	0.06	0.05	0.06	0.05	0.05	0.05
8	0.13	0.13	0.13	0.12	0.13	0.12
12	0.13	0.14	0.14	0.13	0.13	0.13
24	0.14	0.14	0.14	0.14	0.13	0.14

**Negative (unsupplemented MEM $\alpha$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.04	0.02	0.03
4	0.03	0.04	0.04	0.04	0.04	0.05
8	0.11	0.11	0.12	0.12	0.11	0.11
12	0.14	0.14	0.14	0.13	0.14	0.13
24	0.14	0.15	0.14	0.14	0.14	0.14

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.02	0.03
4	0.07	0.06	0.07	0.06	0.06	0.06
8	0.20	0.19	0.17	0.18	0.18	0.20
12	0.25	0.28	0.25	0.26	0.28	0.27
24	0.29	0.28	0.27	0.30	0.29	0.28

**Positive (10  $\mu$ M FeSO $_4$ ), KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.03	0.02	0.02
4	0.05	0.05	0.05	0.06	0.05	0.05
8	0.15	0.16	0.17	0.17	0.15	0.17
12	0.23	0.20	0.20	0.24	0.24	0.25
24	0.28	0.28	0.29	0.27	0.29	0.29

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.02	0.02
4	0.07	0.06	0.06	0.07	0.07	0.07
8	0.18	0.20	0.20	0.16	0.16	0.16
12	0.30	0.29	0.29	0.26	0.27	0.27
24	0.30	0.32	0.32	0.30	0.31	0.31

**0.27  $\mu$ M human ferri-Lf, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.02	0.02	0.02	0.02	0.02
4	0.04	0.05	0.04	0.04	0.04	0.04
8	0.14	0.14	0.14	0.09	0.10	0.10
12	0.23	0.24	0.24	0.14	0.16	0.16
24	0.16	0.18	0.18	0.18	0.19	0.18

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.04	0.03	0.03	0.02
4	0.07	0.07	0.08	0.07	0.08	0.07
8	0.21	0.21	0.21	0.22	0.19	0.19
12	0.26	0.29	0.29	0.28	0.28	0.29
24	0.33	0.33	0.34	0.32	0.32	0.34

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, KAR2**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.04	0.03	0.03	0.02	0.02
4	0.05	0.05	0.05	0.05	0.05	0.05
8	0.15	0.12	0.13	0.12	0.16	0.15
12	0.24	0.22	0.23	0.20	0.21	0.22
24	0.29	0.29	0.29	0.28	0.27	0.28

**Mean of 0.27  $\mu$ M human ferri-Lf, KAR2 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, KAR2 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0032
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 17.50$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1167
95% confidence interval	-0.1454 to -0.08798
R squared	0.9935

**Mean of 0.27  $\mu$ M human ferri-Lf, KAR2 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, KAR2 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0051
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 14.00, df = 2
Number of pairs	3
<u>How big is the difference?</u>	
Mean of differences	-0.09333
95% confidence interval	-0.1220 to -0.06465
R squared	0.9899

**Figure 6.5d.**NCTC 11168 and CEM5 ( $\Delta$ *cfrA::aphA-3*) with human ferri-Lf.**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.03	0.02	0.02	0.02
4	0.06	0.05	0.06	0.05	0.05	0.05
8	0.13	0.13	0.13	0.12	0.13	0.12
12	0.13	0.14	0.14	0.13	0.13	0.13
24	0.14	0.14	0.14	0.14	0.13	0.14

**Negative (unsupplemented MEM $\alpha$ ), CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.02	0.03	0.04
4	0.05	0.05	0.05	0.04	0.05	0.04
8	0.13	0.12	0.14	0.12	0.12	0.13
12	0.13	0.14	0.14	0.14	0.13	0.13
24	0.14	0.14	0.14	0.13	0.13	0.14

**Positive (10  $\mu$ M FeSO $_4$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.02	0.02	0.03	0.02	0.03
4	0.07	0.06	0.07	0.06	0.06	0.06
8	0.20	0.19	0.17	0.18	0.18	0.20
12	0.25	0.28	0.25	0.26	0.28	0.27
24	0.29	0.28	0.27	0.30	0.29	0.28

**Positive (10  $\mu\text{M}$   $\text{FeSO}_4$ ), CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.03	0.02	0.03	0.03
4	0.05	0.06	0.06	0.06	0.06	0.05
8	0.17	0.18	0.18	0.18	0.19	0.17
12	0.31	0.29	0.26	0.28	0.27	0.30
24	0.33	0.32	0.35	0.30	0.32	0.29

**0.27  $\mu\text{M}$  human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.02	0.02
4	0.07	0.06	0.06	0.07	0.07	0.07
8	0.18	0.20	0.20	0.16	0.16	0.16
12	0.30	0.29	0.29	0.26	0.27	0.27
24	0.30	0.32	0.32	0.30	0.31	0.31

**0.27  $\mu\text{M}$  human ferri-Lf, CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.03	0.03	0.03
4	0.06	0.06	0.06	0.06	0.06	0.06
8	0.16	0.16	0.16	0.14	0.14	0.14
12	0.21	0.22	0.22	0.18	0.19	0.18
24	0.19	0.20	0.20	0.22	0.23	0.24

**0.27  $\mu\text{M}$  human ferri-Lf with 100  $\mu\text{M}$  NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.04	0.03	0.03	0.02
4	0.07	0.07	0.08	0.07	0.08	0.07
8	0.21	0.21	0.21	0.22	0.19	0.19
12	0.26	0.29	0.29	0.28	0.28	0.29
24	0.33	0.33	0.34	0.32	0.32	0.34

**0.27  $\mu\text{M}$  human ferri-Lf with 100  $\mu\text{M}$  NA, CEM5**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.03	0.02	0.03	0.03
4	0.04	0.05	0.04	0.04	0.05	0.05
8	0.15	0.15	0.16	0.14	0.15	0.16
12	0.28	0.26	0.27	0.27	0.27	0.25
24	0.38	0.40	0.37	0.39	0.39	0.42

**Mean of 0.27  $\mu$ M human ferri-Lf, CEM5 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, CEM5 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0022
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 21.17$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1867
95% confidence interval	-0.2246 to -0.1487
R squared	0.9956

**Mean of 0.27  $\mu$ M human ferri-Lf, CEM5 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, CEM5 optical density values at 24 h from experimental run 2.**

Paired t test

P value	0.0012
P value summary	**
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 29.44$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.1700
95% confidence interval	-0.1948 to -0.1452
R squared	0.9977

**Figure 6.5e.**

**NCTC 11168 and 11168  $\Delta$ *feoB::ermC'* with human ferri-Lf.**

**Negative (unsupplemented MEM $\alpha$ ), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.01	0.02	0.02	0.03
4	0.05	0.04	0.05	0.04	0.04	0.05
8	0.09	0.09	0.09	0.10	0.10	0.10
12	0.14	0.15	0.14	0.13	0.14	0.14
24	0.16	0.15	0.15	0.14	0.14	0.15

**Negative (unsupplemented MEMa),  $\Delta feoB::ermC'$  11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.02	0.02	0.03	0.01	0.02
4	0.05	0.05	0.05	0.05	0.05	0.04
8	0.12	0.12	0.10	0.11	0.10	0.12
12	0.14	0.14	0.15	0.14	0.13	0.14
24	0.15	0.16	0.15	0.13	0.12	0.13

**Positive (10  $\mu$ M FeSO<sub>4</sub>), 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.03	0.03	0.01	0.02	0.02
4	0.05	0.05	0.06	0.06	0.05	0.05
8	0.12	0.12	0.12	0.15	0.15	0.16
12	0.27	0.29	0.30	0.30	0.29	0.28
24	0.29	0.31	0.30	0.32	0.30	0.30

**Positive (25  $\mu$ M haem),  $\Delta feoB::ermC'$  11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.03	0.02	0.03	0.01
4	0.06	0.06	0.09	0.08	0.08	0.07
8	0.18	0.18	0.20	0.22	0.23	0.22
12	0.36	0.36	0.37	0.37	0.39	0.36
24	0.38	0.37	0.39	0.35	0.32	0.38

**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.03	0.02	0.02	0.01	0.02
4	0.05	0.05	0.05	0.05	0.05	0.05
8	0.15	0.15	0.15	0.16	0.17	0.18
12	0.33	0.31	0.31	0.30	0.30	0.30
24	0.31	0.33	0.33	0.29	0.31	0.29

**0.27  $\mu$ M human ferri-Lf,  $\Delta feoB::ermC'$  11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.01
4	0.05	0.05	0.05	0.05	0.05	0.04
8	0.13	0.13	0.13	0.12	0.13	0.12
12	0.18	0.18	0.17	0.17	0.17	0.17
24	0.22	0.23	0.24	0.21	0.22	0.23

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.03	0.02	0.02	0.02	0.02	0.01
4	0.05	0.06	0.05	0.05	0.06	0.06
8	0.16	0.16	0.16	0.18	0.18	0.19
12	0.28	0.28	0.34	0.33	0.33	0.33
24	0.31	0.30	0.30	0.31	0.32	0.32

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA,  $\Delta$ feoB::ermC' 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.01	0.01	0.01	0.01	0.02	0.02
4	0.05	0.06	0.06	0.06	0.05	0.05
8	0.16	0.15	0.15	0.15	0.14	0.15
12	0.17	0.16	0.18	0.16	0.19	0.16
24	0.25	0.22	0.25	0.23	0.24	0.25

**Mean of 0.27  $\mu$ M human ferri-Lf,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0351
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 5.196, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.0300
95% confidence interval	-0.05484 to -0.005157
R squared	0.9310

**Mean of 0.27  $\mu$ M human ferri-Lf,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA,  $\Delta$ feoB::ermC' 11168 optical density values at 24 h from experimental run 2.**

Paired t test

P value	<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t = 4011000, df = 2
Number of pairs	3

How big is the difference?

Mean of differences	-0.0200
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**0.27  $\mu$ M human ferri-Lf, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.04	0.03	0.03	0.01	0.03
4	0.05	0.04	0.04	0.04	0.05	0.05
8	0.19	0.16	0.15	0.16	0.17	0.17
12	0.32	0.29	0.29	0.29	0.30	0.30
24	0.33	0.31	0.31	0.30	0.30	0.31

**0.27  $\mu$ M human ferri-Lf, CEM12**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.02	0.01
4	0.02	0.02	0.02	0.03	0.03	0.03
8	0.07	0.08	0.08	0.09	0.08	0.08
12	0.14	0.14	0.14	0.15	0.15	0.15
24	0.14	0.16	0.15	0.16	0.15	0.15

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, 11168**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.04	0.03	0.04	0.02	0.02	0.02
4	0.06	0.05	0.06	0.05	0.06	0.06
8	0.18	0.18	0.19	0.17	0.18	0.17
12	0.28	0.28	0.28	0.31	0.31	0.29
24	0.28	0.29	0.30	0.33	0.34	0.33

**0.27  $\mu$ M human ferri-Lf with 100  $\mu$ M NA, CEM12**

Hours at 37°C	Experimental Run 1 (optical density at 600 nm)			Experimental Run 2 (optical density at 600 nm)		
0	0.02	0.04	0.03	0.02	0.01	0.03
4	0.02	0.03	0.02	0.03	0.04	0.04
8	0.07	0.09	0.08	0.09	0.09	0.10
12	0.15	0.14	0.15	0.13	0.14	0.16
24	0.17	0.16	0.17	0.16	0.17	0.18

**Mean of 0.27  $\mu$ M human ferri-Lf, CEM12 optical density values at 24 h from experimental run 1 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, CEM12 optical density values at 24 h from experimental run 1.**

Paired t test

P value	0.0153
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 8.000$ , $df = 2$
Number of pairs	3

### Appendix 3. Raw Growth Curve Data and Statistical Testing of Differences

#### How big is the difference?

Mean of differences	-0.02667
95% confidence interval	-0.04101 to -0.01232
R squared	0.9697

**Mean of 0.27  $\mu$ M human ferri-Lf, CEM12 optical density values at 24 h from experimental run 2 vs. mean of 0.27  $\mu$ M human ferri-Lf with NA, CEM12 optical density values at 24 h from experimental run 2.**

#### Paired t test

P value	0.0377
P value summary	*
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 5.000$ , $df = 2$
Number of pairs	3

#### How big is the difference?

Mean of differences	-0.03333
95% confidence interval	-0.06202 to -0.004647
R squared	0.9259

**Appendix 4.**

**Raw Promoter Activity Data and Statistical Testing of Differences**

Promoter activity levels were determined from  $\beta$ -galactosidase activity of iron-limited (20  $\mu$ M Desferal<sup>TM</sup>) or iron-replete (40  $\mu$ M FeSO<sub>4</sub>) *C. jejuni* 480 cultures containing reporter constructs grown in MH broth for 5 h at 37 °C. All conditions were tested in triplicate (technical replicates) in two independent assays (biological replicates).

**$\beta$ -galactosidase assay to determine activity levels of promoter regions across the *cj0173c-tonB1* region of the *C. jejuni* NCTC 11168 genome.**

**Figure 5.11.**

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (final optical density at 420 nm). Iron-replete conditions.				Experimental Run 2 (final optical density at 420 nm). Iron-replete conditions.			
pMW10	0.074	0.090	0.085		0.042	0.048	0.036	
<i>pmetK</i>	0.297	0.317	0.309	0.301	0.670	0.652	0.692	0.696
<i>pchuA</i>	0.013	0.013	0.015	0.019	0.055	0.051	0.068	0.058
<i>pcj0178</i>	0.084	0.072	0.070		0.052	0.045	0.055	
<i>pexbB1</i>	0.075	0.072	0.076		0.045	0.064	0.056	
pMW10	0.016	0.009	0.015	0.012	0.024	0.020	0.019	0.025
<i>pcj0176c</i>	0.028	0.032	0.033	0.030	0.093	0.080	0.078	0.078
<i>pcj0177</i>	0.018	0.023	0.018	0.025	0.039	0.041	0.044	0.043

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (final optical density at 420 nm). Iron-limited conditions.				Experimental Run 2 (final optical density at 420 nm). Iron-limited conditions.			
pMW10	0.080	0.082	0.093		0.054	0.043	0.047	
<i>pmetK</i>	0.290	0.296	0.295	0.295	0.695	0.711	0.701	0.699
<i>pchuA</i>	0.875	0.909	0.897	0.896	1.094	1.000	1.000	1.011
<i>pcj0178</i>	0.078	0.069	0.077		0.043	0.045	0.035	
<i>pexbB1</i>	0.092	0.098	0.099		0.059	0.045	0.047	
pMW10	0.008	0.007	0.008	0.009	0.038	0.037	0.035	0.039
<i>pcj0176c</i>	0.082	0.090	0.089	0.085	0.227	0.200	0.211	0.227
<i>pcj0177</i>	0.062	0.074	0.060	0.068	0.077	0.084	0.083	0.078

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (initial optical density at 600 nm). Iron-replete conditions.				Experimental Run 2 (initial optical density at 600 nm). Iron-replete conditions.			
pMW10	0.40				0.41			
<i>pmetK</i>	0.38				0.40			
<i>pchuA</i>	0.37				0.39			
<i>pcj0178</i>	0.40				0.42			
<i>pexbB1</i>	0.43				0.41			
pMW10	0.35				0.44			
<i>pcj0176c</i>	0.32				0.43			
<i>pcj0177</i>	0.38				0.41			

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (initial optical density at 600 nm). Iron-limited conditions.	Experimental Run 2 (initial optical density at 600 nm). Iron-limited conditions.
pMW10	0.41	0.43
<i>pmetK</i>	0.35	0.42
<i>pchuA</i>	0.36	0.44
<i>pcj0178</i>	0.44	0.40
<i>pexbB1</i>	0.43	0.42
pMW10	0.32	0.41
<i>pcj0176c</i>	0.36	0.42
<i>pcj0177</i>	0.37	0.44

$$\text{Miller units} = 1000 \times \frac{\text{OD}_{420}}{t \times v \times \text{OD}_{600}}$$

where  $t$  = time of the reaction in minutes (60),  $v$  = volume of culture in the assay in ml (0.05),  $\text{OD}_{600}$  = the cell density reading taken just before the assay began and  $\text{OD}_{420}$  = the absorbance of the reaction mixture at the end of the assay.

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (Miller units). Iron-replete conditions.				Experimental Run 2 (Miller units). Iron-replete conditions.			
pMW10	61.67	75.00	70.83		34.15	39.02	29.27	
<i>pmetK</i>	260.5	278.1	271.1	264.0	558.3	543.3	576.6	580.0
<i>pchuA</i>	11.7	11.7	13.5	17.1	47.01	43.58	58.12	49.57
<i>pcj0178</i>	70.00	60.00	58.33		41.27	35.71	42.06	
<i>pexbB1</i>	58.14	62.02	58.92		36.58	52.03	45.53	
pMW10	15.24	8.57	14.29	11.43	18.18	15.15	14.39	18.94
<i>pcj0176c</i>	29.2	33.3	34.4	31.3	72.09	62.02	60.47	60.47
<i>pcj0177</i>	15.8	20.2	15.8	21.9	31.71	33.33	35.77	34.96

Promoter in pMW10 in <i>C. jejuni</i> 480	Experimental Run 1 (Miller units). Iron-limited conditions.				Experimental Run 2 (Miller units). Iron-limited conditions.			
pMW10	65.04	66.67	75.61		41.86	33.33	36.43	
<i>pmetK</i>	276.2	281.9	280.9	280.9	551.6	564.3	556.3	554.8
<i>pchuA</i>	810.2	841.6	830.5	829.6	828.8	757.6	757.6	765.9
<i>pcj0178</i>	59.09	52.27	58.33		35.83	37.50	29.17	
<i>pexbB1</i>	71.32	75.97	76.74		46.83	35.71	37.30	
pMW10	8.3	7.3	8.3	9.4	30.89	30.08	28.46	31.70
<i>pcj0176c</i>	76.0	83.3	83.4	78.7	180.2	158.7	167.5	180.2
<i>pcj0177</i>	55.9	66.6	54.1	61.3	58.33	63.64	62.88	59.09

**Mean of iron-replete pMW10 Miller Units from experimental run 1 vs. mean of iron-limited pMW10 Miller Units from experimental run 1.**

Paired t test

P value	0.9898
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.01444$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-0.06000
95% confidence interval	-17.94 to 17.82
R squared	0.0001043

**Mean of iron-replete pMW10 Miller Units from experimental run 2 vs. mean of iron-limited pMW10 Miller Units from experimental run 2.**

Paired t test

P value	0.5569
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.6990$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	3.060
95% confidence interval	-15.78 to 21.90
R squared	0.1963

**Mean of iron-replete pmetK Miller Units from experimental run 1 vs. mean of iron-limited pmetK Miller Units from experimental run 1.**

Paired t test

P value	0.2415
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 1.456$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	6.550
95% confidence interval	-7.768 to 20.87
R squared	0.4139

**Mean of iron-replete *pmetK* Miller Units from experimental run 2 vs. mean of iron-limited *pmetK* Miller Units from experimental run 2.**Paired t test

P value	0.5065
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.7524$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	-7.800
95% confidence interval	-40.79 to 25.19
R squared	0.1587

**Mean of iron-replete *pchuA* Miller Units from experimental run 1 vs. mean of iron-limited *pchuA* Miller Units from experimental run 1.**Paired t test

P value	<0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 125.8$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	814.5
95% confidence interval	793.9 to 835.1
R squared	0.9998

**Mean of iron-replete *pchuA* Miller Units from experimental run 2 vs. mean of iron-limited *pchuA* Miller Units from experimental run 2.**Paired t test

P value	<0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 39.68$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	727.9
95% confidence interval	669.5 to 786.3
R squared	0.9981

**Mean of iron-replete *pcj0178* Miller Units from experimental run 1 vs. mean of iron-limited *pcj0178* Miller Units from experimental run 1.**

Paired t test

P value	0.9476
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.07427$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	0.4533
95% confidence interval	-25.81 to 26.72
R squared	0.002750

**Mean of iron-replete *pcj0178* Miller Units from experimental run 2 vs. mean of iron-limited *pcj0178* Miller Units from experimental run 2.**

Paired t test

P value	0.4114
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 1.030$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-2.180
95% confidence interval	-11.29 to 6.930
R squared	0.3465

**Mean of iron-replete *pexbB1* Miller Units from experimental run 1 vs. mean of iron-limited *pexbB1* Miller Units from experimental run 1.**

Paired t test

P value	0.1166
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.667$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	8.317
95% confidence interval	-5.104 to 21.74
R squared	0.7805



**Mean of iron-replete *pexbB1* Miller Units from experimental run 2 vs. mean of iron-limited *pexbB1* Miller Units from experimental run 2.**

Paired t test

P value	0.6060
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 0.6062$ , $df = 2$
Number of pairs	3

How big is the difference?

Mean of differences	-4.767
95% confidence interval	-38.60 to 29.07
R squared	0.1552

**Mean of iron-replete pMW10 Miller Units from experimental run 1 vs. mean of iron-limited pMW10 Miller Units from experimental run 1.**

Paired t test

P value	0.0661
P value summary	ns
Are means signif. different? ( $P < 0.05$ )	No
One- or two-tailed P value?	Two-tailed
t, df	$t = 2.832$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	-4.025
95% confidence interval	-8.548 to 0.4982
R squared	0.7277

**Mean of iron-replete pMW10 Miller Units from experimental run 2 vs. mean of iron-limited pMW10 Miller Units from experimental run 2.**

Paired t test

P value	0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 25.26$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	13.62
95% confidence interval	11.90 to 15.33
R squared	0.9953

**Mean of iron-replete *pcj0176c* Miller Units from experimental run 1 vs. mean of iron-limited *pcj0176c* Miller Units from experimental run 1.**

Paired t test

P value	<0.0001
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 55.32$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	47.83
95% confidence interval	45.07 to 50.58
R squared	0.9990

**Mean of iron-replete *pcj0176c* Miller Units from experimental run 2 vs. mean of iron-limited *pcj0176c* Miller Units from experimental run 2.**

Paired t test

P value	0.0002
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.89$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	107.9
95% confidence interval	92.89 to 122.9
R squared	0.9943

**Mean of iron-replete *pcj0177* Miller Units from experimental run 1 vs. mean of iron-limited *pcj0177* Miller Units from experimental run 1.**

Paired t test

P value	0.0002
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 22.25$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	41.08
95% confidence interval	35.20 to 46.95
R squared	0.9940

**Mean of iron-replete *pcj0177* Miller Units from experimental run 2 vs. mean of iron-limited *pcj0177* Miller Units from experimental run 2.**

Paired t test

P value	0.0002
P value summary	***
Are means signif. different? ( $P < 0.05$ )	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t = 21.30$ , $df = 3$
Number of pairs	4

How big is the difference?

Mean of differences	27.04
95% confidence interval	23.00 to 31.08
R squared	0.9934

## **Appendix 5.**

### **Viable Count Data for Selected Growth Assays**

## Appendix 5. Viable Count Data

Assuming that a count of between 30 and 300 colonies is statistically viable, a number of key growth curves were completed and samples of cells (1 ml) were taken at 0, 8 and 24 hours, optical density was confirmed and the cells were used to count viable numbers. Each sample was appropriately serially diluted (100 µl culture taken and mixed with 900 µl phosphate buffered saline (PBS), 100 µl of this mixture taken and mixed with 900 µl PBS etc.) and 100 µl of each chosen final dilution was evenly spread onto Mueller-Hinton agar plates containing vancomycin and trimethoprim (Table 2.1). Plates were incubated for up to 5 days until single colony growth was clear enough to count.

### Raw Data

#### 0 h

Strain	Condition	Dilution Factor			
		$10^1$	$10^2$	$10^3$	$10^4$
NCTC 11168	Unsupplemented		~12-15,000	~12-1500	120
	MEM $\alpha$ (no iron)				
	10 µM FeSO <sub>4</sub>		~14,000	~1400	125
	0.27 µM human ferri-lactoferrin (Lf)		~13-15,000	~12-1400	123
	0.27 µM human ferri-transferrin (Tf)		~14,000	~1300	117
	0.27 µM ferri-ovotransferrin (ovo-Tf)		~14,000	~13-1400	139
	0.27 µM human apo-Lf		~14,000	~1300	128
	0.27 µM human apo-Tf		~13,000	~13-1500	132
	0.27 µM apo-ovo-Tf		~13-15000	~1300	119

#### 8 h

Strain	Condition	Dilution Factor					
		$10^1$	$10^2$	$10^3$	$10^4$	$10^5$	$10^6$
NCTC 11168	Unsupplemented	~25,000	~2-3000	214			
	MEM $\alpha$ (no iron)						
	10 µM FeSO <sub>4</sub>				~6-7000	~600	69
	0.27 µM human ferri-Lf				~6-7000	~6-700	72
	0.27 µM human ferri-Tf				~6-7000	~6-700	70
	0.27 µM ferri-ovo-Tf				~6-7000	~6-700	75
	0.27 µM human apo-Lf	~20,000	~2000	246			
	0.27 µM human apo-Tf	45	5	0			
	0.27 µM apo-ovo-Tf	48	4	0			

**24 h**

Strain	Condition	Dilution Factor						
		Neat	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
NCTC 11168	Unsupplemented	~700	74	6				
	MEM $\alpha$ (no iron)							
	10 $\mu$ M FeSO <sub>4</sub>					~1000	100	7
	0.27 $\mu$ M human ferri-Lf					~1000	119	15
	0.27 $\mu$ M human ferri-Tf					~1000	98	9
	0.27 $\mu$ M ferri-ovo-Tf					~1000	102	10
	0.27 $\mu$ M human apo-Lf	~20,000	~2000	282				
	0.27 $\mu$ M human apo-Tf	32	1	0				
	0.27 $\mu$ M apo-ovo-Tf	34	2	0				

**0 h**

Strain	Condition	Dilution Factor			
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>
81-176	Unsupplemented		~15,000	~14-1500	143
	MEM $\alpha$ (no iron)				
	10 $\mu$ M FeSO <sub>4</sub>		~15,000	~1500	155
	0.27 $\mu$ M human ferri-Lf		~14,000	~13-1400	130
	0.27 $\mu$ M human ferri-Tf		~14,000	~13-1400	131
	0.27 $\mu$ M ferri-ovo-Tf		~14,000	~13-1400	145

**8 h**

Strain	Condition	Dilution Factor					
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
81-176	Unsupplemented	~20,000	~1500 - 2000	203			
	MEM $\alpha$ (no iron)						
	10 $\mu$ M FeSO <sub>4</sub>				~6-7000	~6-700	68
	0.27 $\mu$ M human ferri-Lf				~7000	~6-700	79
	0.27 $\mu$ M human ferri-Tf				~6000	~600	66
	0.27 $\mu$ M ferri-ovo-Tf				~7000	~6-700	71

**24 h**

Strain	Condition	Dilution Factor						
		Neat	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
81-176	Unsupplemented MEMα (no iron)	~7-800	82	5				
	10 μM FeSO <sub>4</sub>					~1000	112	10
	0.27 μM human ferri-Lf					~1000	124	12
	0.27 μM human ferri-Tf					~1000	119	8
	0.27 μM ferri-ovo-Tf					~1000	129	9

**0 h**

Strain	Condition	Dilution Factor			
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>
JDR5 (Δ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEMα (no iron)		~15,000	~13-1500	159
	10 μM FeSO <sub>4</sub>		~17-20,000	~17-2000	170
	0.27 μM human ferri-Lf		~15-17,000	~15-1700	162

**8 h**

Strain	Condition	Dilution Factor					
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
JDR5 (Δ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEMα (no iron)	~25,000	~2-3000	220			
	10 μM FeSO <sub>4</sub>				~6-7000	~6-700	73
	0.27 μM human ferri-Lf				~6-7000	~6-700	77

**24 h**

Strain	Condition	Dilution Factor						
		Neat	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
JDR5 (Δ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEMα (no iron)	~5-600	51	5				
	10 μM FeSO <sub>4</sub>					~1000	102	9
	0.27 μM human ferri-Lf					~1000-1200	126	11

**0 h**

Strain	Condition	Dilution Factor			
		$10^1$	$10^2$	$10^3$	$10^4$
KAR3 ( <i>p19</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)		~14,000	~13-1400	135
	10 $\mu$ M FeSO <sub>4</sub>		~13-14,000	~13-1400	142
	0.27 $\mu$ M human ferri- Lf		~12-13,000	~1300	127

**8 h**

Strain	Condition	Dilution Factor					
		$10^1$	$10^2$	$10^3$	$10^4$	$10^5$	$10^6$
KAR3 ( <i>p19</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	~20,000	~2000	203			
	10 $\mu$ M FeSO <sub>4</sub>				~6000	~600	60
	0.27 $\mu$ M human ferri- Lf				~6000	~600	63

**24 h**

Strain	Condition	Dilution Factor						
		Neat	$10^1$	$10^2$	$10^3$	$10^4$	$10^5$	$10^6$
KAR3 ( <i>p19</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	~500	47	3				
	10 $\mu$ M FeSO <sub>4</sub>					~1000	98	7
	0.27 $\mu$ M human ferri- Lf					~1000	101	10

**0 h**

Strain	Condition	Dilution Factor			
		$10^1$	$10^2$	$10^3$	$10^4$
KAR2 ( <i>cj0178</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)		~16,000	~15-1700	169
	10 $\mu$ M FeSO <sub>4</sub>		~15-17,000	~15-1700	152
	0.27 $\mu$ M human ferri- Lf		~15-17,000	~15-1700	159
	0.27 $\mu$ M human ferri- Tf		~15-17,000	~15-1700	162
	0.27 $\mu$ M ferri-ovo-Tf		~15-17,000	~15-1700	165



**8 h**

Strain	Condition	Dilution Factor					
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
KAR2 ( <i>cj0178</i> :: <i>aphA-3</i> ) 11168	Unsupplemented	~20,000	~2000	225			
	MEMα (no iron)						
	10 μM FeSO <sub>4</sub>				~6000	~6-700	76
	0.27 μM human ferri-Lf	~6000	~600	65			
	0.27 μM human ferri-Tf				~6-700	67	5
	0.27 μM ferri-ovo-Tf	~9000	~8-1000	104			

**24 h**

Strain	Condition	Dilution Factor						
		Neat	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
KAR2 ( <i>cj0178</i> :: <i>aphA-3</i> ) 11168	Unsupplemented	~600	61	5				
	MEMα (no iron)							
	10 μM FeSO <sub>4</sub>					~800	86	8
	0.27 μM human ferri-Lf		~1000	115	13			
	0.27 μM human ferri-Tf			~800	81	8		
	0.27 μM ferri-ovo-Tf		~2000	208	21			

**0 h**

Strain	Condition	Dilution Factor			
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>
CEM3 ( <i>Δcj0174c</i> :: <i>aphA-3</i> ) 11168	Unsupplemented		~15-20,000	~2000	171
	MEMα (no iron)				
	10 μM FeSO <sub>4</sub>		~20,000	~17-2000	175
	0.27 μM human ferri-Lf		~20,000	~17-2000	178

**8 h**

Strain	Condition	Dilution Factor					
		10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>
CEM3 ( <i>Δcj0174c</i> :: <i>aphA-3</i> ) 11168	Unsupplemented	~20,000	~2000	194			
	MEMα (no iron)						
	10 μM FeSO <sub>4</sub>				~7000	~6-700	79
	0.27 μM human ferri-Lf				~5000	~5-600	56

**24 h**

<b>Strain</b>	<b>Condition</b>	<b>Dilution Factor</b>						
		<b>Neat</b>	<b>10<sup>1</sup></b>	<b>10<sup>2</sup></b>	<b>10<sup>3</sup></b>	<b>10<sup>4</sup></b>	<b>10<sup>5</sup></b>	<b>10<sup>6</sup></b>
CEM3 ( <i>Δcj0174c</i> :: <i>aphA-3</i> ) 11168	Unsupplemented	~1500	158	15				
	MEM $\alpha$ (no iron)							
	10 $\mu$ M FeSO <sub>4</sub>					~10- 1300	136	15
	0.27 $\mu$ M human ferri- Lf					~1000	103	9

**Correction** = 100 µl plated, so multiply value by 10 to increase to cells in original 1 ml volume.

**Correction** = Multiply value by dilution factor, i.e. a value from a dilution of  $10^4$  needs to be multiplied by 10000 to calculate the original number of cells in the sample prior to dilution.

### Viable Count Calculations

#### 0 h

Strain	Condition	Original number of viable cells
NCTC 11168	Unsupplemented MEMα (no iron)	$120 \times 10$ (vol correction) $\times 10^4$ (dilution factor) = $1.2 \times 10^7$
	10 µM FeSO <sub>4</sub>	$125 \times 10 \times 10^4 = 1.25 \times 10^7$
	0.27 µM human ferri-Lf	$123 \times 10 \times 10^4 = 1.23 \times 10^7$
	0.27 µM human ferri-Tf	$117 \times 10 \times 10^4 = 1.17 \times 10^7$
	0.27 µM ferri-ovo-Tf	$139 \times 10 \times 10^4 = 1.39 \times 10^7$
	0.27 µM human apo-Lf	$128 \times 10 \times 10^4 = 1.28 \times 10^7$
	0.27 µM human apo-Tf	$132 \times 10 \times 10^4 = 1.32 \times 10^7$
	0.27 µM apo-ovo-Tf	$119 \times 10 \times 10^4 = 1.19 \times 10^7$

#### 8 h

Strain	Condition	Original number of viable cells
NCTC 11168	Unsupplemented MEMα (no iron)	$214 \times 10 \times 10^3 = 2.14 \times 10^6$
	10 µM FeSO <sub>4</sub>	$69 \times 10 \times 10^6 = 6.9 \times 10^8$
	0.27 µM human ferri-Lf	$72 \times 10 \times 10^6 = 7.2 \times 10^8$
	0.27 µM human ferri-Tf	$70 \times 10 \times 10^6 = 7 \times 10^8$
	0.27 µM ferri-ovo-Tf	$75 \times 10 \times 10^6 = 7.5 \times 10^8$
	0.27 µM human apo-Lf	$246 \times 10 \times 10^3 = 2.46 \times 10^6$
	0.27 µM human apo-Tf	$45 \times 10 \times 10^1 = 4.5 \times 10^3$
	0.27 µM apo-ovo-Tf	$48 \times 10 \times 10^1 = 4.8 \times 10^3$

**24 h**

<b>Strain</b>	<b>Condition</b>	<b>Original number of viable cells</b>
NCTC 11168	Unsupplemented MEM $\alpha$ (no iron)	$74 \times 10 \times 10^1 = 7.4 \times 10^3$
	10 $\mu$ M FeSO <sub>4</sub>	$100 \times 10 \times 10^5 = 1 \times 10^8$
	0.27 $\mu$ M human ferri- Lf	$119 \times 10 \times 10^5 = 1.19 \times 10^8$
	0.27 $\mu$ M human ferri- Tf	$98 \times 10 \times 10^5 = 9.8 \times 10^7$
	0.27 $\mu$ M ferri-ovo-Tf	$102 \times 10 \times 10^5 = 1.02 \times 10^8$
	0.27 $\mu$ M human apo- Lf	$282 \times 10 \times 10^2 = 2.82 \times 10^5$
	0.27 $\mu$ M human apo- Tf	$32 \times 10 = 3.2 \times 10^2$
	0.27 $\mu$ M apo-ovo-Tf	$34 \times 10 = 3.4 \times 10^2$

**0 h**

<b>Strain</b>	<b>Condition</b>	<b>Original number of viable cells</b>
81-176	Unsupplemented MEM $\alpha$ (no iron)	$143 \times 10 \times 10^4 = 1.43 \times 10^7$
	10 $\mu$ M FeSO <sub>4</sub>	$155 \times 10 \times 10^4 = 1.55 \times 10^7$
	0.27 $\mu$ M human ferri- Lf	$130 \times 10 \times 10^4 = 1.30 \times 10^7$
	0.27 $\mu$ M human ferri- Tf	$131 \times 10 \times 10^4 = 1.31 \times 10^7$
	0.27 $\mu$ M ferri-ovo-Tf	$145 \times 10 \times 10^4 = 1.45 \times 10^7$

**8 h**

<b>Strain</b>	<b>Condition</b>	<b>Original number of viable cells</b>
81-176	Unsupplemented MEM $\alpha$ (no iron)	$203 \times 10 \times 10^3 = 2.03 \times 10^5$
	10 $\mu$ M FeSO <sub>4</sub>	$68 \times 10 \times 10^6 = 6.8 \times 10^8$
	0.27 $\mu$ M human ferri- Lf	$79 \times 10 \times 10^6 = 7.9 \times 10^8$
	0.27 $\mu$ M human ferri- Tf	$66 \times 10 \times 10^6 = 6.6 \times 10^8$
	0.27 $\mu$ M ferri-ovo-Tf	$71 \times 10 \times 10^6 = 7.1 \times 10^8$

**24 h**

Strain	Condition	Original number of viable cells
81-176	Unsupplemented MEM $\alpha$ (no iron)	$82 \times 10 \times 10^1 = 8.2 \times 10^3$
	10 $\mu$ M FeSO <sub>4</sub>	$112 \times 10 \times 10^5 = 1.12 \times 10^8$
	0.27 $\mu$ M human ferri-Lf	$124 \times 10 \times 10^5 = 1.24 \times 10^8$
	0.27 $\mu$ M human ferri-Tf	$119 \times 10 \times 10^5 = 1.19 \times 10^8$
	0.27 $\mu$ M ferri-ovo-Tf	$129 \times 10 \times 10^5 = 1.29 \times 10^8$

**0 h**

Strain	Condition	Original number of viable cells
JDR5 ( $\Delta$ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEM $\alpha$ (no iron)	$159 \times 10 \times 10^4 = 1.59 \times 10^7$
	10 $\mu$ M FeSO <sub>4</sub>	$170 \times 10 \times 10^4 = 1.70 \times 10^7$
	0.27 $\mu$ M human ferri-Lf	$162 \times 10 \times 10^4 = 1.62 \times 10^7$

**8 h**

Strain	Condition	Original number of viable cells
JDR5 ( $\Delta$ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEM $\alpha$ (no iron)	$220 \times 10 \times 10^3 = 2.2 \times 10^6$
	10 $\mu$ M FeSO <sub>4</sub>	$73 \times 10 \times 10^6 = 7.3 \times 10^8$
	0.27 $\mu$ M human ferri-Lf	$77 \times 10 \times 10^6 = 7.7 \times 10^8$

**24 h**

Strain	Condition	Original number of viable cells
JDR5 ( $\Delta$ <i>chuA</i> :: <i>cat</i> ) 11168	Unsupplemented MEM $\alpha$ (no iron)	$51 \times 10 \times 10^1 = 5.1 \times 10^3$
	10 $\mu$ M FeSO <sub>4</sub>	$102 \times 10 \times 10^5 = 1.02 \times 10^8$
	0.27 $\mu$ M human ferri-Lf	$126 \times 10 \times 10^5 = 1.26 \times 10^8$

**0 h**

Strain	Condition	Original number of viable cells
KAR3 ( <i>p19</i> :: <i>aphA</i> -3) 11168	Unsupplemented MEM $\alpha$ (no iron)	$135 \times 10 \times 10^4 = 1.35 \times 10^7$
	10 $\mu$ M FeSO <sub>4</sub>	$142 \times 10 \times 10^4 = 1.42 \times 10^7$
	0.27 $\mu$ M human ferri-Lf	$127 \times 10 \times 10^4 = 1.27 \times 10^7$

**8 h**

Strain	Condition	Original number of viable cells
KAR3 ( <i>p19</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	$203 \times 10 \times 10^3 = 2.03 \times 10^6$
	10 $\mu$ M FeSO <sub>4</sub>	$60 \times 10 \times 10^6 = 6 \times 10^8$
	0.27 $\mu$ M human ferri- Lf	$63 \times 10 \times 10^6 = 6.3 \times 10^8$

**24 h**

Strain	Condition	Original number of viable cells
KAR3 ( <i>p19</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	$47 \times 10 \times 10^1 = 4.7 \times 10^3$
	10 $\mu$ M FeSO <sub>4</sub>	$98 \times 10 \times 10^5 = 9.8 \times 10^7$
	0.27 $\mu$ M human ferri- Lf	$101 \times 10 \times 10^5 = 1.01 \times 10^8$

**0 h**

Strain	Condition	Original number of viable cells
KAR2 ( <i>cj0178</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	$169 \times 10 \times 10^4 = 1.69 \times 10^7$
	10 $\mu$ M FeSO <sub>4</sub>	$152 \times 10 \times 10^4 = 1.52 \times 10^7$
	0.27 $\mu$ M human ferri- Lf	$159 \times 10 \times 10^4 = 1.59 \times 10^7$
	0.27 $\mu$ M human ferri- Tf	$162 \times 10 \times 10^4 = 1.62 \times 10^7$
	0.27 $\mu$ M ferri-ovo-Tf	$165 \times 10 \times 10^4 = 1.65 \times 10^7$

**8 h**

Strain	Condition	Original number of viable cells
KAR2 ( <i>cj0178</i> :: <i>aphA</i> - 3) 11168	Unsupplemented MEM $\alpha$ (no iron)	$225 \times 10 \times 10^3 = 2.25 \times 10^6$
	10 $\mu$ M FeSO <sub>4</sub>	$76 \times 10 \times 10^6 = 7.6 \times 10^8$
	0.27 $\mu$ M human ferri- Lf	$65 \times 10 \times 10^3 = 6.5 \times 10^5$
	0.27 $\mu$ M human ferri- Tf	$67 \times 10 \times 10^5 = 6.7 \times 10^7$
	0.27 $\mu$ M ferri-ovo-Tf	$104 \times 10 \times 10^3 = 1.04 \times 10^6$

**24 h**

Strain	Condition	Original number of viable cells
KAR2 ( <i>cj0178</i> ::aphA- 3) 11168	Unsupplemented	$61 \times 10 \times 10^1 = 6.1 \times 10^3$
	MEM $\alpha$ (no iron)	
	10 $\mu$ M FeSO <sub>4</sub>	$86 \times 10 \times 10^5 = 8.6 \times 10^7$
	0.27 $\mu$ M human ferri-Lf	$115 \times 10 \times 10^2 = 1.15 \times 10^5$
	0.27 $\mu$ M human ferri-Tf	$81 \times 10 \times 10^3 = 8.1 \times 10^5$
	0.27 $\mu$ M ferri-ovo-Tf	$208 \times 10 \times 10^2 = 2.08 \times 10^5$

**0 h**

Strain	Condition	Original number of viable cells
CEM3 ( $\Delta$ <i>cj0174c</i> ::aphA-3) 11168	Unsupplemented	$171 \times 10 \times 10^4 = 1.71 \times 10^7$
	MEM $\alpha$ (no iron)	
	10 $\mu$ M FeSO <sub>4</sub>	$175 \times 10 \times 10^4 = 1.75 \times 10^7$
	0.27 $\mu$ M human ferri-Lf	$178 \times 10 \times 10^4 = 1.78 \times 10^7$

**8 h**

Strain	Condition	Original number of viable cells
CEM3 ( $\Delta$ <i>cj0174c</i> ::aphA-3) 11168	Unsupplemented	$194 \times 10 \times 10^3 = 1.94 \times 10^6$
	MEM $\alpha$ (no iron)	
	10 $\mu$ M FeSO <sub>4</sub>	$79 \times 10 \times 10^6 = 7.9 \times 10^8$
	0.27 $\mu$ M human ferri-Lf	$56 \times 10 \times 10^6 = 5.6 \times 10^8$

**24 h**

Strain	Condition	Original number of viable cells
CEM3 ( $\Delta$ <i>cj0174c</i> ::aphA-3) 11168	Unsupplemented	$158 \times 10 \times 10^1 = 1.58 \times 10^4$
	MEM $\alpha$ (no iron)	
	10 $\mu$ M FeSO <sub>4</sub>	$136 \times 10 \times 10^5 = 1.36 \times 10^8$
	0.27 $\mu$ M human ferri-Lf	$103 \times 10 \times 10^5 = 1.03 \times 10^8$

## **Appendix 6.**

### **Conference Abstracts and Papers**

#### Conference attendance:

- **2006 Canadian *Campylobacter* Conference**, November 2<sup>nd</sup>-3<sup>rd</sup>, 2006, Vancouver, British Columbia, oral and poster presentation.
- **American Society for Microbiology (ASM) 107<sup>th</sup> General Meeting**, May 21<sup>st</sup>-25<sup>th</sup>, 2007, Toronto, Ontario, Canada, poster presentation.
- **14<sup>th</sup> International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (CHRO)**, September 2<sup>nd</sup>-5<sup>th</sup>, 2007, Rotterdam, The Netherlands, poster presentation.

#### Papers

- **Miller, C. E.**, Rock, J. D., Ridley, K. A., Williams, P. H. and Ketley, J. M. (2008). Utilization of lactoferrin-bound and transferrin-bound iron by *Campylobacter jejuni*. *Journal of Bacteriology* **190**, 1900-1911.



**2006 Canadian *Campylobacter* Conference**

**Characterization of a lactoferrin and transferrin-bound iron uptake system in *Campylobacter jejuni***

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A major determinant of intestinal colonisation by *Campylobacter jejuni* is iron acquisition. Haem/haemoglobin supports *C. jejuni* NCTC11168 growth under iron-limited conditions (*chuABCD*) and NCTC11168 can acquire iron from enterochelin by siderophore piracy (*cfrA*, *ceuBCDE*). There are two additional uncharacterised iron uptake systems in the NCTC11168 genome, Cj1658-Cj1663 and Cj0173c-Cj0178, but no siderophore biosynthesis capability. Brucella agar plate assays supplemented with lactoferrin (Lf) or transferrin (Tf)-bound iron cannot sustain growth, therefore *C. jejuni* was considered incapable of exploiting this potential iron source. Using an established defined iron-limited medium (MEM $\alpha$ ), NCTC11168 growth with holo-Lf and holo-Tf was reviewed. Growth of NCTC11168 in unsupplemented MEM $\alpha$  was poor, while the addition of at least 2 $\mu$ M FeSO<sub>4</sub> stimulated growth. Holo-Lf, holo-Tf or holo-conalbumin (Ca) enabled growth to levels comparable with FeSO<sub>4</sub>. In contrast, supplementation of MEM $\alpha$  with equivalent concentrations of apo-Lf, apo-Tf or apo-Ca resulted in growth levels lower than unsupplemented medium. Competition between apo- and holo-protein resulted in decreased growth compared to holo-protein alone and growth was not inhibited when BSA was competed against each holo-protein thus iron acquisition from Lf, Tf and Ca appears to be specific. Defined mutants in all of the major iron uptake systems (*chuA*, *cfrA*, p19, Cj0178) were assayed for growth in MEM $\alpha$  with holo-Lf; only the Cj0178 mutant demonstrated attenuated growth compared to wildtype under these conditions. Deletion mutants in the adjacent ABC transporter system (Cj0173c-Cj0174c) showed no effect when grown in MEM $\alpha$  with holo-Lf. Iron uptake was reduced by partitioning the holo-protein away from NCTC11168 implying that the acquisition of iron from Lf may be contact dependent. In conclusion, we have demonstrated that NCTC11168 can acquire iron from members of the Tf family, further highlighting the diversity of iron sources that *C. jejuni* can successfully exploit in the host environment.

**American Society for Microbiology (ASM) 107<sup>th</sup> General Meeting**

***Campylobacter jejuni* Utilizes Lactoferrin and Transferrin-Bound Iron for Growth**

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**Background:** Iron acquisition is vital for intestinal colonization by *Campylobacter jejuni*. Under iron-limited conditions, a range of iron sources are capable of sustaining *C. jejuni* NCTC11168 growth, including haem/haemoglobin (*chuABCD*) and ferric-enterochelin (*cfrA*, *ceuBCDE*). There are two additional uncharacterized iron uptake systems in the NCTC11168 genome, Cj1658-Cj1663 and Cj0173c-Cj0178. As Brucella agar plate assays supplemented with lactoferrin (Lf) or transferrin (Tf)-bound iron cannot sustain growth, *C. jejuni* was considered incapable of exploiting this potential iron source. Using a defined iron-limited medium (MEM $\alpha$ ), NCTC11168 growth with holo-Lf and holo-Tf was reviewed. **Methods:** *C. jejuni* strains were grown microaerobically in MEM $\alpha$  at 37°C with agitation over 24 h. Growth was assayed in MEM $\alpha$  with 10  $\mu$ M FeSO<sub>4</sub> or with holo/apo- Lf, Tf or ovo-transferrin (Ovo-Tf) to concentrations of 0.27  $\mu$ M or 1.11  $\mu$ M. Growth was also analyzed with competing concentrations of apo- and holo-proteins (3:1 ratio) and with the bacteria partitioned away from the holo-proteins. **Results:** Growth of NCTC11168 in unsupplemented MEM $\alpha$  was poor. Holo-Lf, holo-Tf or holo-Ovo-Tf enabled growth to levels comparable with FeSO<sub>4</sub>. Supplementation of MEM $\alpha$  with apo-Lf, apo-Tf or apo-Ovo-Tf resulted in growth levels lower than unsupplemented MEM $\alpha$ . Competition (apo- vs. holo-protein) decreased growth, but growth was not inhibited when BSA was competed against each holo-protein. Defined major iron uptake system mutants (*chuA*, *cfrA*, p19, Cj0178) were grown in MEM $\alpha$  with holo-Lf. Cj0178 demonstrated the most significantly attenuated growth compared to wildtype and deletion mutants in the adjacent ABC transporter system (Cj0173c-Cj0174c) showed a small but significant effect. Growth was reduced by partitioning the holo-Lf, holo-Tf or holo-Ovo-Tf away from NCTC11168. **Conclusions:** *C. jejuni* NCTC11168 can acquire iron from members of the Tf family for growth. Iron acquisition from holo-Lf, holo-Tf and holo-Ovo-Tf appears to be a receptor specific, contact dependent process. The phenotype observed indicates a primary role for Cj0178 in this process.

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**The acquisition of iron from lactoferrin and transferrin for growth by *Campylobacter jejuni* is enhanced in the presence of norepinephrine**

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The iron sources haem (*chuABCD*), and ferric-enterochelin (*cfrA*, *ceuBCDE*) sustain *Campylobacter jejuni* NCTC 11168 during colonisation in the iron-limited host. Within the genome, Cj1658-Cj1663 and Cj0173c-Cj0178 encode putative iron-uptake systems, but there are no apparent siderophore biosynthesis genes. Lactoferrin (Lf) and transferrin (Tf)-bound iron cannot sustain NCTC 11168 growth in Brucella agar plate assays. Using a defined iron-limited medium (MEM $\alpha$ ), NCTC 11168 growth with holo-Lf and holo-Tf was reviewed. Growth in unsupplemented MEM $\alpha$  was poor, with the addition of at least 2  $\mu$ M FeSO<sub>4</sub> promoting growth. Holo-Lf, holo-Tf or holo-ovotransferrin (Ovo-Tf) (0.27 $\mu$ M), stimulated growth to levels comparable with FeSO<sub>4</sub>. Equivalent concentrations of apo-Lf, apo-Tf or apo-Ovo-Tf limited growth to levels below unsupplemented medium. Competition (apo- vs. holo-protein) decreased growth, but no growth inhibition occurred when BSA was competed against each holo-protein, suggesting receptor specificity. Iron uptake was significantly reduced by separating the holo-protein from NCTC 11168 within dialysis membrane, indicating that iron acquisition from Lf, Tf and Ovo-Tf is contact dependent. Of the defined major iron-uptake system mutants (*chuA*, *cfrA*, p19, Cj0178) grown in MEM $\alpha$  with holo-Lf, Cj0178 demonstrated the most significantly reduced growth compared to wildtype. A deletion mutant in the adjacent ABC transporter system (Cj0174c) showed a small but significant decrease, indicating a role for Cj0173c-Cj0178 in this process. Under iron-limited conditions, the catecholamine neurotransmitter norepinephrine (NE) forms complexes with holo-Lf and holo-Tf, liberating tightly bound iron for *Escherichia coli* growth. The addition of NE (100  $\mu$ M) to unsupplemented MEM $\alpha$  significantly increased NCTC 11168 growth. This increase was not significant under iron-replete conditions. Addition of NE to holo-Lf, holo-Tf or holo-Ovo-Tf supplemented media also increased growth significantly. In conclusion, NCTC 11168 can acquire iron from members of the Tf protein family, with this process augmented by, but not dependent upon, the catecholamine hormone NE.