Analysis of vaccine potency by monoplex and biplex qPRC assay

CÓDIGO: analise\_dados\_JM\_2018a-v01

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**Data:**  dd/mm/aaaa

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| 01 | Versão inicial |

# Assinaturas

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| --- | --- | --- | --- | --- |
| Papel | Nome | Função | Assinatura | Data |
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| Aprovação final |  |  | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | \_\_\_\_\_\_\_\_\_\_\_\_\_ |

# Introduction

# Methods

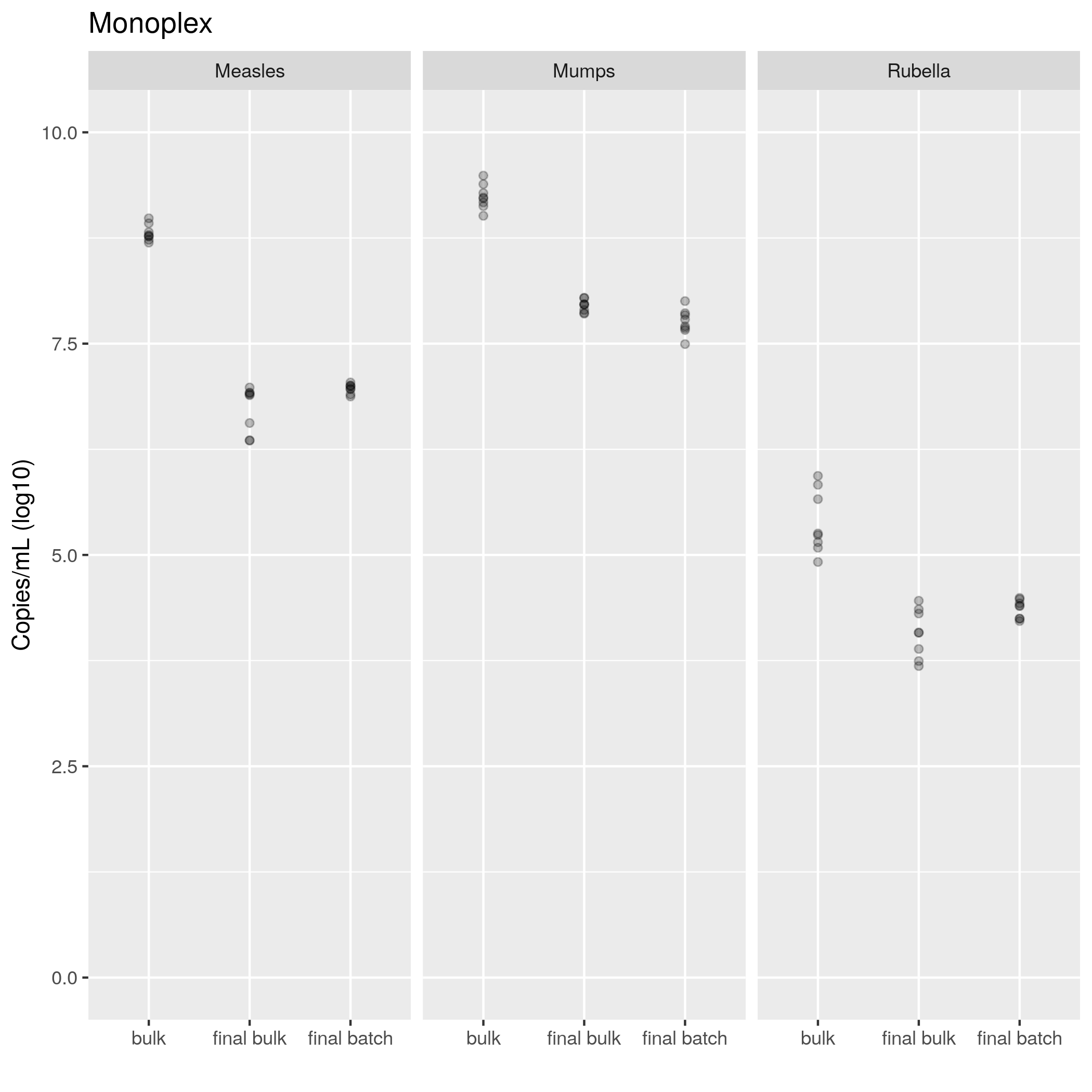
In an initial exploratory analysis, the inspection of the residuals in a classical ANOVA showed the assumption of normality was not strongly violated (no significant values were obtained from the Shapiro-Wilk test, results not shown). The Levene test, however, indicated the assumption of homogeneity 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.

# 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 |

## 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).

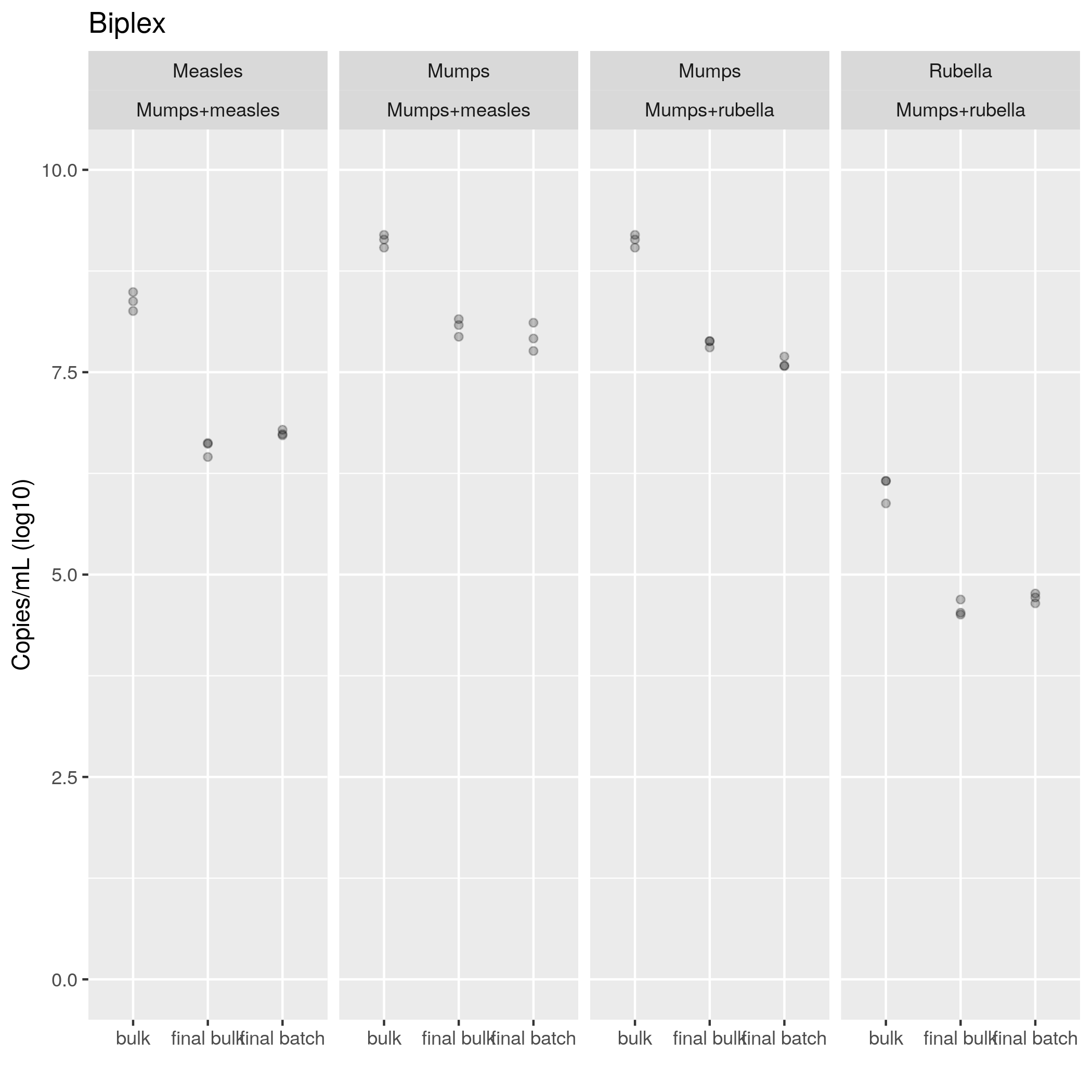
**Mumps**

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).

## Biplex evaluation



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

**Measles**

**Mumps**

**Rubella**

# Conclusions

# References