Analysis of vaccine potency by monoplex and multiplex qPRC assay

RELATÓRIO: analise_dados_JM_2018a-v01

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Data: 19/12/2019

SUMÁRIO

Histórico do documento

Versão	Alterações
01	Versão inicial

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

1 INTRODUCTION
2 METHODS

Evaluation of monoplex and multiplex 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 multiplex 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 multiplex assays

As well as vertical comparisons between different per-virus formulations (Table 6), we performed a global horizontal comparison between the two mixtures tested in this study (Table 7) using the same methodology described above. This was decided in order to allow for a reference of comparison between all results in this study.

3 RESULTS
3.1 Evaluation of monoplex and multiplex 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 Multiplex 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 Multiplex 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 Multiplex 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 multiplex 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 multiplex 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 Multiplex 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 multiplex 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 Multiplex 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 Multiplex 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 multiplex mixture with Mumps virus (Games-Howell test, p = 0.06272 and p = 0.25792, respectively).

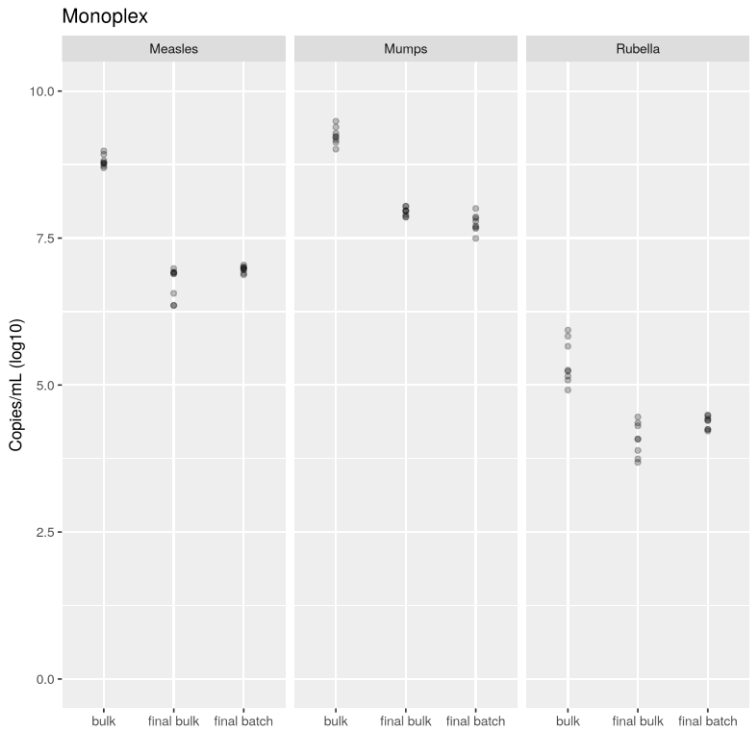

Figure 2: Viral titers observed in monoplex qPCR in the different vaccine formulations.

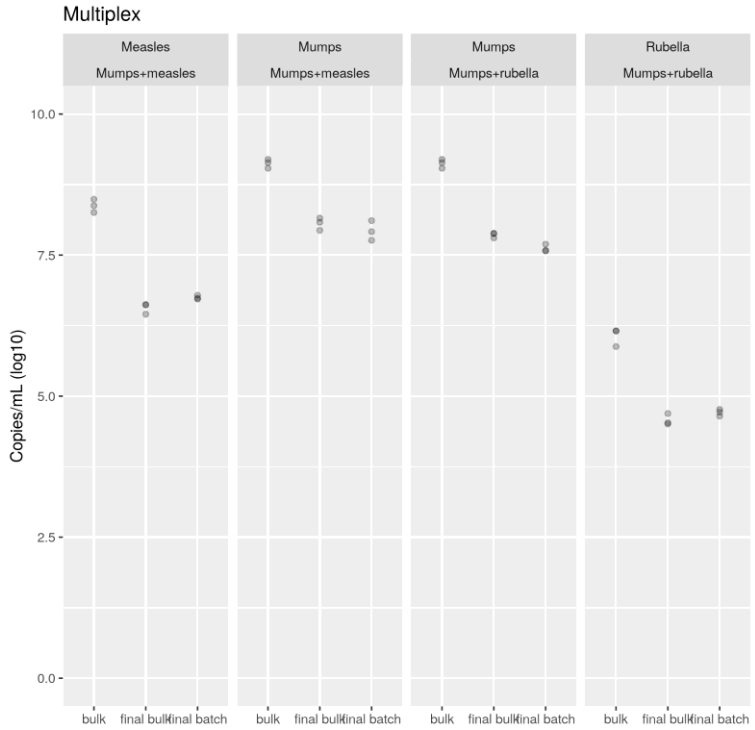

Figure 3: Viral titers observed in multiplex qPCR mixtures in the different vaccine formulations.

3.2 Performance comparison between monoplex and multiplex assays

Table 7 Comparison of vaccine potency by qPCR assay method. The p-values presented correspond to Welch-ANOVA tests comparing the monoplex assays with the multiplex assays, per Virus.

Virus Target	Monoplex Assay	Multiplex Assay	p
Measles	7.504	7.229	0.44265
Mumps	8.314	8.285	0.88968
Rubella	4.607	5.116	0.08458

When comparing the performance of the monoplex and multiplex mixtures (as seen in Table 7) there were no significant evidence of difference in mean titers for the measles virus (Welch-ANOVA p = 0.44265), the mumps virus (Welch-ANOVA p = 0.88968) and the rubella virus (Welch-ANOVA p = 0.08458), so no post-hoc tests were performed. This result indicates that both the monoplex and multiplex PCR mixtures may

have similar performances when used to test for vaccine formulation potency. If larger experiments confirm this initial result, the choice on what mixture to use could be then performed solely on laboratory logistics issues, as opposed to experimental performance issues.

4 CONCLUSIONS
5 REFERENCES