Quantoom Biosciences

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Rabies mRNA Vaccine Case Study:
Quality and Manufacturing of the IVT Performance using Ntensify™ Technology

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1) Introduction

Univercells was founded in 2013 with the mission of making biologics available for all by pursuing radical innovation in production technologies to achieve a dramatic reduction in the cost of goods and capital investment. *Quantoom Biosciences*, one of Univercells group's affiliates, was founded in 2021 in the wake of the COVID-19 pandemic to pursue Univercells' mission in the sector of nucleic acid-based medicines. Quantoom's Nfinity™ platform encompasses a comprehensive suite of technologies to enable affordable end-to-end production of RNA-based products, from DNA to bulk drug products. Their first product, Ntensify™ is a fully automated compact manufacturing system that integrates the complete RNA manufacturing process in a single continuous process train, delivering up to 100 million 50 µg doses of RNA per year per system, with smaller models for candidate screening and for production of batch sizes from 500 µg up to 200 g.

With financial support from their local government (the Walloon Region), *Quantoom* is developing an mRNA vaccine for pre- and post-exposure prophylaxis of rabies. This case study discusses the quality of the material produced by IVT in the context of this ongoing vaccine development program as an illustration of the performance of the Ntensify™ manufacturing technology.

2) Methodology

a) Production of RNA constructs

Thirteen constructs were designed encoding different antigens (i.e., different virus proteins as well as variations of those proteins) and their combinations, ranging in sequence length between 1999 and 4060 ribonucleotides. One or more batches of each construct were manufactured per the Ntensify manufacturing method (depicted in Figure 1), starting from a linearized and purified DNA plasmid. Samples were taken of each batch and analyzed.

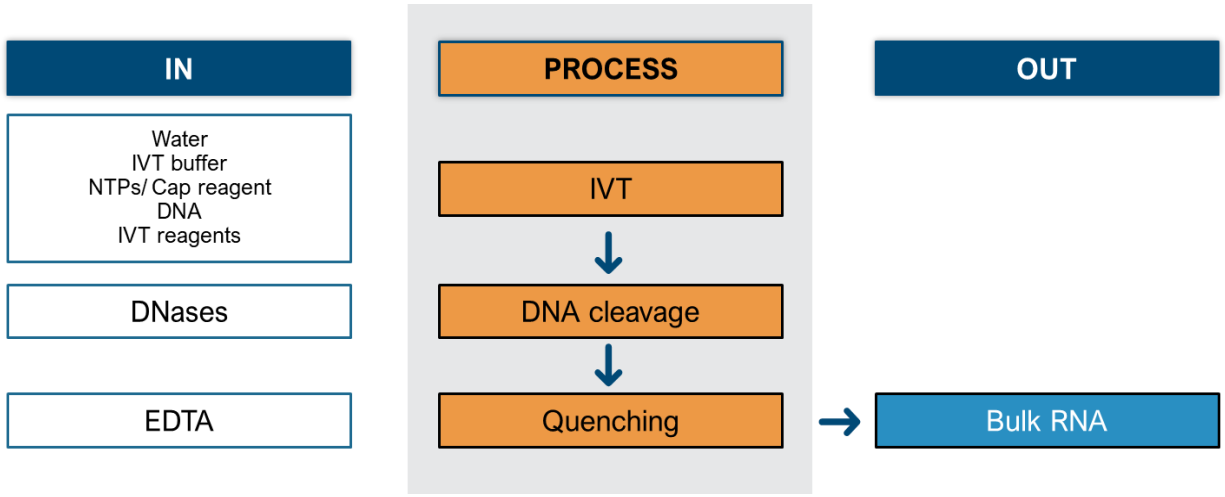


Figure 1: Outline of the Ntensify™ Manufacturing Process

b) Testing of RNA

The quality of each batch of mRNA and the performance of the manufacturing process were analytically measured as described in Table 1.

Table 1: Summary of Analytical Testing

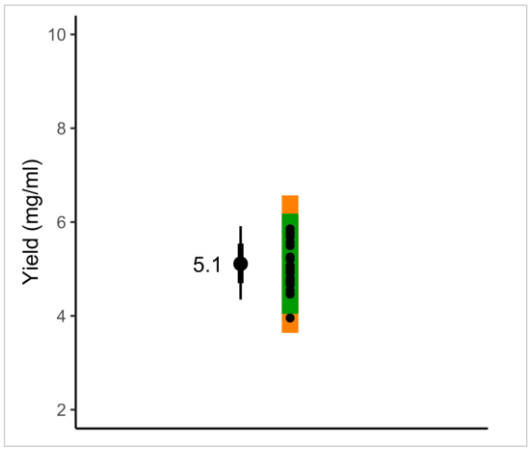
Test attribute	Analytical method	Target or acceptance criteria
Reaction yield	Spectrophotometry	For information only
Residual pDNA	qPCR	≤ 100 ng/mg mRNA
mRNA integrity	Fragment Analyzer	≥ 80%
Capping efficiency	LC-MS	≥ 80%
dsRNA content	ELISA	≤ 500 ng/mg mRNA

3) Results and discussion

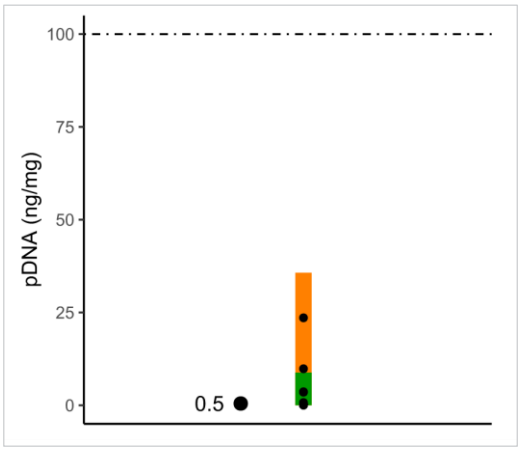
a) Physicochemical analyses

The results from physicochemical analysis of the different batches are provided in figure 2. In all cases, A Bayesian model was fitted to the data (shown in black dots) from different constructs were individually fitted to a Bayesian model for computation of statistical parameters such as the sample mean with its 95% credible interval and the posterior predictive distribution. Where necessary for the analysis, the data were first subjected to a suitable mathematical transformation (i.e., a log transformation for residual pDNA and residual protein, a logit transformation for capping efficiency).

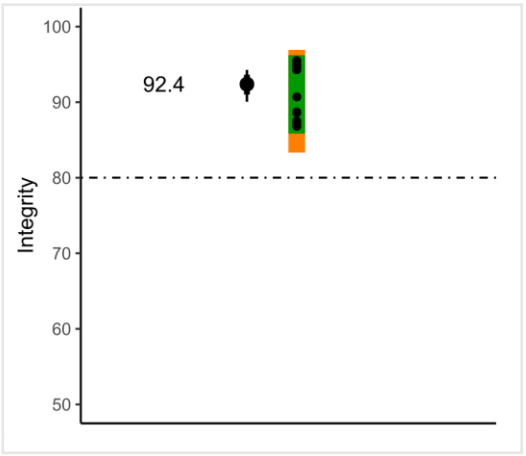
A. Reaction yield



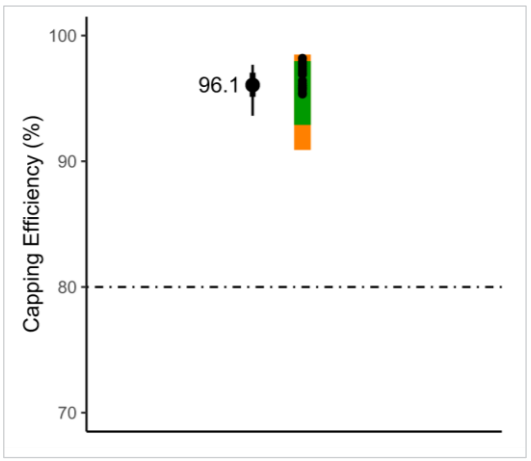
B. Residual pDNA



C. mRNA integrity



D. Capping efficiency



E. dsRNA content

All results were below both lower limit of quantification (78.7 ng/mgRNA) and thus below specifications

Figure 2: Results from physicochemical analysis of mRNA constructs. In each plot, the estimated mean and 95% credible interval are depicted by a dot and line. The colored bar represents the 5%-95% (orange) and 10%-90% (green) quantiles of the predictive distribution of the test attribute. Where shown, the horizontal dotted line represents the specification limit.

Analysis of the data shows that all batches met the specification limits, where defined. Statistical analysis furthermore asserts that future results will meet the defined specification limits with 90% probability, as evidenced by the green bar being fully above/below specification in all cases. Measurement of the performance of the process reveals that with a 90% probability, the mRNA reaction yield is above 4 mg/mL (with an average yield of approximately 5 mg/mL).

4) Conclusions and perspectives

The results from this study confirm that the IVT of the Ntensify™ manufacturing process consistently delivers material meeting predefined quality criteria regardless of construct size.

Additional Rabies constructs are being designed, in order to be tested on the IVT and the purification processes of the Ntensify™ Technology. These data will assess the IVT process robustness and purification performances.

Acknowledgments

Vandekerckhove K., Roelandts G., Lamoral-Theys D., Hop M., Finet O., Garcia A.

