Immune protection in cholera and immune responses to oral cholera vaccination: knowledge from challenged volunteer model studies

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CVD volunteer model of experimental cholera

- **Established in 1976** at the behest of the US Cholera Panel of NIH to test a toxoid vaccine.
- Healthy adult **community volunteers**.
- Performed on CVD Research Isolation Ward under **QUARANTINE**.
- High attack rate of diarrhea.
- **Objective outcomes** measured (diarrheal volume, vomiting, fever).
- Precise **quantitation of diarrheal stool volumes**.
- A proportion of subjects develop copious purging (0.5 - 1.1 liters per hour). ($\geq$ 5 liter purge = “severe” cholera; $\geq$ 3 liter purge = “moderate” cholera).
- Aggressive oral & IV rehydration & early antibiotic therapy.
- Prior cholera & some vaccines are highly protective in this model.
- This model has proved invaluable for studying pathogenesis of and immunity to cholera, relevant to vaccine development.

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Vibrio cholerae

Autochthonous flora of brackish aquatic environments

- > 200 O serogroups
- Two serogroups cause epidemic cholera:
  - O1 > 99% of all cases globally
    - Biotypes
      - El Tor & Classical
      - Hybrid – El Tor expressing Classical cholera toxin
    - Serotypes – Inaba, Ogawa (and, rarely, Hikojima)
  - O139 (rare)

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Experimental cholera challenge of US volunteers immunized with 3 monthly 8 mg enteral doses of purified glutaraldehyde-treated cholera toxoid

	Vaccinees	Controls
Clinical attack rate	6/8	8/8
Mean incubation	47 hours	36½ hours
(Range)	(15-110 hours)	(15-104 hours)
Mean stool output	2·8 litres	3·7 litres
(Range)	(0·3-5·6 litres)	(0·5-10·9 litres)
Significant antibody rise+:		
Vibriocidal -	8/8	8/8
Antitoxic - -	7/8	6/8

* 10^6 *Vibrio cholerae* classical Ogawa 395
+ 4-fold or greater

Levine MM et al, Trans Roy Soc Trop Med Hyg 1979

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Immunity to cholera

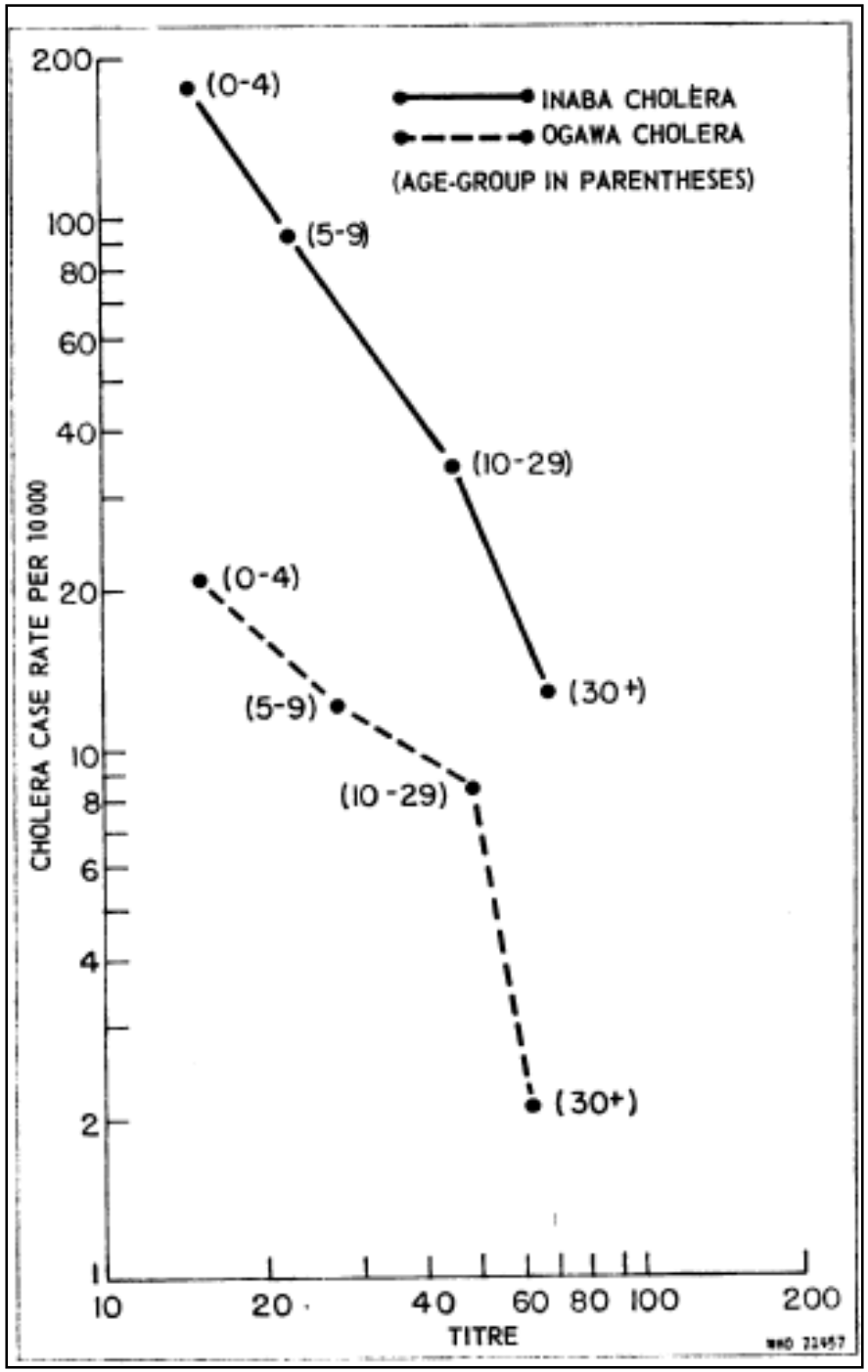
Challenging dogma:
an episode of cholera <u>does</u>
confer significant long-lived!)
protection against diarrhea
upon subsequent re-
challenge

Cholera Reinfection in Man

William E. Woodward

JOURNAL OF INFECTIOUS DISEASES
1971; 123:61-65

"The duration of immunity derived from cholera infection is short, especially in persons whose subsequent reinfection is due to heterologous organisms. The risk of reinfection with *V. cholerae* is probably only slightly less than the risk of initial infection. The relatively high frequency of reinfection indicates that an effective cholera vaccine will need to stimulate greater immunity than does the natural disease."



Relationship between serum reciprocal vibriocidal antibody titer and cholera case rate per 10^3, Matlab Bazar

(Mosley WH et al, Bull WHO 1968; 38:327-334)

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The Relationship of Vibriocidal Antibody Titre to Susceptibility to Cholera in Family Contacts of Cholera Patients*

W. H. MOSLEY,[1] SHAMSA AHMAD, A. S. BENENSON[2] & ANSARUDDIN AHMED

Bull WHO 1968; 38:777-785

Baseline vibriocidal titer	Total no. of family contacts	No. of contacts with culture-confirmed cholera diarrhea
<20	190	28 (14.7%
20	65	4 (6.2%)
40	65	4)6.2%
80	42	1 (2.4%)
≥ 160	59	1 (1.7%)

Protective immunity conferred by clinical classical Ogawa cholera upon subsequent homologous challenge of US volunteers with *V. cholerae* O1 classical biotype serotype Ogawa

Volunteer group	Inoculum*	Clinical attack rate	Mean stool output (Range)	Excretion of vibrios
Re-challenge group				
Initial challenge	10^6	4/4	4·4 litres (1·4-10·9 litres)	4/4
Re-challenge	10^6	0/4	0	0/4
Control group No. 1	10^6	5/5	5·0 litres (0·2-18·2 litres)	5/5
Control group No. 2	10^5	4/6	21·8 litres (1·4-44 litres)	6/6

Levine MM et al, Trans Roy Soc Trop Med Hyg 1979

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Protective immunity conferred by clinical classical Ogawa cholera upon subsequent heterologous challenge of US volunteers with *V. cholerae* O1 classical biotype serotype Inaba

Volunteer group	Clinical attack rate	Mean stool output (Range)	Excretion of vibrios
Re-challenge group			
Initial challenge (Ogawa)	7/7	16·7 litres (0·8-44)	7/7
Re-challenge (Inaba)	0/7	0	1*/7
Control group (Inaba)	10/12	5·1 litres (0·7-11·4)	12/12

Levine MM et al, Trans Roy Soc Trop Med Hyg 1979

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Protective immunity conferred by clinical classical Inaba cholera upon subsequent heterologous challenge of volunteers with *V. cholerae* O1 classical biotype serotype Ogawa

Volunteer group	Clinical attack rate	Mean stool output (Range)	Excretion of vibrios
Re-challenge group			
Initial challenge (Inaba)	5/5	5·3 litres (0·7-11·4)	5/5
Re-challenge (Ogawa)	0/5	0	0/5
Control group (Ogawa)	9/10	3·9 litres (0·3-8·5)	9/10

Levine MM et al, Trans Roy Soc Trop Med Hyg 1979

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Immunity following clinical cholera in U.S. volunteers

	Attack Rate			Positive Coprocultures	
Biotype	**Ctrls**	**Vets**	**Efficacy**	**Ctrls**	**Vets**
Classical	24/27	0/16	100%	26/27	0/16
					p = 0.012
El Tor	32/37	2/22	90%	34/37	8/22

* Levine et al, 1978, 1981, 1983

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Serum vibriocidal antibody (Clements ML et al, J Infect Dis)

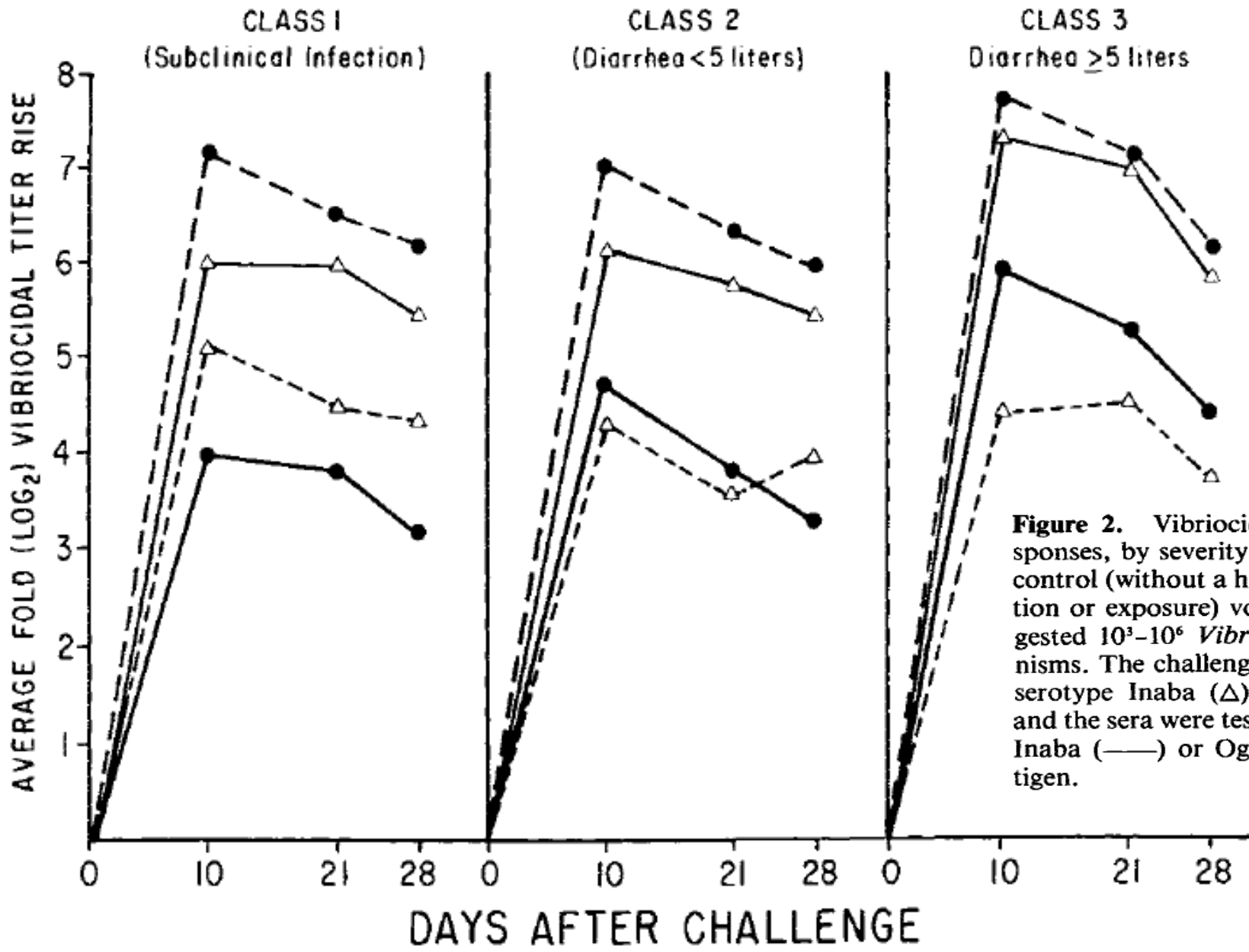

Figure 2. Vibriocidal antibody responses, by severity of illness, in 107 control (without a history of vaccination or exposure) volunteers who ingested 10^3–10^6 *Vibrio cholerae* organisms. The challenge organisms were serotype Inaba (△) or Ogawa (●), and the sera were tested with serotype Inaba (——) or Ogawa (- - -) antigen.

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Long-term immunity in North Americans elicited by prior clinical cholera

	Attack Rate*
Controls	4/5
	p=0.04
Re-challenge veterans**	0/4
	p<0.001
Cumulative controls	26/28

* Attack rate following challenge with 10^6 classical Ogawa 395.

** These four subjects experienced classical biotype cholera 33-36 months earlier following experimental challenge (3 had Ogawa and 1 Inaba). (**Data from Levine et al, J Infect Dis 1981**)

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Immunologic responses of cholera "veterans" & controls to challenge with 10^6 *V. cholerae* O1 classical Ogawa

Subj.	Diarrhea Volume	Excret. of V. ch.	Serum V'dal Titer Pre	Serum V'dal Titer Peak	Serum IgG anti-CT Pre	Serum IgG anti-CT Peak	Intestinal SIgA anti-CT Pre	Intestinal SIgA anti-CT Peak
Veterans		(Levine et al, J Infect Dis 1981)						
-5	0	0	80	80	0.36	0.39	<8	<8
-14	0	0	20	20	1.06	1.00	<8	<8
-10	0	0	80	320	0.18	0.89	<8	32
-9	0	10^4	160	5120	0.68	1.18	<8	>64
Controls								
-1	7.1 liters	10^7	<20	1280	0.06	1.35	<8	<8
-7	2.1 "	10^8	<20	1280	0.07	0.51	<8	<8
-8	7.2 "	10^7	<20	320	0.09	1.23	<8	<8
-13	5.3 "	10^6	20	10240	0.12	1.13	<8	<8
-6	0	10^5	160	5120	0.40	1.34	<8	16

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Natural infection-derived immunity in Bangladesh following clinical cholera caused by different biotypes: field studies corroborate volunteer data

Study	Initial Infection	Subsequent Infection	Protective Efficacy
Glass 1982	Mostly Classical	Mostly Classical	90%
Clemens 1991	Classical	Classical	100%
	Classical	El Tor	100%
	El Tor	El Tor	29%
	El Tor	Classical	0%

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Pathogenesis:
ingestion of cholera enterotoxin alone can cause cholera gravis

TABLE 1. Response of healthy adult volunteers after ingestion of various doses of cholera toxin[a]

Volunteer	Cholera toxin dose (μg)	Incubation (h)	Total diarrheal stool vol (ml)	No. of diarrheal stools	Duration of diarrhea (h)
6004-1	0.5				
6004-3	0.5				
6004-7	0.5				
6006-2	2.5				
6006-3	2.5				
6006-4	2.5				
6006-5	2.5				
6006-6	2.5				
6008-1	5.0	13.5	1,695	5	31.5
6008-2	5.0	9.5	1,281	5	36.0
6008-3	5.0				
6008-5	5.0	6	6,023	33	89.0
6008-6	5.0	5.5	1,020	6	41.5
6004-5	25	5	21,649	50	94
6004-9	25	7	22,074	47	91.5

[a] To diminish gastric acidity and protect toxin from digestion during its transit through the stomach, volunteers received cimetidine (300 mg) 3 h before and 2.0 g of $NaHCO_3$ concomitant with ingestion of cholera toxin.

Insights on mechanisms of immunity against cholera

89% vaccine efficacy of *V. cholerae* O1 El Tor Inaba vaccine strain JBK 70 (*ctx* deletion) in protecting against challenge with virulent El Tor Inaba N16961				
Group	**Diarrhea Attack rate**	**Mean diarrheal stool volume per ill volunteer (range)**	**No. with positive direct stool cultures**	**Geometric mean excretion (vibrios per g stool)**
Controls	7/8	4.5 liters (1.1-7.9 l)	8	$4x10^6$
	P<0.003			P<0.001
Vaccinees	1/10	1.6 liters	2	$4x10^3$
Challenge with 10^6 cfu of virulent El Tor Inaba N16961 one month after ingestion of a single oral dose of vaccine Levine et al. Infect Immun 1988				

Cholera challenges in volunteers to assess efficacy of candidate vaccines in preventing cholera

Serum vibriocidal antibody correlates with protection against cholera

- Serum vibriocidal antibody is a **proxy for a protective intestinal immune response**.
- **Most vibriocidal antibody is anti-LPS**, some is directed against (poorly characterized) protein antigens
- In general, the stronger the vibriocidal response, the greater the protective effect.
- In US volunteers, vibriocidal antibody titers drop rapidly towards (but do not reach) baseline after clinical cholera (or oral vaccine) but protection endures long thereafter.
- **Vibriocidal antibody response is particularly useful for assessing the relative immunogenicity of oral vaccines in non-immune hosts.**

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CVD 103-HgR live oral cholera vaccine

- *Vibrio cholerae* O1
- Classical biotype, Inaba serotype
- *ctxA* deleted, *ctxB* intact
- Hg++ resistance gene inserted into *hlyA* locus
- Makes toxin co-regulated pili (TCP)

Immunogenicity of CVD 103-HgR in US adult subjects*

	Levine & Kaper 1993	Kotloff 1992
No. subjects	182	94
≥ 4-fold v-dal rise	93%	97%
V'dal titers ≥ 2560	50%	67%
Reciprocal GMT (Inaba)	1699	2656
IgG antitoxin rises	81%	72%

* A single $5x10^8$ CFU dose

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Efficacy of single-dose Orochol®/Mutacol® CVD 103-HgR in preventing cholera

Severity	Vacc	Ctrls	Efficacy	p
≥ 5.0 liters	1/103	10/86	92%	<.0029
≥ 3.0 liters	1/103	16/86	95%	<.0001
≥ 1.0 liter	8/103	41/86	84%	<.0001
Any	19/103	73/86	78%	<.001

Composite of 8 separate challenges with El Tor Inaba, El Tor Ogawa, classical Inaba and classical Ogawa

Significant protection is already present 8 days after ingesting the single dose

Efficacy of CVD 103-HgR (Mutacol®) in preventing moderate and severe El Tor cholera when challenged > 3 months after ingestion of a single oral dose

Cholera

Attack Rate	Vacc	Ctrls	Efficacy
Moderate/severe	1/28	9/23	91%
(i.e., > 3.0 liters)	3.6%	39.1%	(51-99%)*

Challenge with 10^5 CFU of NIH El Tor Inaba N16961 frozen inoculum

Data from Tacket, Cohen et al, Infect Immun 1999 * (95% CI)

This study design was requested by the FDA

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PaxVax Vaxchora™ (CVD 103-HgR) protects against experimental challenge with *V. cholerae* O1

Parameter measured after challenge	Vaccine 10-Day post N=35	Vaccine 3-Month post N=33	Placebo N=66
Attack Rate ≥ 3 Liter liquid stool	2/35 (6%)	4/33 (12%)	39/66 (59%)
Vaccine Efficacy	90%	80%	
Lower Bound of 95% CI	63%	50%	

Challenge with 10^5 CFU of NIH El Tor Inaba N16961 frozen inoculum
This study was designed in conjunction with the FDA

Chen WH et al, Clin Infect Dis 2016

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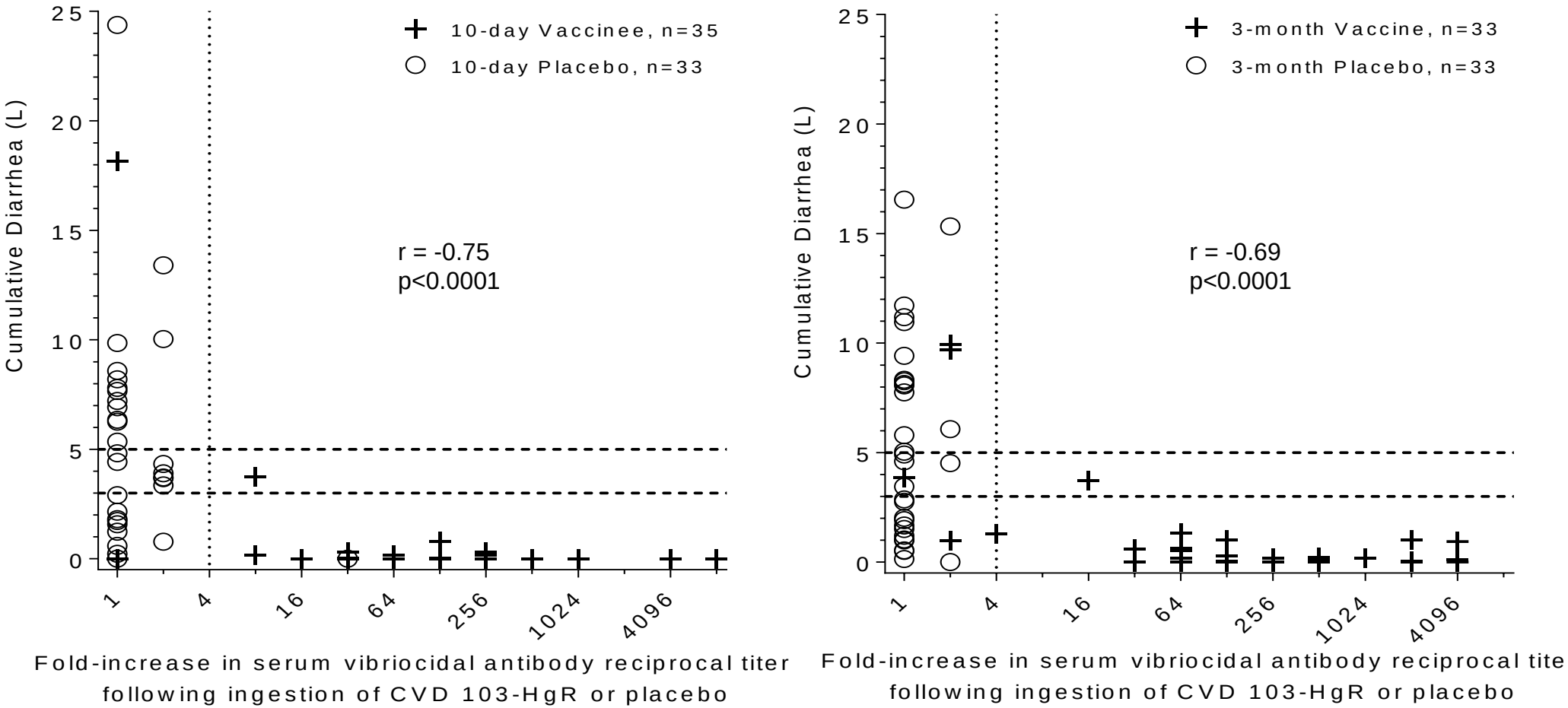
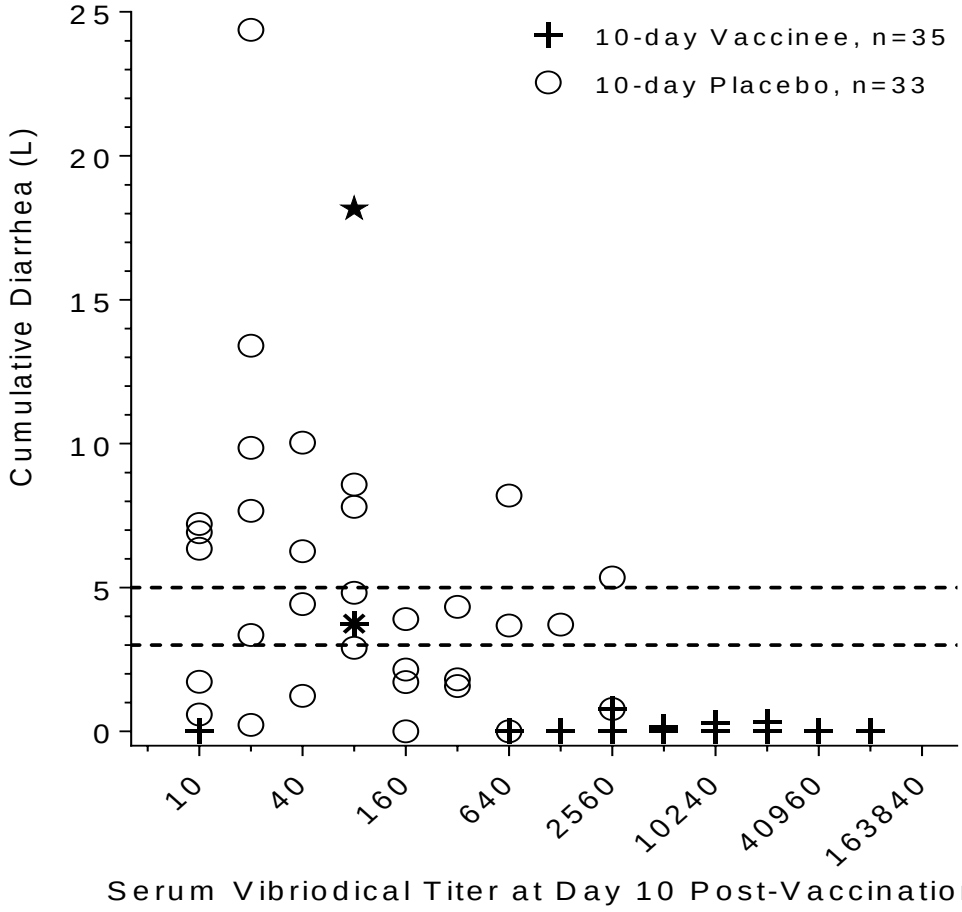
Figure 1. Correlation of serum vibriocidal antibody titer fold-increase in response to vaccination and cumulative diarrheal purge volume following cholera challenge

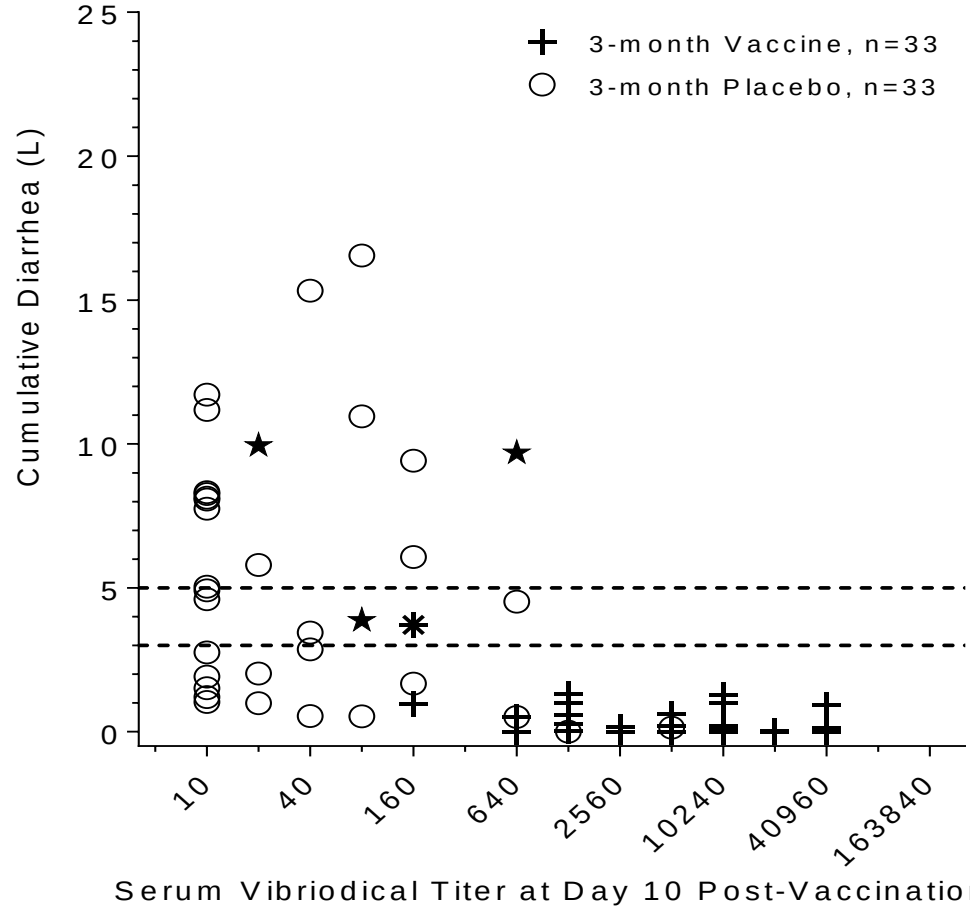


4 of 6 vaccinees who did not seroconvert (67%) got moderate-to-severe cholera versus 2 of 62 who seroconverted (3.2%), p=0.00026

Chen WH et al. Clin Infect Dis 2016

Fig. 2. Correlation of serum vibriocidal antibody endpoint titer at Day 10 post-vaccination and cumulative diarrheal purge volume following cholera challenge (Chen WH et al. Clin Infect Dis 2016)

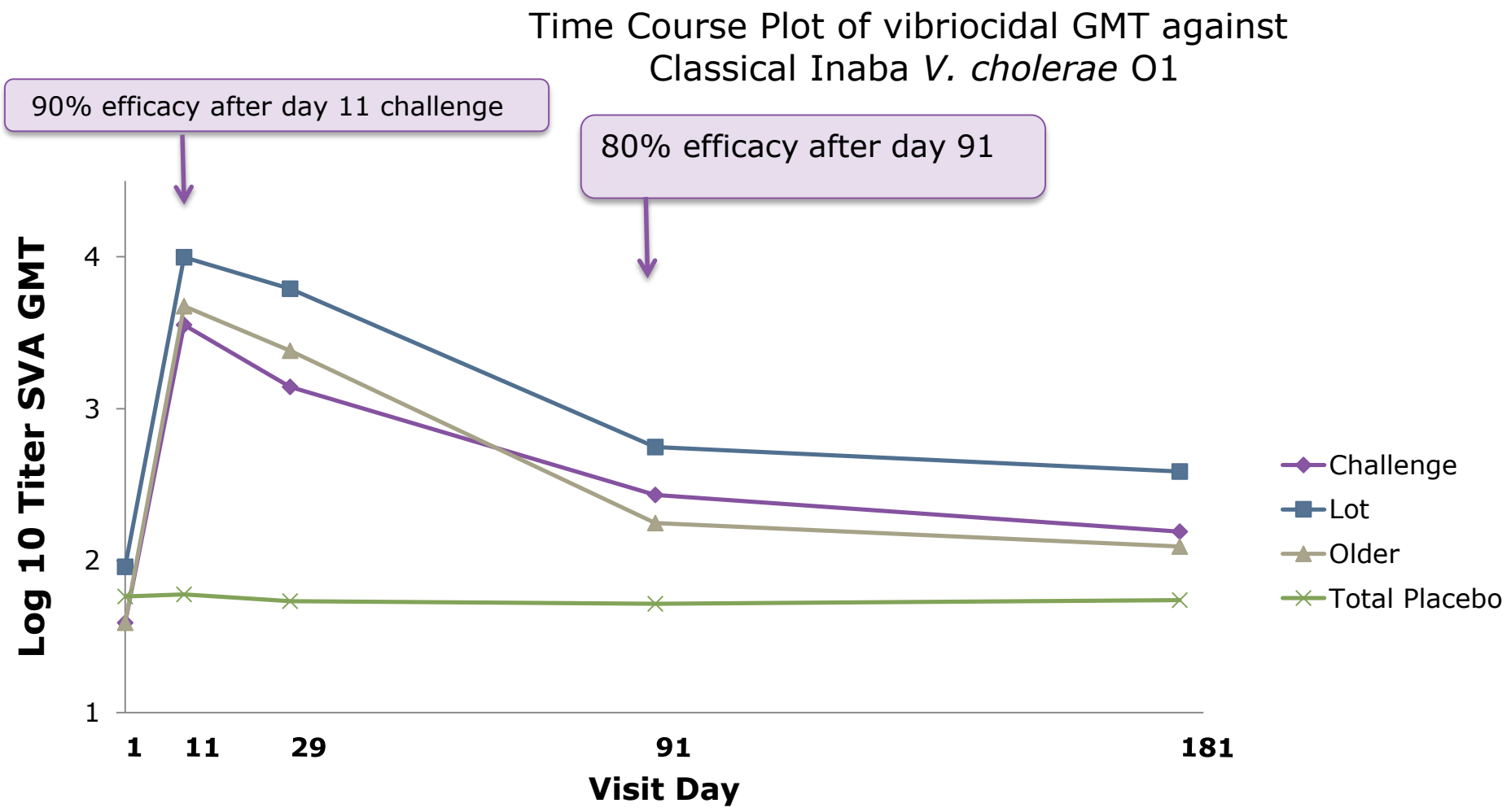




Two vaccinees who seroconverted but had moderate-to-severe cholera are indicated by the eight-point star (✳). Four vaccinees who failed to seroconvert in response to vaccination had moderate-to-severe cholera and are indicated by the five-point star (★).

Vaxchora: Efficacy Demonstrated in Human Challenge Correlates with Immune Response

PaxVax



Anti-LPS IgA Memory B Cell after vaccination or challenge in Study 003 healthy adults

PaxVax

Mean percent anti-LPS IgG memory B cell/total IgG memory B cells

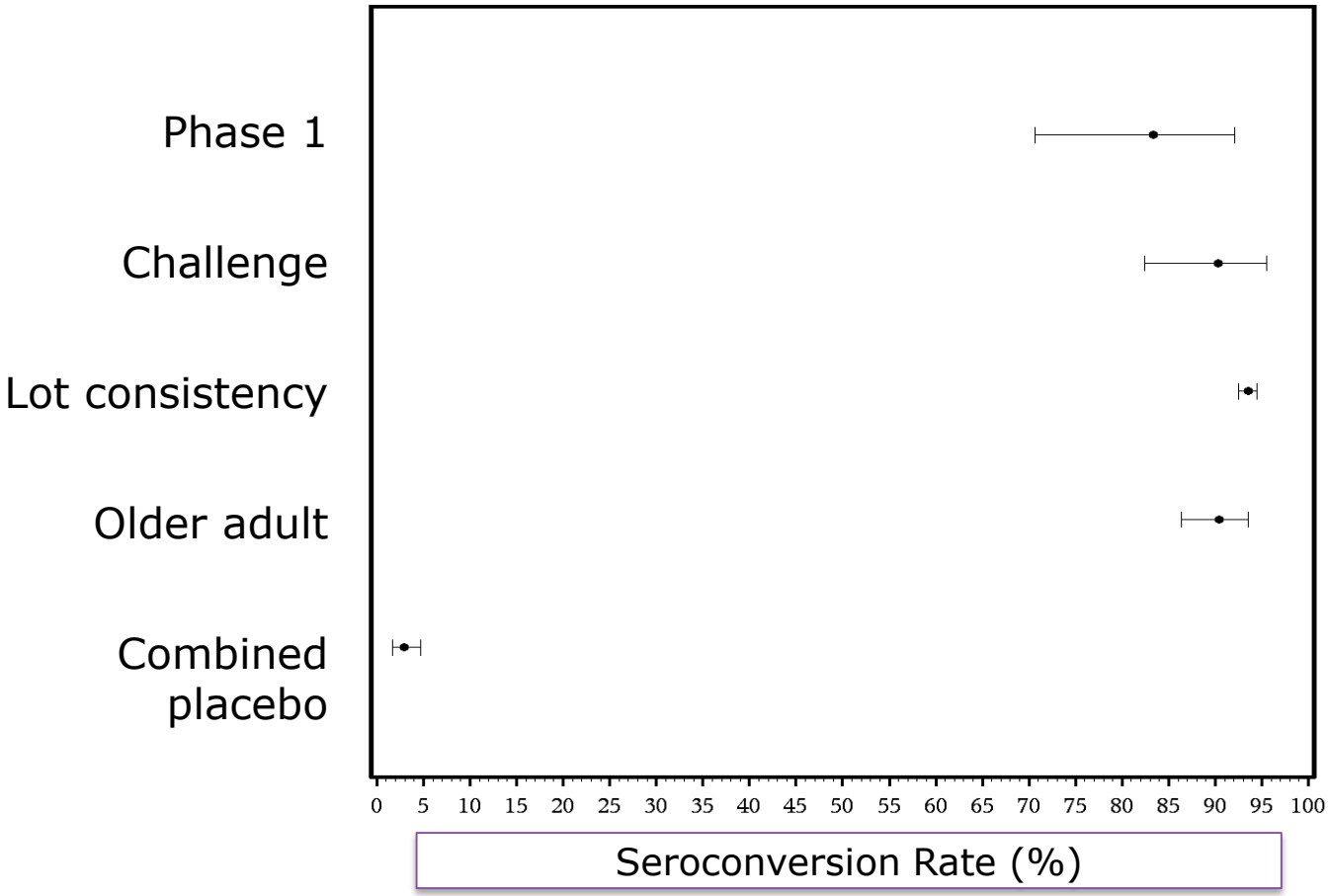
	Unchallenged Vaccinees	Vaccine group pre-challenge	Placebo group 170 days post-cholera challenge
	N=22	N=33	N=26
Day 1	0.089	0.086	0.077
Day 91	n/a	0.153*	n/a
Day 181	0.135*	n/a	0.191*

*p<0.05 Wilcoxon signed rank test when compared to Day 1. n/a = not assessed

Anti-LPS IgG memory B cells increase and remain elevated at Day 181

The memory B cell immunogenicity endpoints were assessed using a qualified Enzyme-Linked ImmunoSpot (ELISPOT) method performed by PaxVax using assays developed in collaboration with the Cellular Immunology Section of CVD, UMB

PXVX0200 (Vaxchora) is immunogenic ~90% seroconversion in Phase 3 studies



Forest Plot of Vibriocidal Antibody Seroconversion (95% CI) against Classical Inaba *V. cholerae* Through Day 11 Immunogenicity Evaluable Population

Note: N analyzable: Phase 1=54; Challenge=93; Lot=2687; Older=291; Combined placebo=544.

Source: Figure 11.4.1; Table 11.3.1.1; Phase 1 (CSR PXVX-VC-200-002); challenge (CSR PXVX-VC-200-003); lot consistency (CSR PXVX-VC-200-004); older adult (CSR PXVX-VC-200-005).

Serum vibriocidal antibody – mechanistic or non-mechanistic ICOP?

- A single clinical infection caused by wild-type *V. cholerae* O1 confers significant protection against cholera upon subsequent exposure to wild-type *V. cholerae* O1 (classical is more immunizing than El Tor). The protection is long-lived.
- Expression of cholera enterotoxin is a necessity for the profuse purging of rice water stools that is characteristic of cholera gravis.
- The fundamental protective **immunity to cholera is anti-bacterial** (but in the short term antitoxic immunity can synergistically enhance antibacterial immunity).
- **Vibriocidal antibody** seroconversion following infection with wild-type *V. cholerae* O1 or ingestion of oral cholera vaccines is a strong correlate for protection against cholera and antibacterial immunity.
- Do *V. cholerae* O1 antigens besides LPS contribute to the vibriocidal protective repertoire? Still the subject of debate.
- IgG anti-LPS B memory cells maintain long-lived protection

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