Supporting Information

Thermostability of Measles and Rubella Vaccines in a Microneedle Patch

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Table S1. Effect of individual excipients on measles and rubella vaccine titer after drying and storage at 40°C for up to one week (data from Figure 3)[1]

Excipient	Measles		Rubella	
	% at Day 0 to Liquid Control	% at Day 7 to Day 0	% at Day 0 to Liquid Control	% at Day 7 to Day 0
Na Lactate	19.1%*	0.0%	28.0%*	1.5%
Histidine	1.0%	35.5%	32.9%*	9.0%**
Dextrose	23.7%*	0.0%	32.9%*	0.0%
Asparigine	3.8%	0.0%	13.9%	7.6%**
Threonine	1.6%	0.0%	55.9%*	1.5%**
Glycine	0.5%	0.0%	35.2%*	1.2%
Adonitol	26.3%*	0.0%	25.7%*	0.0%**
Serine	11.7%	0.0%	20.2%	2.1%
Na Citrate	0.8%	0.0%	4.6%	0.0%
Na Thiosulfate	27.9%*	0.0%	4.8%	0.0%
Sucrose	8.4%	6.9%**	29.4%*	22.7%**
K Gluconate	4.0%	0.0%	116.8%*	4.7%
Sorbitol	9.9%	0.0%	144.3%	4.2%
Na Gluconate	15.2%	0.0%	154.1%	3.6%
Maltose	0.7%	0.0%	87.6%*	0.7%**
Glucose	3.2%	0.0%	53.7%*	0.0%**
Mannitol	0.3%	0.0%	50.6%*	4.2%**
K Citrate	0.0%	0.0%	55.1%*	0.0%
Myo-inositol	1.2%	0.0%	35.7%*	5.0%**
MADPG	0.3%	0.0%	12.0%	0.0%**
Trehalose	3.7%	20.8%	110.3%*	8.5%
Na Phosphate	0.5%	0.0%	0.0%	0.0%**

[1]Green highlight indicates excipients that were selected to progress to combination screen.

* indicates student t-test >0.05 (i.e. no significant change from liquid to Day 0)

** indicates student t-test >0.05 (i.e. no significant change from Day 0 to Day 7)

Table S2. Effect of combinations of two excipients on measles vaccine titer after drying and storage at 40°C for one week. (Data from Figure 4)

Excipient 1	Excipient 2	Code	% Day 0 to liquid control[1]	t-test liquid control and Day 0	Is p>0.05?	% Day 7 to Day 0[2]	t-test Day 0 and Day 7	Is p>0.05?
Histidine	Sucrose	H-S	26 %	0.071	Y	29%	0.126	Y
Histidine	Trehalose	H-T	12%	0.036	N	27%	0.374	Y
Histidine	Asparagine	H-A	3%	0.023	N	8%	0.379	Y
Histidine	Maltose	H-M	13%	0.039	N	0%	0.146	Y
Histidine	Sorbitol	H-So	15%	0.041	N	4%	0.076	Y
Histidine	K Gluconate	K-KG	13%	0.039	N	0%	0.146	Y
Histidine	Dextrose	H-D	7%	0.028	N	0%	0.013	N
Histidine	Na Gluconate	H-NaG	26%	0.071	Y	6%	0.249	Y
Sucrose	Trehalose	S-T	7%	0.028	N	11%	0.019	N
Sucrose	Asparagine	S-A	33%	0.098	Y	44%	0.045	N
Sucrose	Maltose	S-M	7%	0.029	N	0%	0.034	N
Sucrose	Sorbitol	S-So	9%	0.032	N	0%	0.028	N
Sucrose	K Gluconate	S-KG	13%	0.039	N	3%	0.153	Y
Sucrose	Dextrose	S-D	8%	0.03	N	0%	0.12	Y
Sucrose	Na Gluconate	S-NaG	13%	0.039	N	9%	0.171	Y
Trehalose	Asparagine	T-A	117%	0.7	Y	12%	0.282	Y
Trehalose	Maltose	T-M	7%	0.028	N	0%	0.013	N
Trehalose	Sorbitol	T-So	11%	0.034	N	2%	0	N
Trehalose	K Gluconate	T-KG	8%	0.03	N	0%	0.006	N
Trehalose	Dextrose	T-D	4%	0.025	N	0%	0.194	Y
Trehalose	Na Gluconate	T-NaG	1%	0.022	N	0%	0.028	N
Asparagine	Maltose	A-M	4%	0.025	N	0%	0.194	Y
Asparagine	Sorbitol	A-So	4%	0.025	N	12%	0.037	N
Asparagine	K Gluconate	A-KG	3%	0.024	N	21%	0.337	Y
Asparagine	Dextrose	A-D	3%	0.024	N	0%	0.327	Y
Asparagine	Na Gluconate	A-NaG	5%	0.026	N	13%	0.091	Y
Maltose	Sorbitol	M-So	4%	0.025	N	0%	0.346	Y
Maltose	K Gluconate	M-KG	4%	0.025	N	0%	0.315	Y
Maltose	Dextrose	M-D	1%	0.022	N	0%	0.146	Y
Maltose	Na Gluconate	M-NaG	4%	0.025	N	0%	0.009	N
Sorbitol	K Gluconate	So-KG	5%	0.026	N	0%	0.034	N
Sorbitol	Dextrose	So-D	2%	0.022	N	0%	0.07	Y
Sorbitol	Na Gluconate	So-NaG	4%	0.025	N	0%	0.315	Y
K Gluconate	Dextrose	KG-D	5%	0.026	N	0%	0.07	Y
K Gluconate	Na Gluconate	KG-NaG	22%	0.059	Y	0%	0.194	Y
Dextrose	Na Gluconate	D-NaG	3%	0.024	N	0%	0.256	Y
Sucrose	Threonine	S-Thr	5%	0.027	N	36%	0.3	Y

[1]Titer loss due to drying [2]Titer loss due to storage

Table S3. Effect of combinations of two excipients on rubella vaccine titer after drying and storage at 40°C for one week.(Data from Figure 4)

Excipient 1	Excipient 2	Code	% Day 0 to liquid control[1]	t-test liquid control and Day 0	Is p>0.05?	% Day 7 to Day 0[2]	t-test Day 0 and Day 7	Is p>0.05?
Histidine	Sucrose	H-S	8%	0.08	Y	61%	0.296	Y
Histidine	Trehalose	H-T	33%	0.172	Y	59%	0.08	Y
Histidine	Asparagine	H-A	20%	0.116	Y	147%	0.045	N
Histidine	Maltose	H-M	19%	0.11	Y	135%	0.298	Y
Histidine	Sorbitol	H-So	21%	0.118	Y	53%	0.011	N
Histidine	K Gluconate	K-KG	34%	0.181	Y	24%	0.013	N
Histidine	Dextrose	H-D	19%	0.113	Y	0%	0.024	N
Histidine	Na Gluconate	H-NaG	27%	0.144	Y	30%	0.076	Y
Sucrose	Trehalose	S-T	40%	0.215	Y	234%	0.331	Y
Sucrose	Asparagine	S-A	10%	0.083	Y	157%	0.298	Y
Sucrose	Maltose	S-M	38%	0.206	Y	101%	0.98	Y
Sucrose	Sorbitol	S-So	37%	0.196	Y	192%	0.02	N
Sucrose	K Gluconate	S-KG	24%	0.138	Y	226%	0.333	Y
Sucrose	Dextrose	S-D	31%	0.163	Y	0%	0.056	Y
Sucrose	Na Gluconate	S-NaG	13%	0.093	Y	261%	0.091	Y
Trehalose	Asparagine	T-A	6%	0.074	Y	269%	0.136	Y
Trehalose	Maltose	T-M	36%	0.189	Y	73%	0.192	Y
Trehalose	Sorbitol	T-So	36%	0.192	Y	115%	0.264	Y
Trehalose	K Gluconate	T-KG	57%	0.359	Y	5%	0	N
Trehalose	Dextrose	T-D	39%	0.211	Y	0%	0.025	N
Trehalose	Na Gluconate	T-NaG	44%	0.247	Y	13%	0.016	N
Asparagine	Maltose	A-M	28%	0.149	Y	13%	0.027	N
Asparagine	Sorbitol	A-So	44%	0.246	Y	10%	0.036	N
Asparagine	K Gluconate	A-KG	74%	0.576	Y	9%	0.088	Y
Asparagine	Dextrose	A-D	5%	0.072	Y	0%	0.067	Y
Asparagine	Na Gluconate	A-NaG	7%	0.077	Y	0%	0.015	N
Maltose	Sorbitol	M-So	63%	0.432	Y	0%	0.013	N
Maltose	K Gluconate	M-KG	59%	0.383	Y	7%	0.001	N
Maltose	Dextrose	M-D	52%	0.312	Y	0%	0.036	N
Maltose	Na Gluconate	M-NaG	49%	0.285	Y	6%	0.001	N
Sorbitol	K Gluconate	So-KG	44%	0.244	Y	6%	0.001	N
Sorbitol	Dextrose	So-D	49%	0.284	Y	0%	0.045	N
Sorbitol	Na Gluconate	So-NaG	49%	0.281	Y	19%	0.011	N
K Gluconate	Dextrose	KG-D	33%	0.176	Y	0%	0.015	N
K Gluconate	Na Gluconate	KG-NaG	61%	0.404	Y	13%	0.009	N
Dextrose	Na Gluconate	D-NaG	29%	0.155	Y	0%	0.157	Y
Sucrose	Threonine	S-Thr	51%	0.312	Y	43%	0.249	Y

[1]Titer loss due to drying [2]Titer loss due to storage

Additional information about animal study

Justification of the study: This study was carried out after completion of the MR patch optimization in vitro to confirm immunogenicity in animals. We chose rhesus macaques as an accepted model for MR vaccination in a primate. We also chose to use a small cohort of juvenile rhesus macaques to provide initial data in preparation for the follow-on study in a larger cohort of infant rhesus macaques that has been published separately [1].

ARRIVE Guidelines

Study design: This study had two groups of animals. One group received MR vaccination by MN patch and the other received MR vaccination by SC injection.

Sample size: There were four animals in each of the two study groups.

Inclusion and exclusion criteria: Animals were required to be rhesus macaques, ~2 years of age, test seronegative to measles and rubella, have had no previous exposure to measles or rubella and were not previously vaccinated against measles or rubella.

Randomization: The animals were randomly assigned to each of the two groups. Gender was not considered during randomization.

Blinding: Study investigators were not blinded during vaccination. Investigators were also not blinded during blood collection, because the vaccination site was examined at that time and MN patch and SC vaccination sites looked different. Investigators were blinded during serum analysis for antibody titers.

Outcome measures: The primary outcomes measures were measles and rubella titers over time after vaccination. Additional outcome measures were assessment of local reactogenicity at the site of vaccination and general health status of the animals after vaccination.

Statistical methods: The statistical methods are described in the Methods section.

Experimental animals: The animals were outbred male or female rhesus macaques, approximately 2 years old, weighing approximately 5 kg.

Experimental procedures: The experimental procedures are described in the Methods section, comprising vaccinating the animals by MN patch or SC injection, drawing blood on days 0 and 28, measuring serum IgG titers, and visually assessing the animals for vaccination site reactogenicity and general health status.

Results: The results are reported in Figure 7 and the associated text.

Supplementary References

1. Joyce, J.C., et al., *A Microneedle Patch for Measles and Rubella Vaccination Is Immunogenic and Protective in Infant Rhesus Macaques.* The Journal of Infectious Diseases, 2018. **218**(1): p. 124-132.