Non-typhoidal *Salmonella* (NTS) Core-O Polysaccharide (COPS) conjugated to the homologous flagellin as candidate vaccines for protection against invasive NTS infections

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Raphael Simon, PhD
Instructor and Head Antigen Purification Unit
Center for Vaccine Development
University of Maryland, Baltimore

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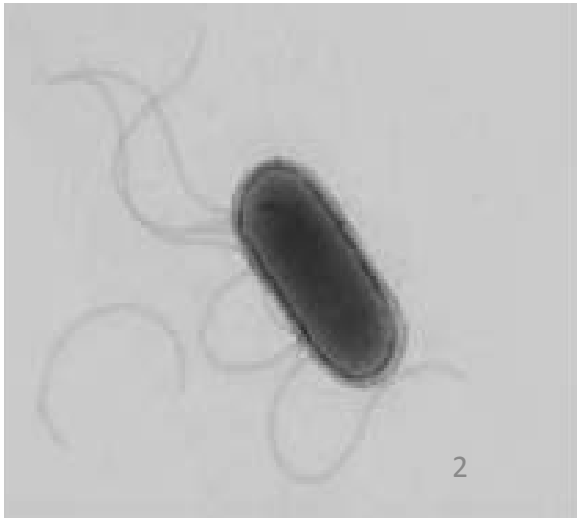
Salmonella COPS:Flagellin Conjugate

- Polysaccharide:Protein Conjugate Vaccines
  - Elicit immunologic memory (T dependent)
  - Immunogenic & protective in young infants (e.g. Hib, pneumo, MCV)

Components:

- **O polysaccharide** plus the **core polysaccharide** (**"COPS"**) of *Salmonella* lipopolysaccharide
- Homologous *Salmonella* Phase 1 **flagellin** subunits serve as the carrier protein
  - Target for immune responses
  - T helper epitopes and immunologic memory relate to carrier protein antigens found on the pathogen
  - Flagellin monomers activate TLR5



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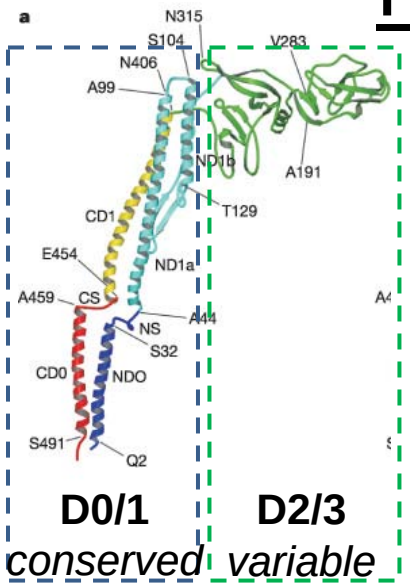
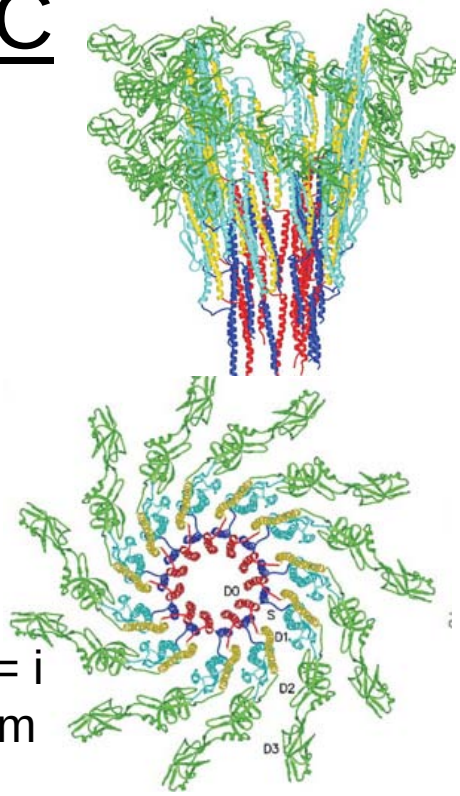
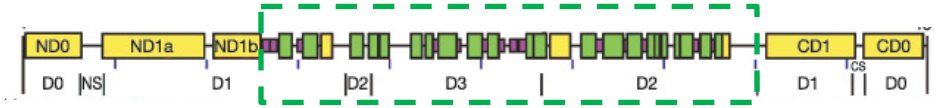
Salmonella COPS:Flagellin Conjugate

- End linkage on polysaccharide to multiple potential sites protein -> **sun-type**
- Random conjugation at multiple potential sites on protein & polysaccharide
  -> intermolecular bridges / **lattice-type**

FliC



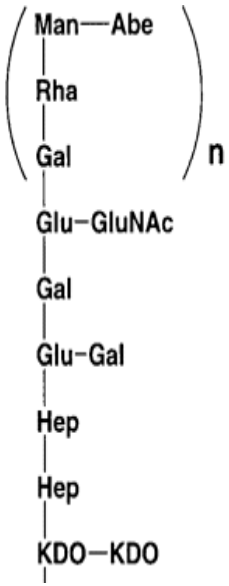




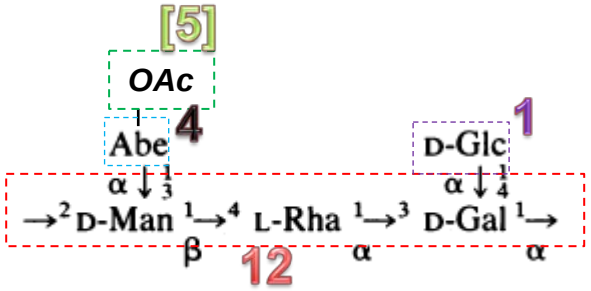
S. Typhimurium FliC = i
S. Enteritidis FliC = g,m

Namba, 2003

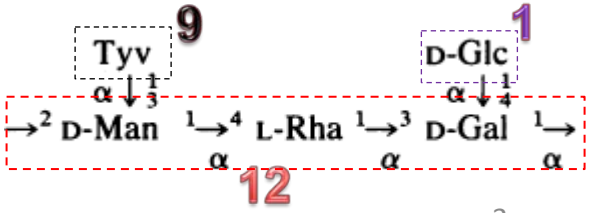
COPS



S. Typhimurium (B) – O:1,**4**,[5],12

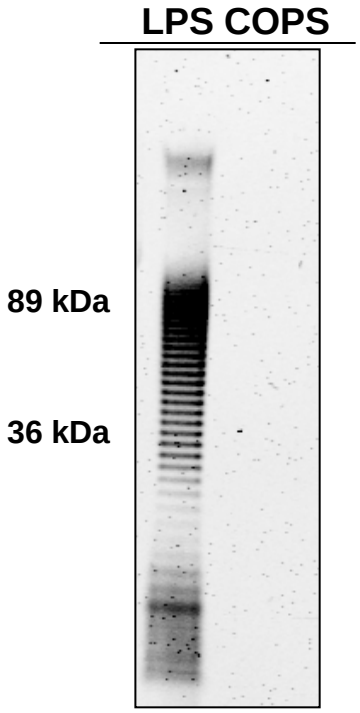


S. Enteritidis (D) – O:1,**9**,12

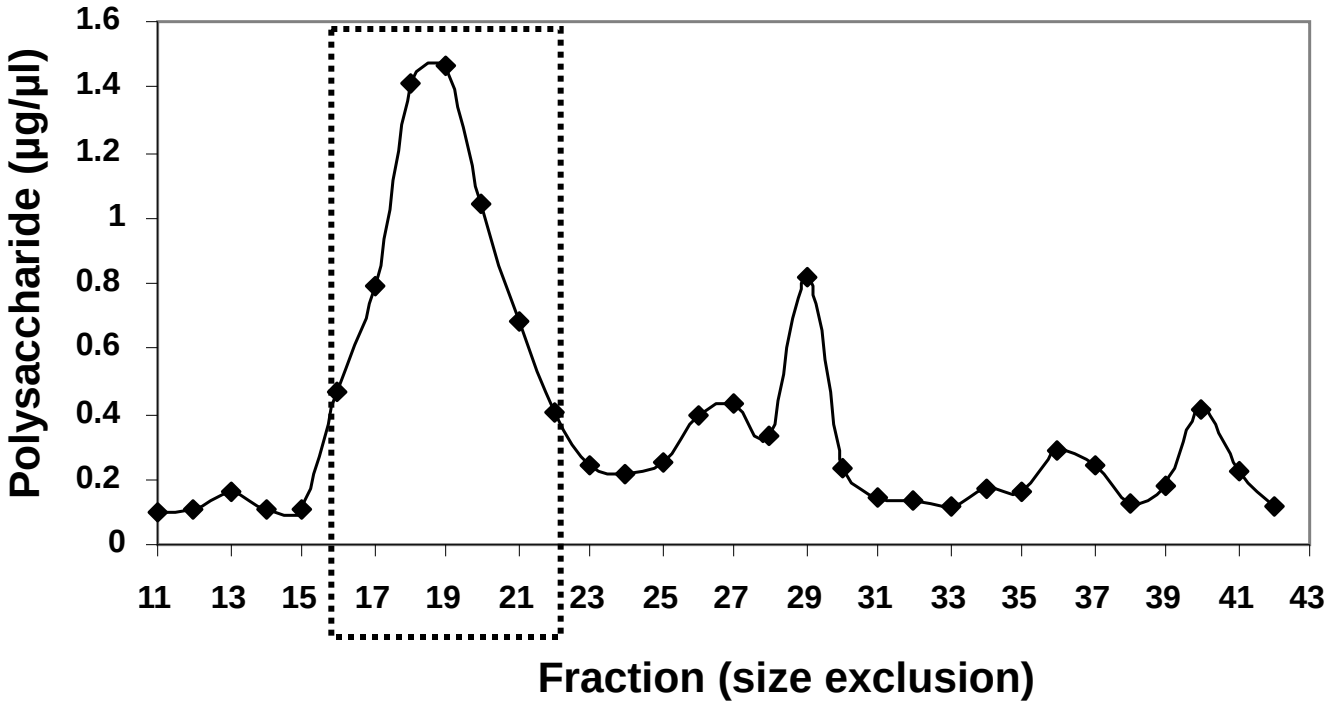


Purification of *S.* Enteritidis CVD 1941 *ΔguaBAΔclpP* LPS & high MW COPS

SDS-PAGE



SEC-FPLC purification of Core-OPS (lipid A removed)



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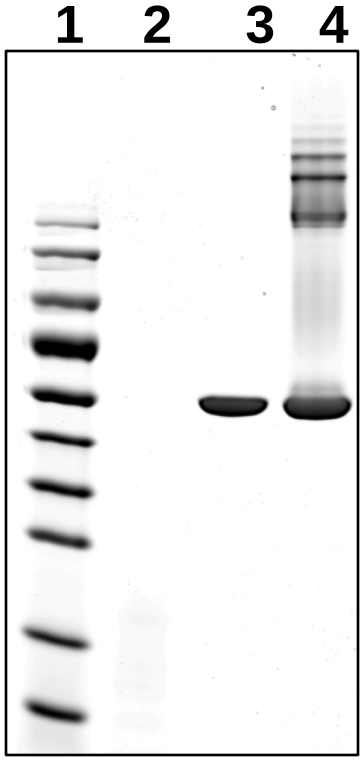
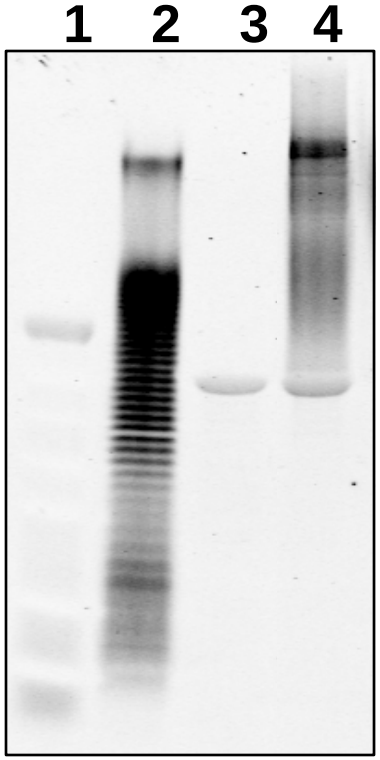
Starting material ➡ Rx with 1% acetic acid @ 100 C ➡ 100,000 x g

S. Enteritidis COPS:FliC conjugate



Coomassie stain (protein)



Pro-Q stain (polysaccharide)

Two-step purification
1. Size (SEC)
2. Charge (IEX)

1: Protein Standards
2: *S.* Enteritidis LPS
3: *S.* Enteritidis Flagella
5: COPS:FliC*

*1:1 conjugation ratio with CDAP

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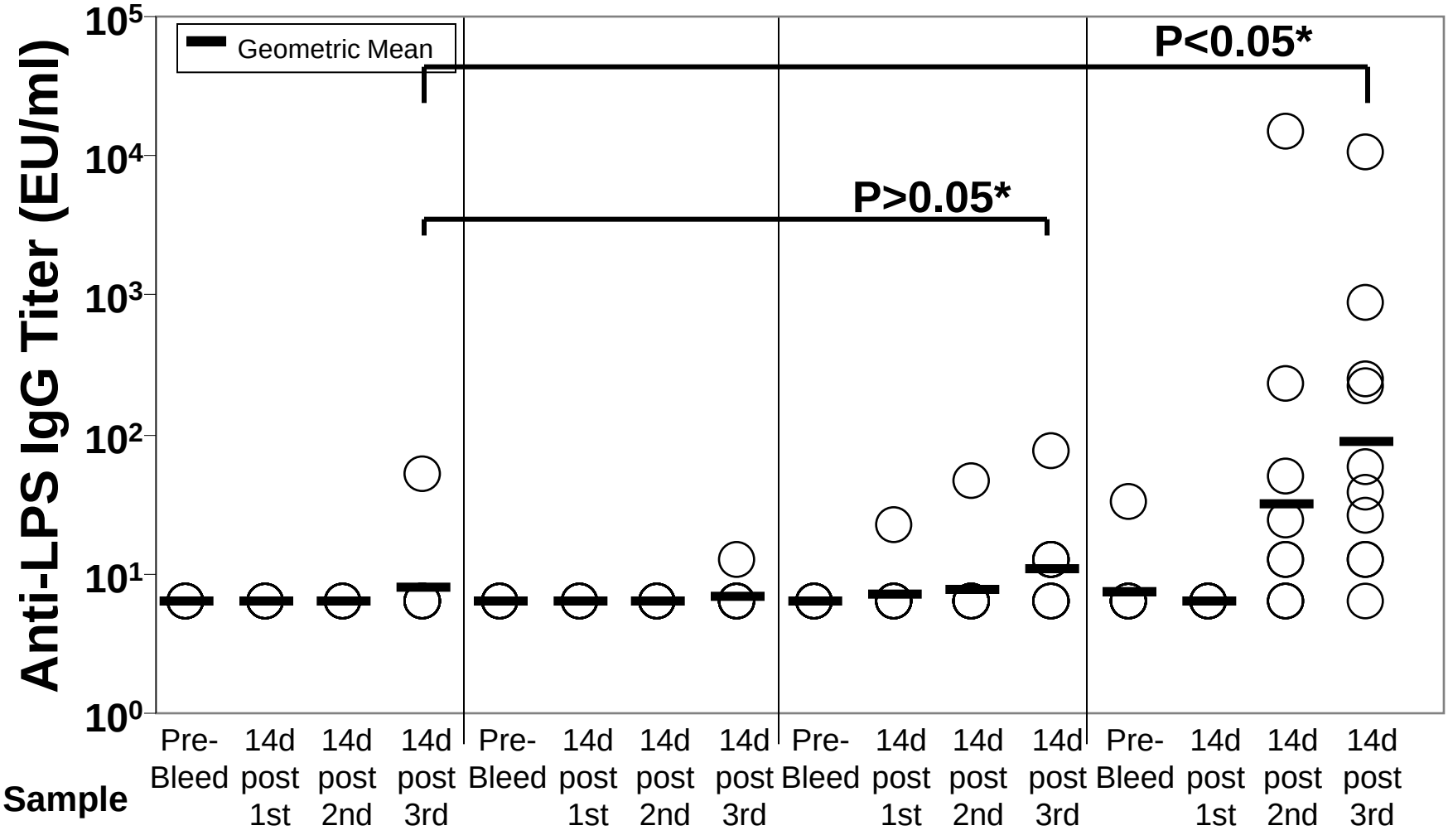
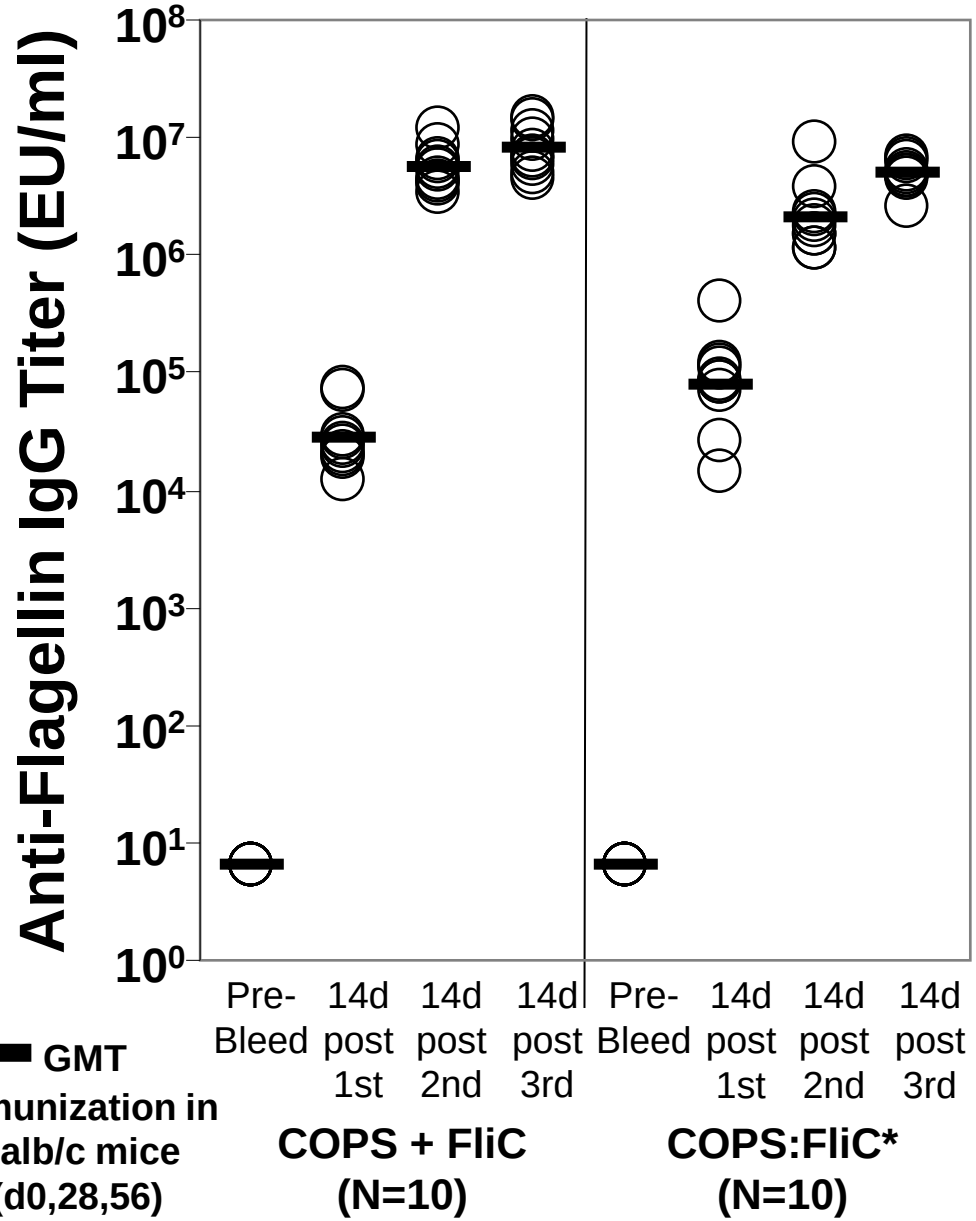
Immunogenicity in BALB/c mice of *S.* Enteritidis COPS:FliC Conjugate



*Mann-Whitney Rank-Sum test

**1:1 conjugation ratio with CDAP

Summary of immunogenicity and protection results obtained with flagellin in mice



Group [a]	Mortality[d]	Vaccine Efficacy[e]
PBS[b]	19/20	-
FliC Monomer[c]	0/20	100%[d]
FliC Polymer[c]	0/20	100%[d]

[a] CD-1 mice immunized with indicated antigen on days 0 & 10; Mice were challenged on day 24

[b] Administration of 50 µl of PBS i.m.

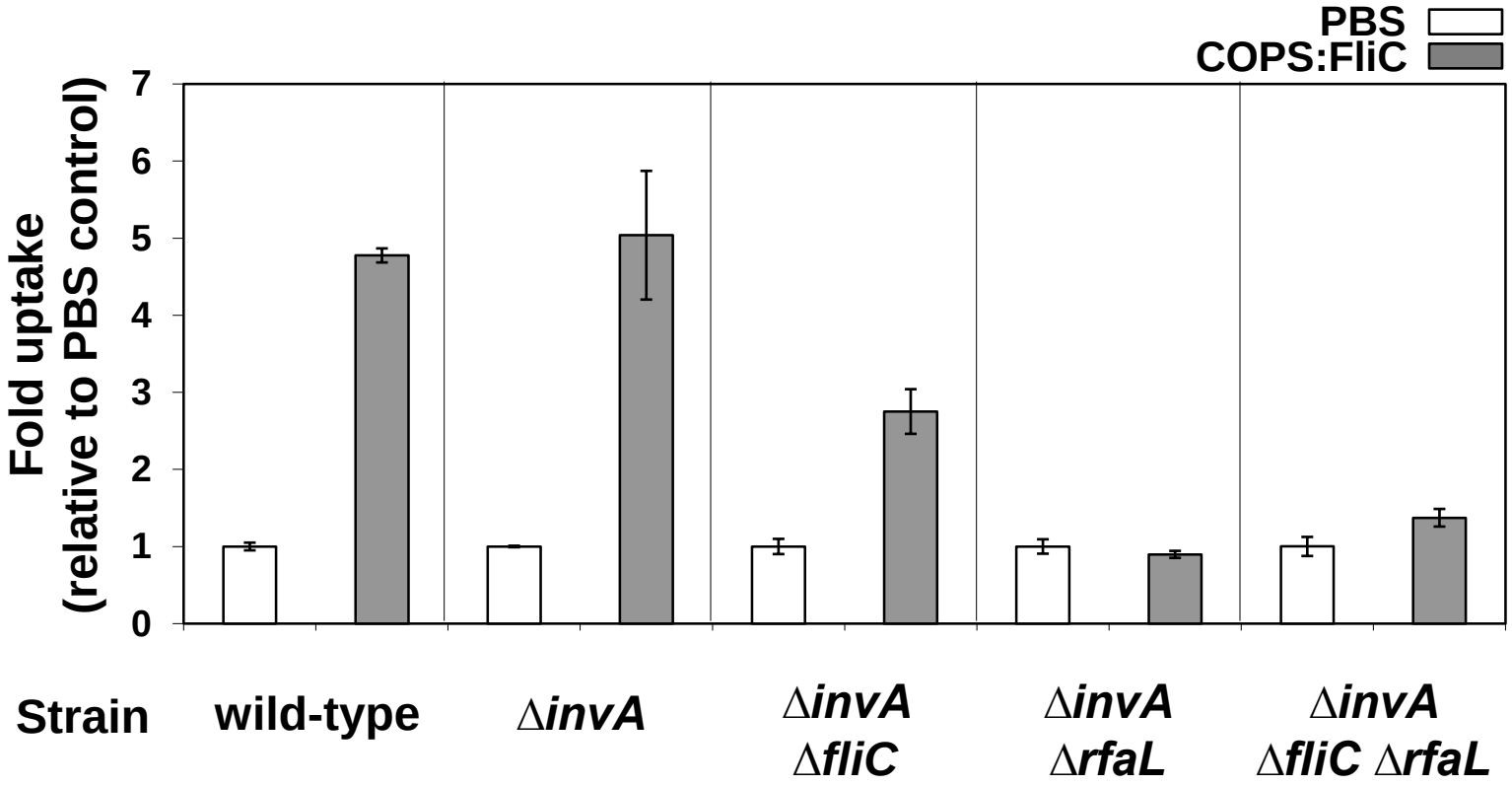
[c] I.m. immunisation with 2.5 µg of monomers or polymers

[d] 21 days after challenge with 5 x 10^5 CFU R11 IP

[e] $p < 0.05$ by Fisher's exact test

*1:1 conjugation ratio with CDAP

Opsonophagocytic assay using sera of immunized mice and J774 cells with invasion, flagellin and OPS mutants of *S.* Enteritidis R11



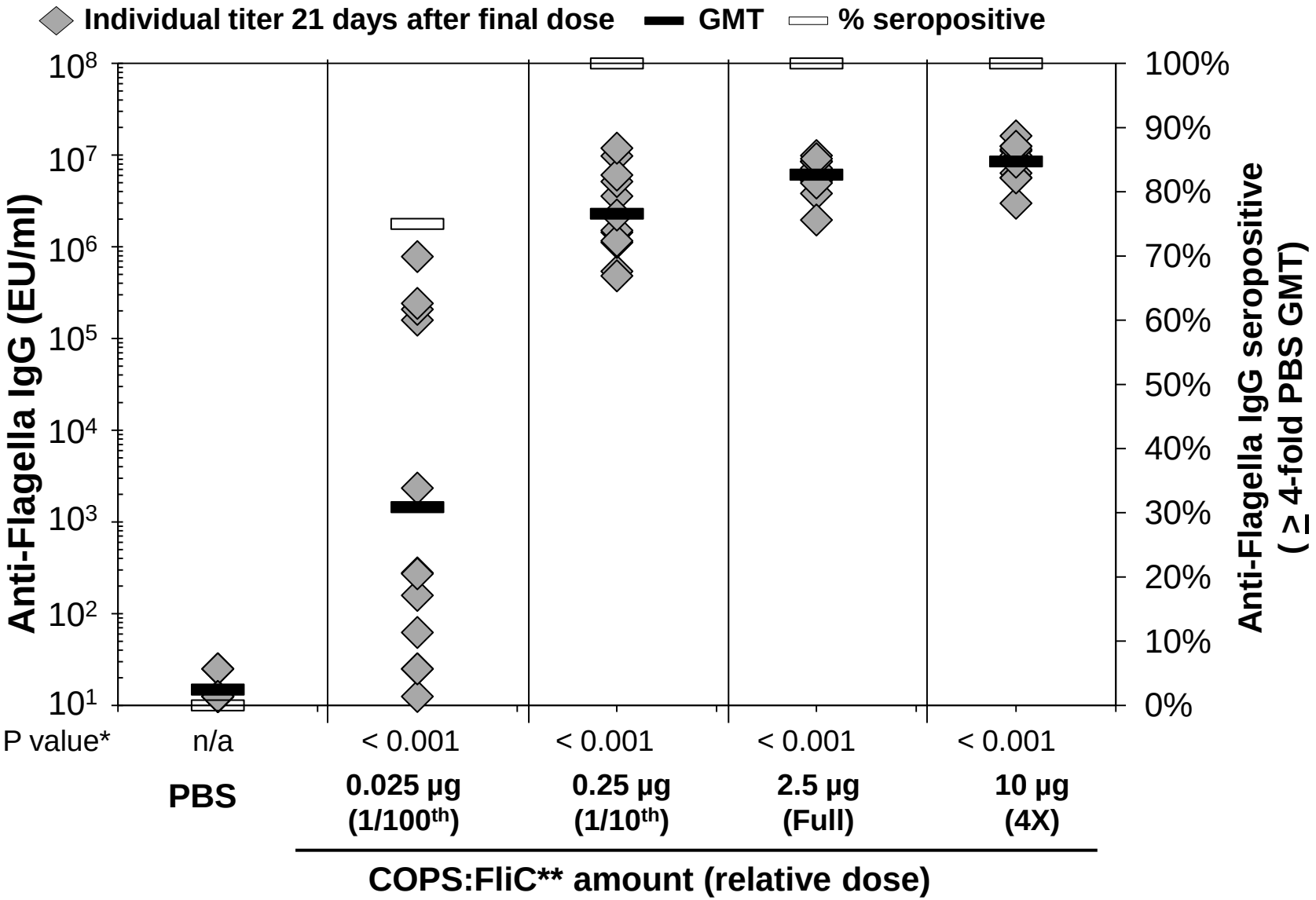
Salmonella bacterial culture → Opsonization with serum → Incubation/uptake into J774 mouse macrophages → Kill extra-cellular bacteria → Lyse J774 cells, enumerate intracellular CFU

Immunogenicity of *S.* Enteritidis Random- (CDAP) vs End- (Amox.) linked COPS:FliC Conjugates

Group	Anti-LPS IgG GMT[a]	Anti-FliC IgG GMT[a]	Mortality Rate[b,c]	Vaccine efficacy
PBS	82	101	12/13	-
COPS:FliC CDAP	227	9,548,869	3/13	75%[d]
COPS:FliC Amox.	392	8,735,020	2/13	83%[d]

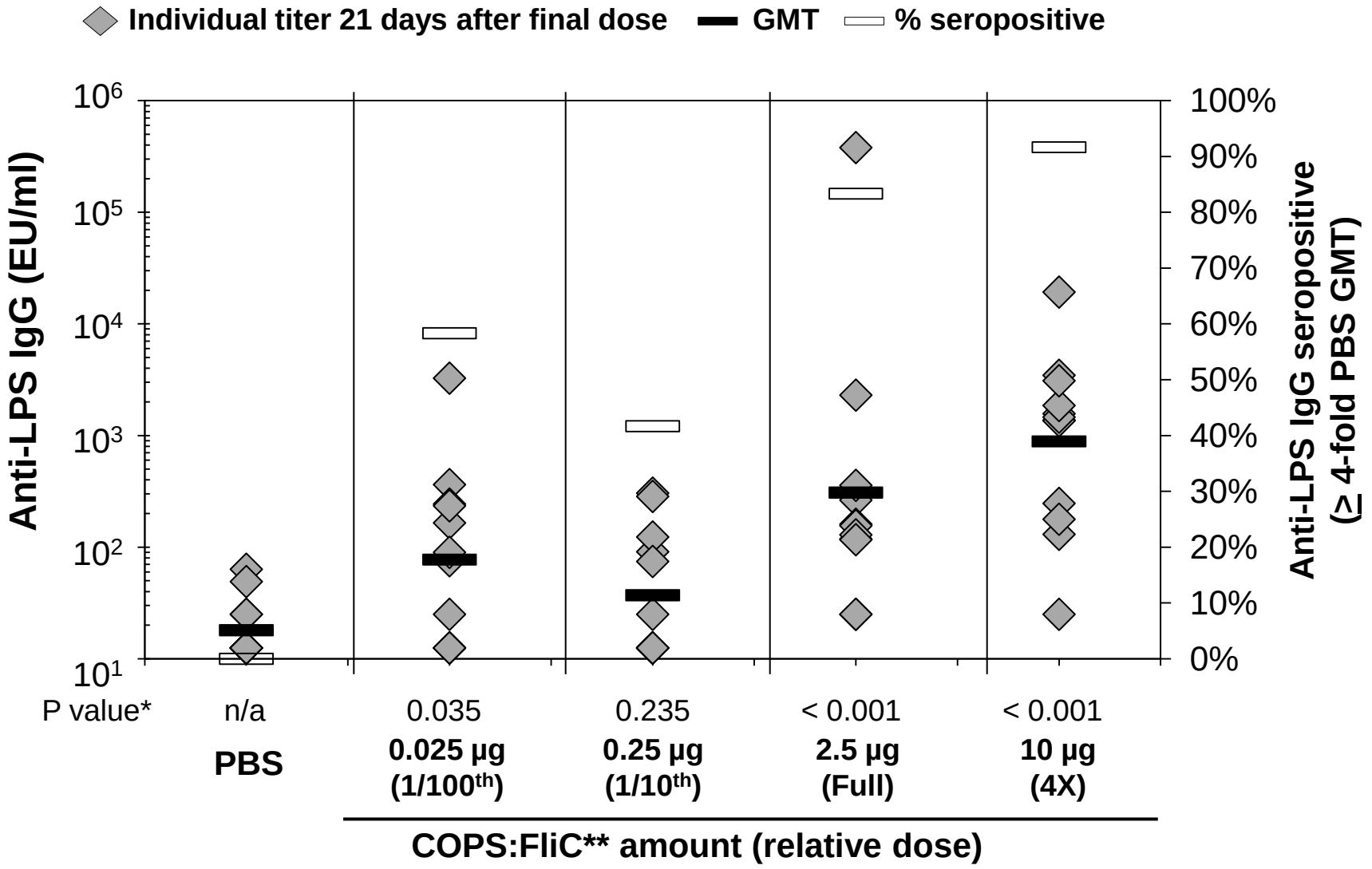
[a] CD-1 Mice (n= 24-28) immunized IM at 0, 28, 56 days
[b] LD_{50} = 2. 2 x 10^5
[c] Challenge at 28d after the 3rd dose with 1 x 10^6 cfu
[d] $p < 0.05$ vs PBS controls by Fisher's exact test

Immunogenicity in mice of different doses of *S. Enteritidis* COPS:FliC conjugates



CD-1 Mice immunized IM at 0, 28, 56 days *vs PBS by Mann-Whitney (n = 12/dose) **CDAP linked

Immunogenicity in mice of different doses of *S.* Enteritidis COPS:FliC CDAP conjugates



CD-1 Mice immunized IM at 0, 28, 56 days *vs PBS by Mann-Whitney (n = 12/dose) **CDAP linked

Efficacy of different doses of *S.* Enteritidis COPS:FliC conjugates in protecting mice from lethal challenge with wild-type *S.* Enteritidis R11

Vaccine	Dose[a]	Mortality (dead/total)[b]	p value[c]	Vaccine efficacy
PBS	-	12/12	-	-
COPS:FliC	0.025 µg	1/12	< 0.0001	92%
COPS:FliC	0.25 µg	0/12	< 0.0001	100%
COPS:FliC	2.5 µg	0/12	< 0.0001	100%
COPS:FliC	10 µg	0/12	< 0.0001	100%

[a] Doses at 0, 28 56 days

[b] 21 days after IP challenge with 1 x 10^6 R11 CFU; LD50 = 2.2 x 10^5

[c] Compared to mice receiving PBS by two-tailed Fisher's exact test

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Protection from a fatal dose of *S.* Enteritidis R11 after passive immunization with sera containing high titers of anti-LPS and anti-FliC IgG

Group/treatment[a]	Mortality[b]
PBS	5/6
Normal sera	7/7
COPS:FliC sera	1/7[c]

a Intravenous transfer of pooled sera from CD-1 mice receiving 3 doses of 10 µgs of COPS:FliC (434 EU anti-LPS IgG; 550,000 EU anti-flagella IgG) or 3 doses of PBS (normal sera)

b 14 days after IP challenge with $5x10^5$ CFU R11 (IP LD_{50} = 2.2×10^5)

c p = 0.005 by Fisher's exact test compared to mice receiving normal sera

Summary of key preclinical results

- Immunization with **flagellin alone** is protective
- COPS **reliably forms covalent linkages** to flagellin by several conjugation strategies
- Conjugates elicit significant **seroconversion (4-fold or > rise) of anti-LPS** & stimulate **very high anti-flagellin** antibody titers
- COPS:H Antibodies show **opsonophagocytic activity** and are **protective by passive transfer**
- COPS:Flagellin conjugates are **protective** against lethal challenge with wild-type virulent *Salmonella* Enteritidis in mice that persists at **1/100th vaccine dose**

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