Table S1: Effect of temperature and formalin concentration on inactivation of *S. flexneri* 2a

Treatment (% formalin in HBSS)	Duration of Treatment with Formalin (hours)						
	0 hr	4 hr	8 hr	10 hr	24 hr	48 hr	72 hr
4°C, 0%	2.3×10^{10} [a]	G	G	2.3×10^{10}	3.1×10^{10}	$>6.0 \times 10^{10}$	$>6.0 \times 10^{10}$
4°C 0.2%	8.0×10^{9}	G	G	G	G	G	G
4°C, 0.4%	2.3×10^{10}	G	G	G	G	NG	NG
4°C, 1.0%	2.3×10^{10}	G	NG	NG	NG	NG	NG
25°C, 0%	8.0×10^{9}	8.0×10^{9}	8.0×10^{9}	8.0×10^{9}	7.0×10^{9}	5.0×10^{9}	6.0×10^{9}
25°C, 0.2%	8.0×10^{9}	G	G	NG	NG	NG	ND
25°C, 1.0%	8.0×10^{9}	NG	NG	NG	NG	NG	ND
37°C, 0%	2.3×10^{10}	G	G	1.7×10^{10}	3.1×10^{9}	5.0×10^{9}	3.5×10^{9}
37°C, 0.2%	2.3×10^{10}	NG	NG	NG	NG	NG	NG
37°C 0.2%	8.0×10^{9}	NG	NG	NG	NG	NG	NG

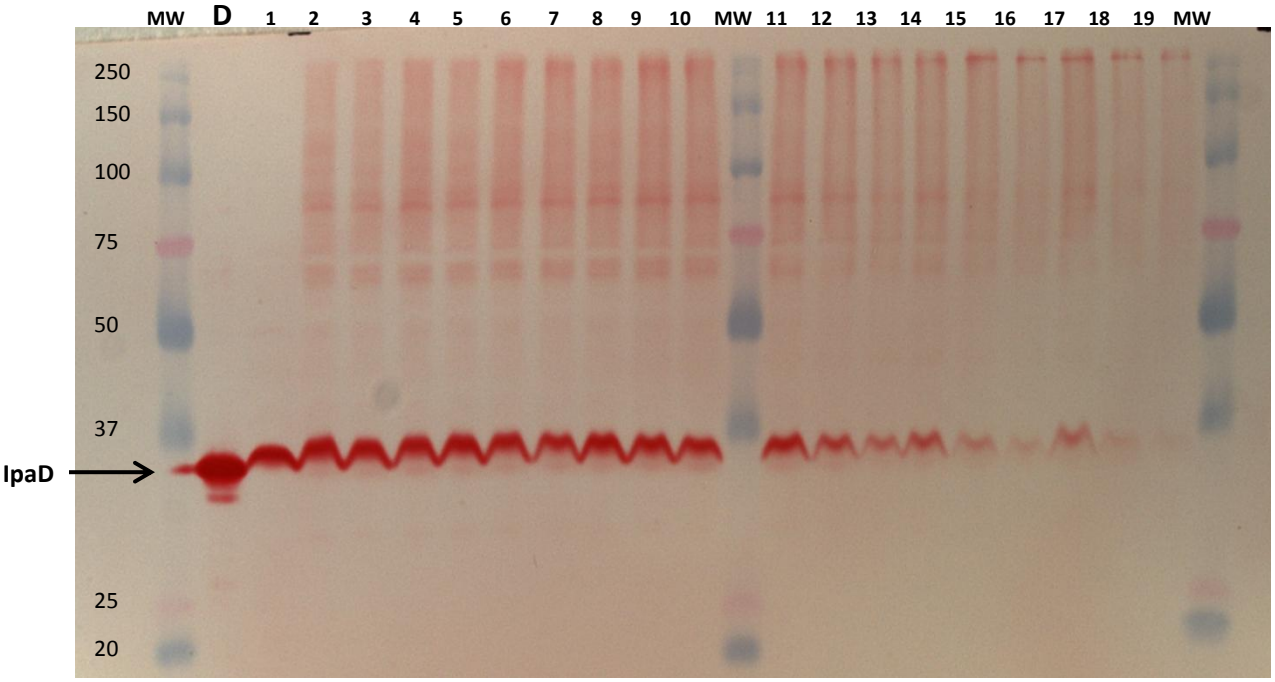
Abbreviations: G, growth; NG, no growth; ND, not done; HBSS, Hank's balanced salt solution
a: cfu/ml
S. flexneri 2a, strain 2457T cells collected from an overnight culture were washed and suspended in HBSS and subsequently incubated with various formalin concentrations for the indicated times and temperatures. At each timepoint a sample of the treated culture was either diluted to determine the titer of viable cells (cfu/ml) on TSA plates or plated directly (undiluted) on three TSA plates to determine if viable *Shigella* were present (G or NG). Inoculated plates were incubated at 37oC for 48 hrs.

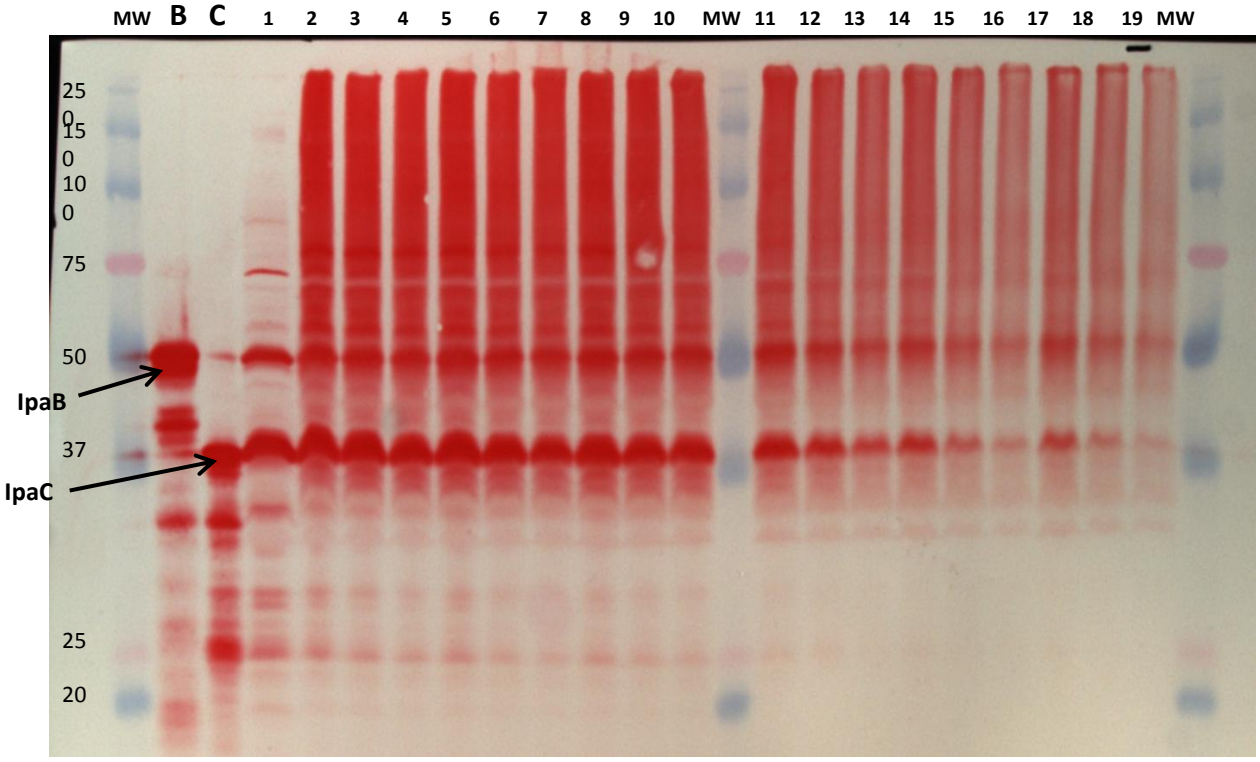
Table S2: Effect of formalin concentration on *S. sonnei* inactivation at 25^{o}C

Treatment	Viability of *S. sonnei* after various times (hrs) of incubation with formalin						
	0 hr	2 hr	4 hr	8 hr	24 hr	48 hr	72 hr
0% formalin	6×10^{9} [a]	8×10^{9}	7×10^{9}	6×10^{9}	8×10^{9}	6.0×10^{9}	5.0×10^{9}
0.2% formalin	6×10^{9}	G	G	G	G	G	G
0.6% formalin	6×10^{9}	G	NG	NG	NG	NG	NG
0.8% formalin	6×10^{9}	G	NG	NG	NG	NG	NG
1.0% formalin	6×10^{9}	NG	NG	NG	NG	NG	NG

Abbreviations: G, growth; NG, no growth; ND, not done; HBSS, Hank's balanced salt solution
a: cfu/ml
S. sonnei, strain Moseley cells collected from an overnight culture were washed and suspended in HBSS and subsequently incubated with various formalin concentrations for the indicated times at 25^{o}C. At each timepoint a sample of the treated culture was either diluted to determine the titer of viable cells (cfu/ml) on TSA plates or plated directly (undiluted) on three TSA plates to determine if viable *Shigella* were present (G or NG). Inoculated plates were incubated at 37^{o}C for 48 hrs.

Supplement Figure #1
Kaminski *et al*

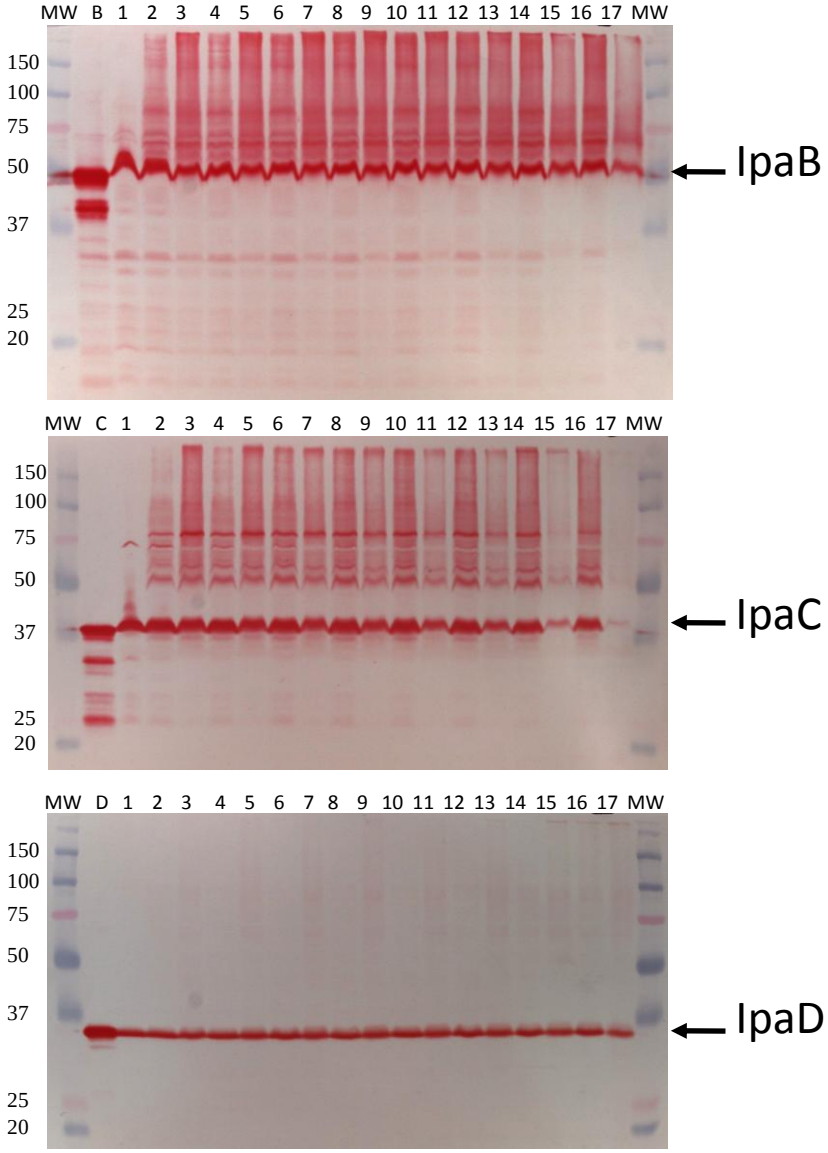




LEGEND

Lane 1: untreated
Lane 2: 0.6% formalin 4h
Lane 3: 0.8% formalin 4h
Lane 4: 1% formalin 4h
Lane 5: 0.6% formalin 8h
Lane 6: 0.8% formalin 8h
Lane 7: 1% formalin 8h
Lane 8: 0.6% formalin 12h
Lane 9: 0.8% formalin 12h
Lane 10: 1% formalin 12h
Lane 11: 0.6% formalin 24h
Lane 12: 0.8% formalin 24h
Lane 13: 1% formalin 24h
Lane 14: 0.6% formalin 48h
Lane 15: 0.8% formalin 48h
Lane 16: 1% formalin 48h
Lane 17: 0.6% formalin 72h
Lane 18: 0.8% formalin 72h
Lane 19: 1% formalin 72h
mw: molecular weight standards
B: IpaB; C: IpaC; D:IpaD

Supplement Figure 1. Western blot analysis of *S. sonnei* cells inactivated with different concentrations of formalin. *S. sonnei* cells were incubated with either 0.6%, 0.8%, or 1.0% formalin for different times up to 72 hrs. At indicated time point (0 or untreated, 4, 8, 12, 24, 48, and 72 hrs) samples were collected and whole cell lysates prepared for western blots for IpaD (top panel) or a combination blot with mAbs to IpaB and IpaC (bottom panel). Treated preparations (lanes 2-19) are compared to purified Ipa protein (indicated as lane B, C, or D) and untreated *S. sonnei* cells (lane 1). The same quantity of cells were added in lanes 1-20. At later time points and higher concentration of formalin (lanes 15 – 19) the reactivity of the Ipa mAbs with the specific Ipa proteins is reduced in the whole cell lysates. MAb reactivity with higher molecular weight bands is likely due to cross-linking events that occur upon incubation with formalin.



LEGEND

Lane 1: untreated
Lane 2: 0.2% formalin 2h
Lane 3: 0.6% formalin 2h
Lane 4: 0.2% formalin 4h
Lane 5: 0.6% formalin 4h
Lane 6: 0.2% formalin 6h
Lane 7: 0.4% formalin 6h
Lane 8: 0.2% formalin 8h
Lane 9: 0.6% formalin 8h
Lane 10: 0.2% formalin 10h
Lane 11: 0.6% formalin 10h
Lane 12: 0.2% formalin 12h
Lane 13: 0.6% formalin 12h
Lane 14: 0.2% formalin 24h
Lane 15: 0.6% formalin 24h
Lane 16: 0.2% formalin 48h
Lane 17: 0.6% formalin 48h

mw: molecular weight standards
B: IpaB; C: IpaC; D:IpaD

Supplement Figure 2. Western blot analysis of *S. flexneri* 3a cells inactivated with different concentrations of formalin. *S. flexneri* 3a cells were incubated with either 0.2% or 0.6% formalin for different times up to 48 hrs. At indicated time point (0 or untreated, 2, 4, 6, 8, 10, 12, 24, and 48 hrs) samples were collected and whole cell lysates prepared for western blots for IpaB, IpaC, and IpaD. Treated preparations (lanes 2-17) are compared to purified recombinant Ipa protein (indicated as lane B, C, or D) and untreated *S. flexneri* 3a cells (lane 1). The same quantities of cells were added in lanes 1-17. At later time points and higher concentration of formalin (lanes 15 and 17) the reactivity of the Ipa mAbs with the Ipa protein is reduced in the whole cell lysates. MAb reactivity with higher molecular weight bands is likely due to cross-linking events that occur upon incubation with formalin.