Analysis of vaccine potency by monoplex and biplex qPRC assay

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SUMÁRIO

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1. ASSINATURAS

Papel	Nome	Função	Assinatura	Data
Elaborador	Felipe Figueiredo	Bioestatístico	_____	_____
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Verificado por			_____	_____
Aprovação final			_____	_____

2. INTRODUCTION

3. METHODS

In an initial exploratory analysis, the inspection of the residuals in a classical ANOVA showed the assumption of normality was not strongly violated (no significant values were obtained from the Shapiro-Wilk test, results not shown). The Levene test, however, indicated the assumption of homogeneity of variances was violated in most cases ($p = 0.00002$ for Measles, $p = 0.25029$ for Mumps and $p = 0.00761$ for Rubella). We therefore opted to use the same procedure of the Welch correction in all analyses performed. In the same manner, the Games-Howell post-hoc test was employed to correct for multiple comparisons in the presence of heteroskedasticity.

4. RESULTS

Table 6 Potency of vaccine presentations quantified by monoplex and multiplex qPCR method. The p-values presented correspond to Welch-ANOVA tests comparing different vaccine formulation stages, per Virus and qPCR mixture.

qPCR Mixture	Virus Target	Monovalent Bulk	Final Vaccine Bulk	Final Vaccine Batch	p
Monoplex	Measles	8.81	6.73	6.97	< 0.00001
Monoplex	Mumps	9.24	7.95	7.75	< 0.00001
Monoplex	Rubella	5.38	4.08	4.36	0.00003
Mumps+measles	Measles	8.37	6.56	6.75	0.00036
Mumps+measles	Mumps	9.12	8.06	7.93	0.00057
Mumps+rubella	Mumps	9.12	7.86	7.62	0.00007
Mumps+rubella	Rubella	6.06	4.58	4.71	0.00097

4.1. Monoplex evaluation

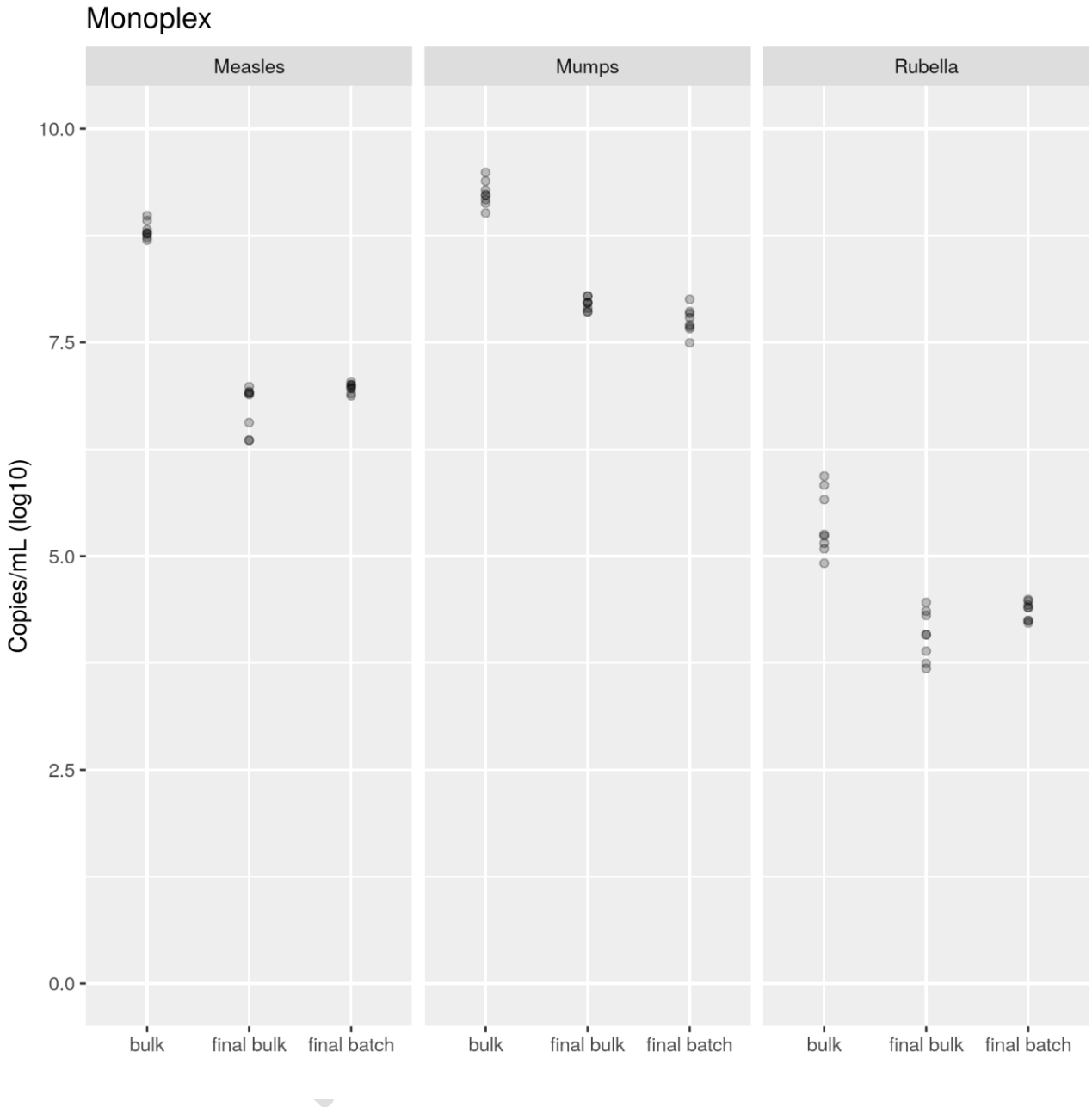

Figure 2: Comparison of viral titer by monoplex qPCR in the different vaccine formulations.

Measles

There were significant differences between the vaccine groups considered (Welch ANOVA, $p < 0.00001$). We observed an average decrease of 2.0745 copies/PCR (log10) in final vaccine bulk, when compared to the bulk vaccine (Games-Howell test, $p < 0.00001$), and an average decrease of 1.8423 copies/PCR (log10) in final vaccine batch, when compared to the bulk vaccine (Games-

Howell test, p < 0.00001). When comparing the two tested vaccine groups (final bulk and final batch), there was no significant difference (Games-Howell test, p = 0.09923).

Mumps

There were significant differences between the vaccine groups considered (Welch ANOVA, p < 0.00001). We observed an average decrease of 1.2904 copies/PCR (log10) in final vaccine bulk, when compared to the bulk vaccine (Games-Howell test, p < 0.00001), and an average decrease of 1.4848 copies/PCR (log10) in final vaccine batch, when compared to the bulk vaccine (Games-Howell test, p < 0.00001). When comparing the two tested vaccine groups, there is also a significant difference (Games-Howell test, p = 0.02198), indicating an average decrease of 0.1945 Copies/PCR (in log10) in the final batch preparation compared to the final bulk preparation.

Rubella

There were significant differences between the vaccine groups considered (Welch ANOVA, p = 0.00003). We observed an average decrease of 1.3083 copies/PCR (log10) in final vaccine bulk, when compared to the bulk vaccine (Games-Howell test, p = 0.00002), and an average decrease of 1.0209 copies/PCR (log10) in final vaccine batch, when compared to the bulk vaccine (Games-Howell test, p = 0.00035). When comparing the two tested vaccine groups (final bulk and final batch), there was no significant difference (Games-Howell test, p = 0.06272).

4.2. Biplex evaluation

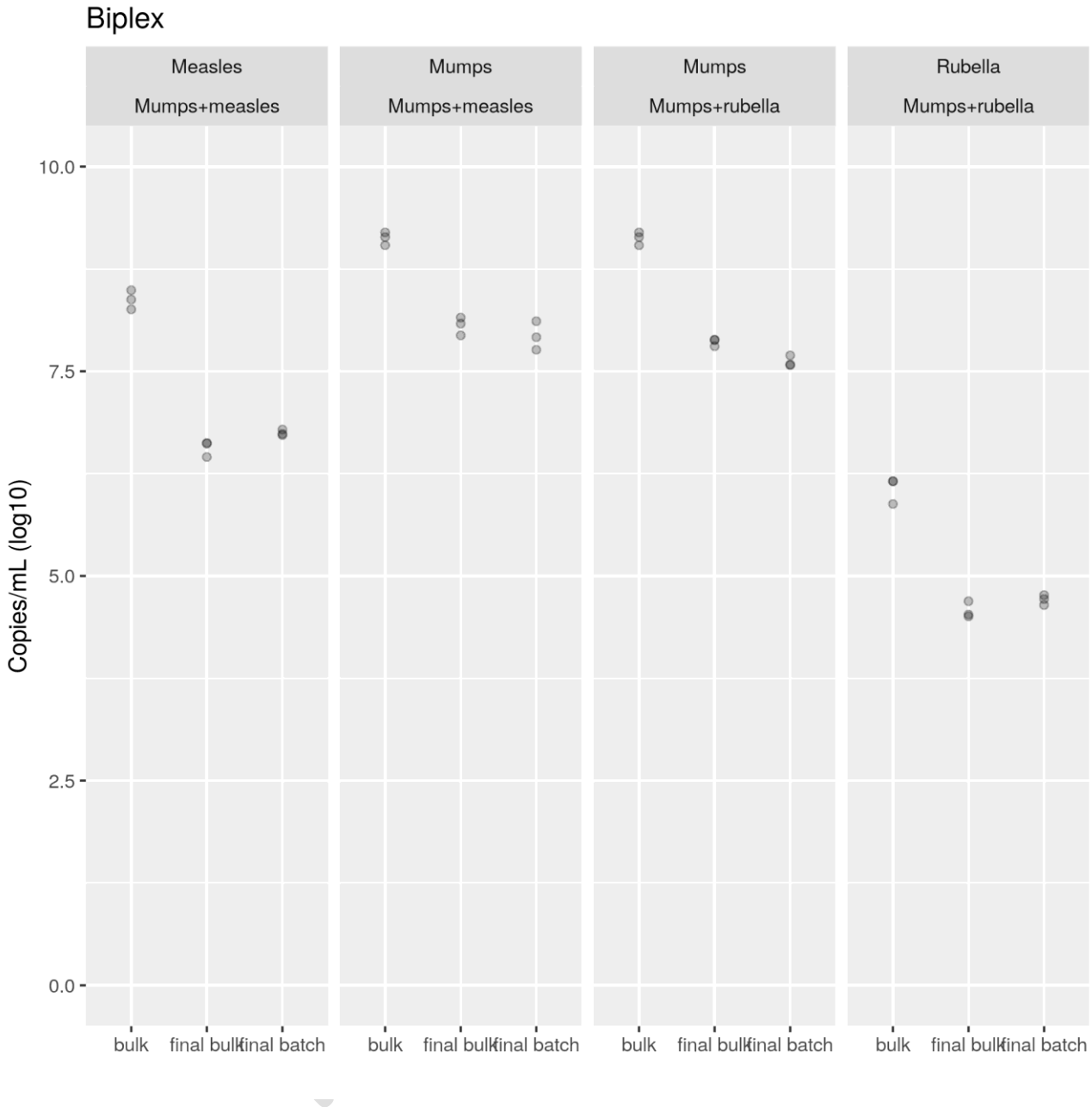

Figure 3: Comparison of viral titer by biplex qPCR mixtures in the different vaccine formulations.

Measles

Mumps

Rubella

5. CONCLUSIONS

6. REFERENCES