Analysis of vaccine potency by monoplex and biplex qPRC assay

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Sumário

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# Introduction

# Methods

**Evaluation of monoplex and biplex assays**

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 monoplex samples tested (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.

For the biplex samples, the Levene test did not show evidence of heterogeneity of variances for either Measles (p = 0.34015), Mumps (p = 0.10204) or Rubella (p = 0.13186). For simplicity sake these experiments were analyzed with the same methodology as described above.

**Performance comparison between monoplex and biplex assays**

# Results

## Evaluation of monoplex and biplex assays

As expected, a significant titer change was observed between the three formulations (bulk, final bulk and final batch), with the three vaccine virus strains. This effect was observed when using both the Monoplex assay and the Biplex assay (table 6). Pairwise post-tests confirm a major titer drop can be consistently detected from the initial bulk formulation the next processing stages in all scenarios evaluated, as described below. Figure 2 shows the results for the Monoplex assay and Figure 3 shows the mixtures tested in the Biplex assay.

**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. Monovalent Bulk indicated in both Mumps Biplex mixtures originate from the same experiment.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 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 |

**Measles**

The Measles sample had significant titer drops from the bulk to both final bulk and final batch formulations in the monoplex assay (Games-Howell test, p < 0.00001 and p < 0.00001, respectively), and in the biplex assay (Games-Howell test, p = 0.00011 and p = 0.00131, respectively). When testing for differences between the final bulk and final batch, we found no significant difference in titers in either the monoplex (Games-Howell test, p = 0.09923) or the biplex assay (Games-Howell test, p = 0.12683).

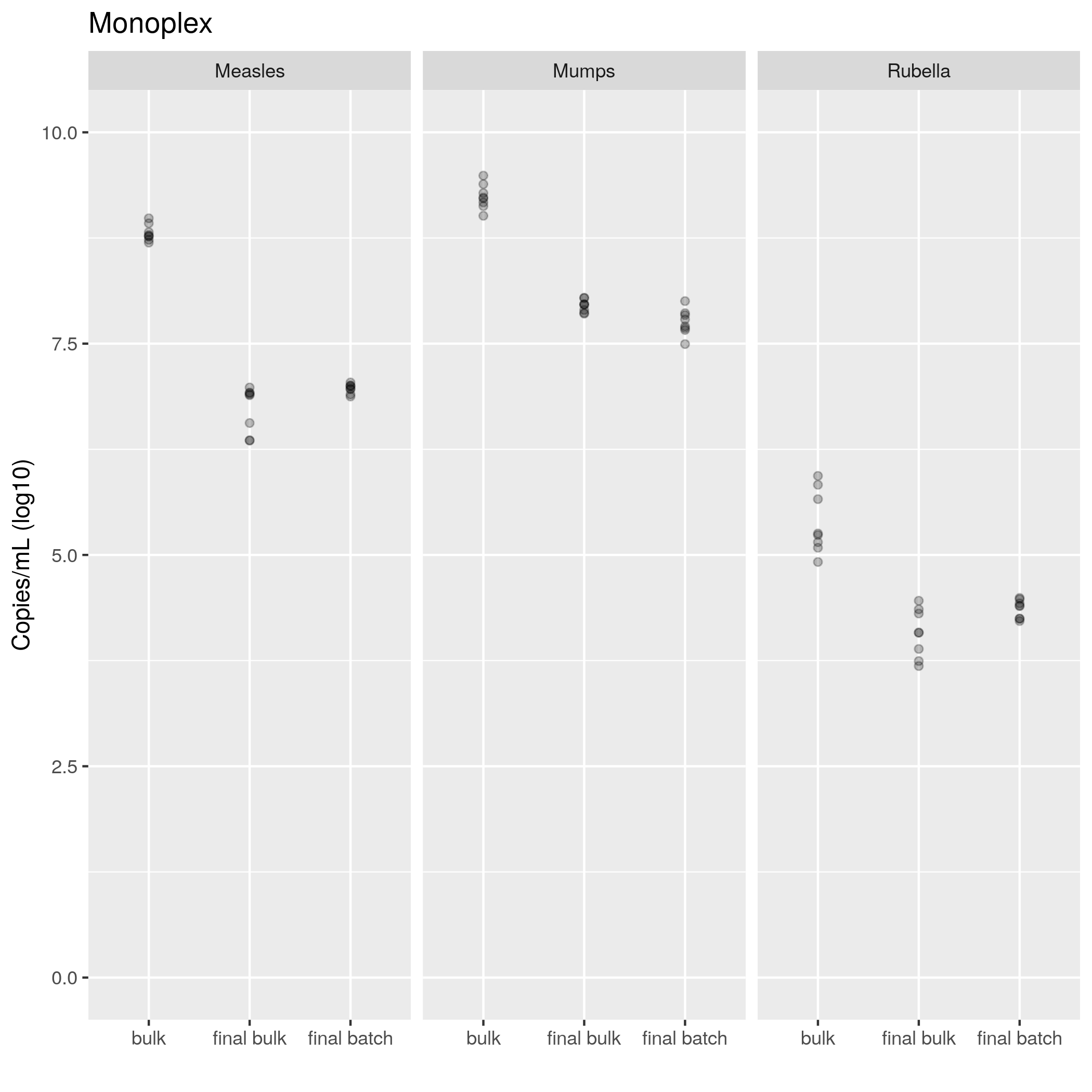
**Mumps**

The Mumps virus Monoplex assay had significant titer drops from the bulk to both final bulk and final batch formulations (Games-Howell test, p < 0.00001 and p < 0.00001, respectively). In both Mumps Biplex mixtures tested, there were significant drops in viral titer from the bulk to other formulations (Games-Howell test, p = 0.00067 and p = 0.00468 for Measles mixture, and p = 0.00025 and p = 0.00006 for Rubella mixture). These two biplex mixtures were compared to a single Monovalent Bulk experiment (table 6 and figure 3).

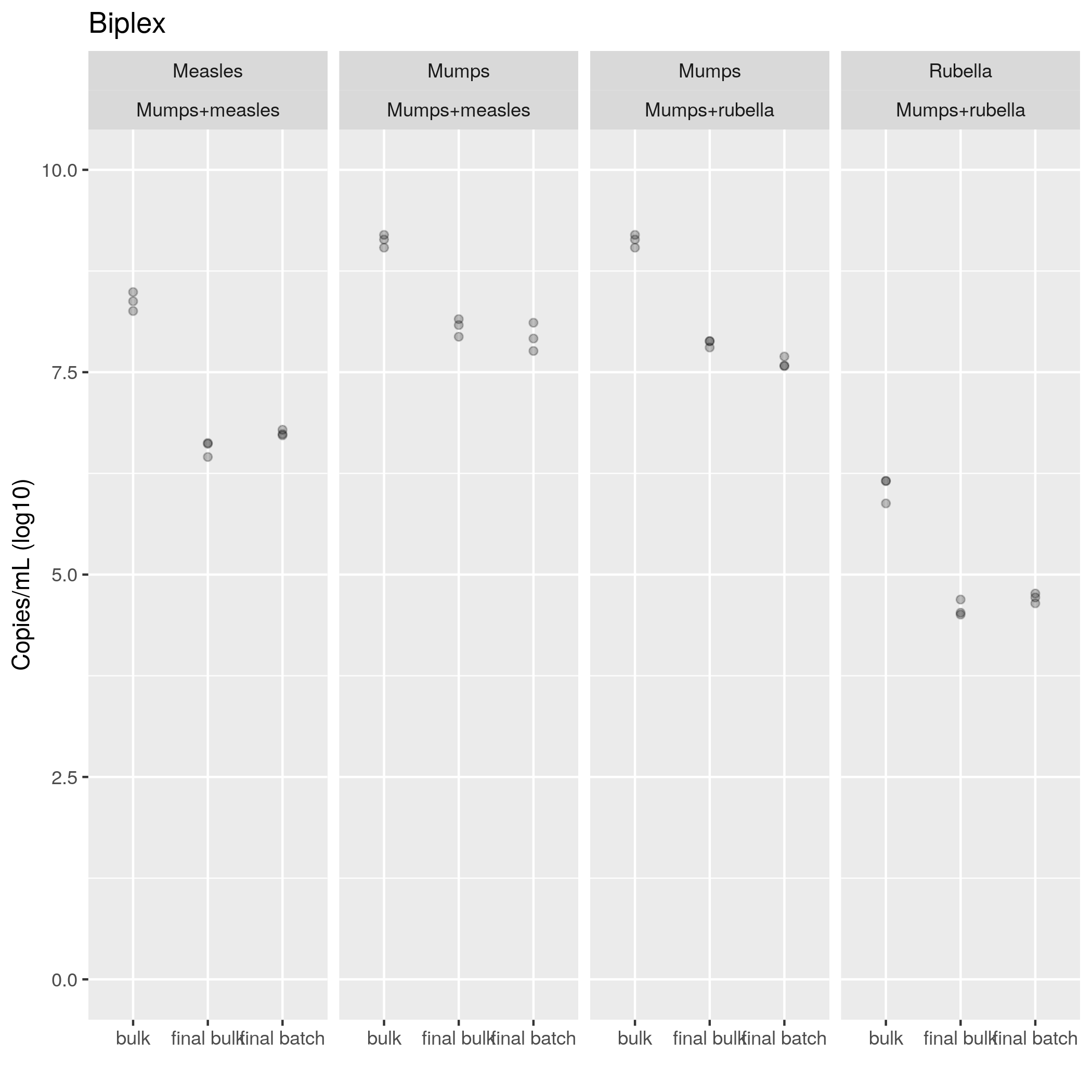
There was an average decrease of 0.1945 log10 Copies/PCR in the final batch preparation when compared to the final bulk (Games-Howell test, p = 0.02198). Such difference in titer was not observed in the Biplex assay for either Measles mixture (Games-Howell test, p = 0.58129) or the for Rubella mixture (Games-Howell test, p = 0.01966).

**Rubella**

The Rubella sample had significant titer drops from the bulk to both final bulk and final batch formulations in the Monoplex assay (Games-Howell test, p = < 0.00001 and p = 0.00017, respectively), and in the Biplex assay (Games-Howell test, p = 0.00099 and p = 0.00361, respectively). When comparing the two tested vaccine groups (final bulk and final batch), there was no significant difference in either the monoplex assay, nor in the biplex mixture with Mumps virus (Games-Howell test, p = 0.06272 and p = 0.25792, respectively).



**Figure 2**: Viral titers observed in monoplex qPCR in the different vaccine formulations.



**Figure 3**: Viral titers observed in biplex qPCR mixtures in the different vaccine formulations.

## Performance comparison between monoplex and biplex assays

# Conclusions

# References