Case Study

CELLREV

Enhancement Potential for a Classical Batch-Mode Upstream Measles Process*: Impact of AggreGuard™

*Full data report available upon request

Summary

Measles is a highly contagious viral infection, with most of the population having contracted it by age 15 until the first vaccine became available in 1963. The subsequent vaccination programs successfully eradicated measles in most geographies by 2000. A resurgence of the virus is being experienced globally (>2023), attributed to divergence from established vaccination programs in high-income countries and insufficient biomanufacturing capacity in low- to middle-income countries. The upstream bioprocess required for the manufacturing of the measles vaccine is well established, using classical batch mode processes and Vero cells as recommended by the World Health Organisation. This African green monkey-derived kidney epithelial cell line is highly permissive to the attenuated Edmonston strain of the measles virus, relatively easy to culture at industrial scale in serum-free media and consistently shows reproducible high virus titres. The cost of manufacturing for measles vaccines is ringfenced as a balance between a minimal acceptable profit margin and meeting high individual dosage demands. Improving any aspect of the measles biomanufacturing process is required to (a) be cost-effective; (b) be robust and reproducible; (c) require minimal technical process change, and (d) have low impact on the existing regulatory process. CellRev has a portfolio of enzymes which target extracellular matrix components, reducing cell-to-cell adhesion to promote cell culture homogeneity and reduce process optimisation. AggreGuard™, a CellRev product, was independently evaluated in a commercially relevant measles vaccine process without altering standard operating protocol, showing:

(i) ↓cell-to-cell adhesion and ↑cell culture homogeneity.
(ii) ↓ glucose/glutamine consumption and ↓lactate/ammonia production.
(iii) ↑viral infection kinetics and ↓time to peak viral titre
(iv) Up to 8% cost reduction in upstream manufacturing per 1L per day

Background

Viral vaccines play an essential role in disease prophylaxis, immunisation and outbreak control for diseases (***i.e., measles, hepatitis B, shingles, chicken pox and influenza***), with human viral vaccines valued at USD42 billion in 2023, with a projected worth of USD86 billion by 2032[1,2]. The measles vaccine[2,3,4] (***+mumps +rubella = MMR***) was valued at USD480 million in 2024, with USD190 million reported purchases by UNICEF for rollout in low-to-middle income countries (***LMIC***) in long-term supply contracts[2]. UNICEF provides the MMR vaccine to LMIC countries to ensure ~108 million mandatory first-time vaccinations annually. Measles was once classified as an eradicated disease in most geographies, but it is now showing a resurgence globally. Measles vaccination is recommended at two dosages, with one dose at 12 – 15 months of age and the second dose at 4 – 6 years of age, to promote immunity. The resurgence can be attributed to biomanufacturing and supply chain logistics challenges in LMICs, leaving millions without their first dose. A reluctance or avoidance of vaccination in other geographies adds to this disease resurgence phenomenon. The WHO reported ~10.5 million new cases of measles globally in 2023 and expects the disease burden to grow.

Biomanufacturing complexity and regulatory requirements result in cost of goods (*COGS*) comprising 20-85% of vaccine market value[4], with estimated cost per dose dependent on the available biomanufacturing capacity. The cost of manufacturing[3,4] in a typical viral vaccine bioprocess is distributed between upstream (**~30%; USP**) and downstream (**~70%; DSP**) processes, with the challenges in developing an USP impacting the full manufacturing value chain, via affecting the development cost and timelines as well as jeopardising envisioned commercialisation. Modern technologies and methodologies can yield a single MMR dose to the patient at ~USD3, where legacy technologies typically reach ~USD6[3], equating to manufacturing costs of USD216 million – USD650 million for first-time MMR vaccinations. However, the cost per dose can differ significantly per geography, manufacturer and end-point distributor. The most prominent MMR manufacturers[3,4] are: (i) Merck & Co (***USA, International***); (ii) GlaxoSmithKline (***Europe***); (iii) Sanofi Pasteur (***LMIC***); (iv) Serum Institute India (***LMIC***) and (v) Biofarma (***Indonesia, South-East Asia***). These companies use biomanufacturing technologies dependent on microcarriers[3,5,6] for adherent Vero cells, which are grown in bioreactors ranging from 1L – 3000L.

Vero cells are a continuous, non-tumorigenic cell line which has been a vaccine-manufacturing workhorse cell line, as it is well-characterised and recommended by the WHO[6] to produce vaccines for diseases such as measles, rabies and polio. This mammalian cell line is regulatory prequalified and well-characterised for industrial applications but presents significant cell-to-cell adhesion (***C2CA***), or cell clumping, challenges resulting in increased cell culture heterogeneity at industrial-relevant scales. The C2CA-induced aggregation causes increased bioprocess complexity[5,6,7], and unfavourable cell culture conditions, which then result in cell loss, and greater batch-to-batch variation during production, or 5-30% of total batches lost.

The evaluation of AggreGuard™ for this communication was done independently by bespark*bio, an Austrian-based contract research and development organisation. A summation of the full report follows in the next few sections.

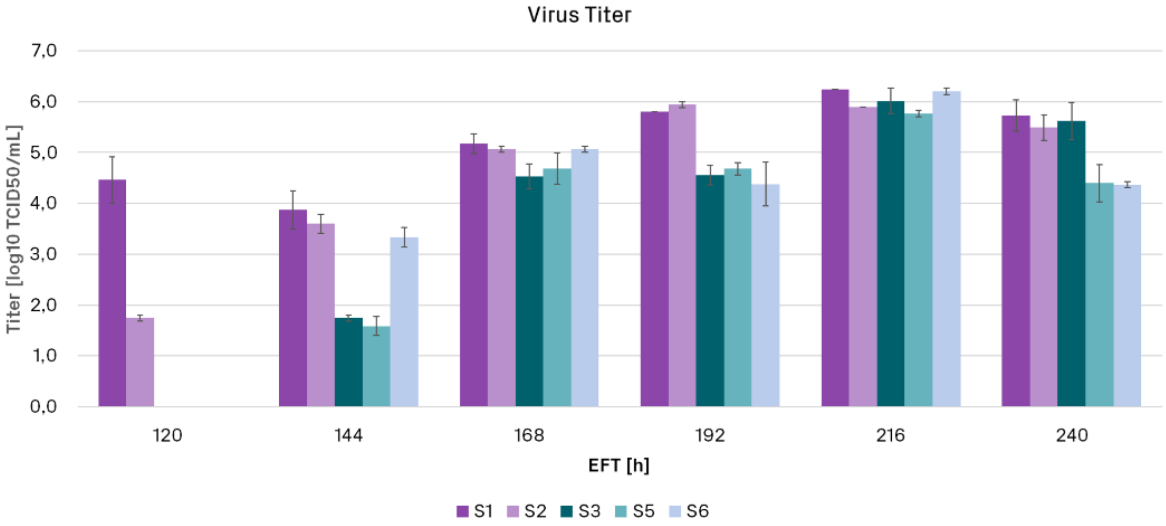
Key Data

Here we report the impact of AggreGuard™, an enzymatic formulation to control C2CA, in an industrially relevant measles biomanufacturing process. A classical batch mode upstream process was used to infect Vero cells with a Schwarz strain virus, with AggreGuard™ introduced to the process prior to infection, which was on process day 4 during a media change. No workflow modification was required to introduce AggreGuard™. Initial dosage optimisation of AggreGuard™ was done previously in spinner flasks, with the best conditions taken to 1L stirred tank bioreactors for final evaluation.

During initial parameter optimisation, AggreGuard™ beneficially influenced viral infection kinetics and showed potential to reduce the time to reach peak viral titre (***192h vs. 240h***) when used with Kolliphor® P188 (***Fig. 2 – S1***). The synergistic effect of AggreGuard™ and Kolliphor® P188 was significant when compared to each component being used independently or not being present at all. Based on initial observations, two sets of conditions were chosen (***Fig. 2 – S1, S2***) to proceed to 1L stirred tank bioreactors (***Eppendorf DASGIP Multi-Bioreactor System***). A combination of the two products showed similar trends (Fig. 2 – U1, U2) as observed in spinner flasks; however, the duration to peak viral titre was more subdued than observed in spinner flasks. It should be noted that no in-depth process optimisation was done as part of the objectives to determine if AggreGuard™ can be integrated into a commercially relevant process. Optimisation of process parameters (***i.e., agitation, aeration***) could show a more pronounced effect. Daily cell counts (data not shown) indicated there

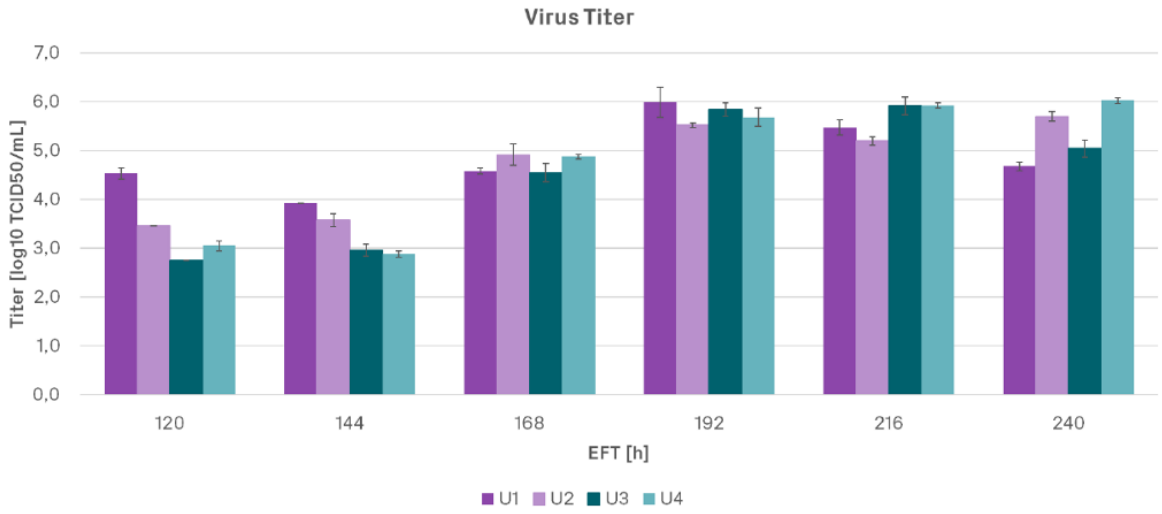
was no significant difference in cells/mL between the different conditions for the spinner flask (*Fig. 1*) or stirred tank bioreactor (Fig. 2) experiments.

AggreGuard™ was fully compatible with Kolliphor® P188, showing synergistic effects on the overall bioprocess, resulting in reduced C2CA (↑*cell culture homogeneity, result not shown*) and improved metabolic profiles (*Fig. 3, A-D*). Lactate dehydrogenase (*LDH*) is a biomarker for cellular stress, only released from cells once the cell membrane integrity becomes compromised. Cells cultured with AggreGuard™ showed lower LDH concentrations (*Fig. 3, E*) overall in similar conditions to cells cultured with Kolliphor® P188 only.



Spinner ID	AggreGuard™ (1U/mL)	Kolliphor (0.2%)	Infection
S1	✓	✓	✓
S2	/	✓	✓
S3	/	/	✓
S4	/	/	/
S5	✓	/	✓
S6	✓	/	✓

Figure 1: Spinner flask parameters investigation to determine how the classical measles bioprocess performs with and without AggreGuard™ and Kolliphor® P188. When used synergistically (S1), the effect on the viral titre is more pronounced than when AggreGuard™ (S5, S6) or Kolliphor® P188 (S2) are used on their own.



Bioreactor ID	AggreGuard™ (1U/mL)	Infection
U1	✓	✓
U2	✓	✓
U3	/	✓
U4	/	✓

Figure 2: 1L Stirred tank bioreactors were used to evaluate AggreGuard™ and Kolliphor® P188 in combination (U1, U2) or Kolliphor® P188 only (U3, U4). Similar trends in viral titre were seen, as for spinner flasks, when both products were used in combination.

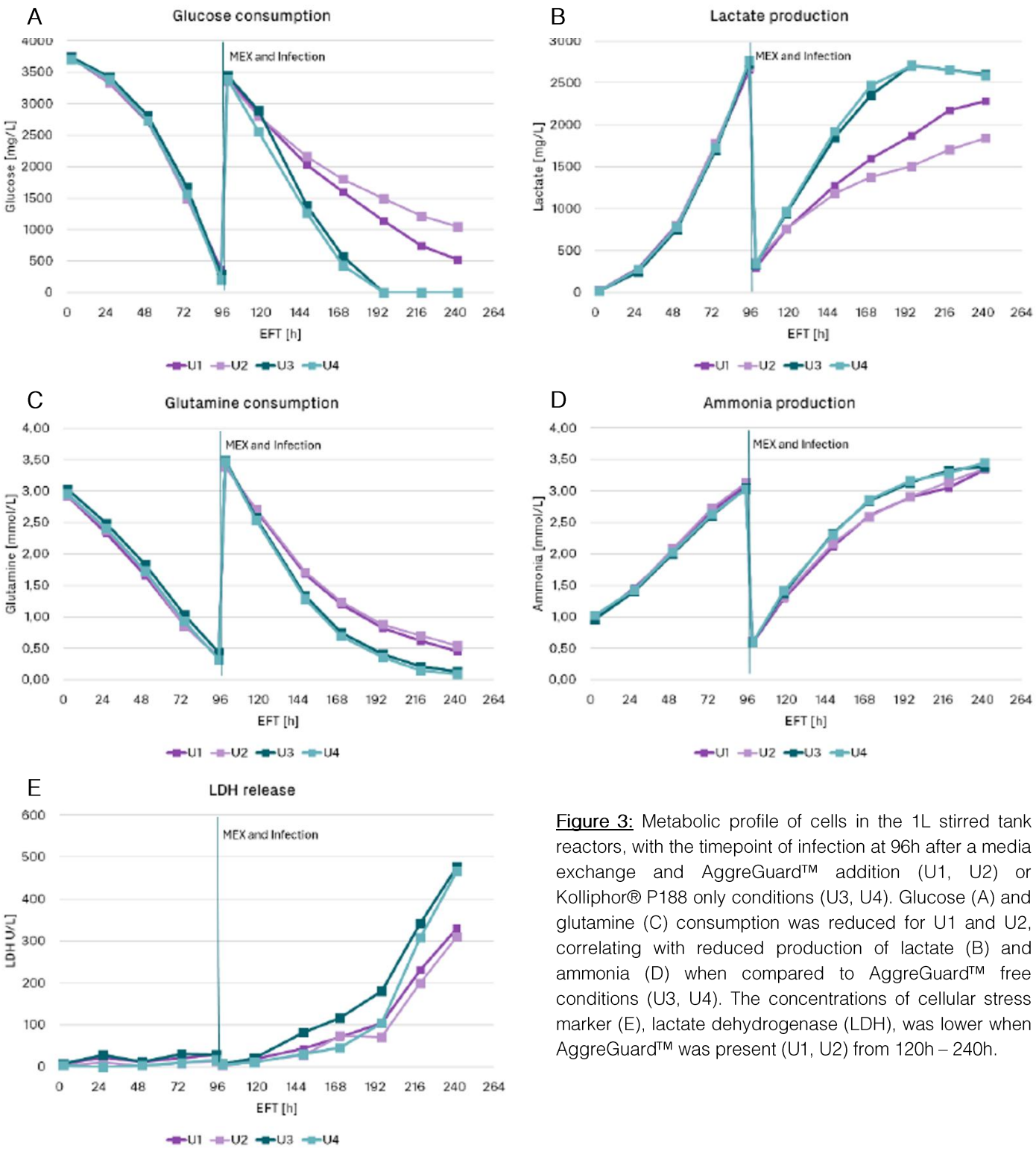


Figure 3: Metabolic profile of cells in the 1L stirred tank reactors, with the timepoint of infection at 96h after a media exchange and AggreGuard™ addition (U1, U2) or Kolliphor® P188 only conditions (U3, U4). Glucose (A) and glutamine (C) consumption was reduced for U1 and U2, correlating with reduced production of lactate (B) and ammonia (D) when compared to AggreGuard™ free conditions (U3, U4). The concentrations of cellular stress marker (E), lactate dehydrogenase (LDH), was lower when AggreGuard™ was present (U1, U2) from 120h – 240h.

Table 1: Assumptions* for determining the overall cost of manufacturing and an estimated commercial value of the 1L bioreactor production reported for this study.

Assumptions	Value	Rationale
Upstream process cost contribution	30 %	Based on averages for upstream processes
Downstream process cost contribution	70 %	Based on averages for downstream processes
Plaque forming units (P.F.U.) available	500 x 10^6	Total virus particles produced was 1.0 x 10^6 /mL (= 1.0 x 10^9). A 50% yield was estimated based on overall losses during downstream processing as well not all viral particles able to form P.F.U.
Total dosages available	20 000	Dosages vary (1.0 x 10^3 – 50 x 10^3 P.F.U.) depending on vaccine type and formulation used, where a median dosage of 25 x 10^3 P.F.U. was chosen.
Commercial value of the batch	£60 000 (≈ US$82 000)	Cost per dose varies (£2.00 - £90.00) depending on the manufacturer, distributor and the geography of sale. A cost per dose of £3 was used to assign an estimated value to this measles batch. A commercial margin of 15% was assumed.
Scaling assumptions	N/A	Cost of manufacturing per litre as the process scales was determined through interviews with industry insiders, consultants and advisors

*These values are not absolutes, only closest possible estimation for simplicity. The viral vaccine industry presents with high variation in overall process design, scale of production, vaccine types within a disease class, cost per dose per geography.

Achieving peak viral titre within 192h (*6 log_{10}, Fig. 1 + Fig. 2*) can have significant implications for reducing the cost of manufacturing in the upstream process. When basic assumptions (*Table 1*) are made for the cost of manufacturing and overall value of the process at 1L scale, a single day reduction in upstream manufacturing can have an overall cost reduction of £ 1,500. Peak viral titre was achieved two days earlier than with a standard measles process. Biomanufacturing costs are highly expensive at low volumes, with a significant drop in production cost per litre as the process scales volumetrically.

Table 2: An estimated scaling model for production volume (*1L – 1000L*), showing cost (*£*) saving possible with a reduction of a single process day.

Parameter	Values			
Volume (L) of production per batch	1	50	500	1000
Cost of manufacturing (£) / L	60 000	12 000	1 200	720
Total Saving (£) / L	1 500	300	30	18
Total Saving (£) / Volume of production	**1 500**	**15 000**	**15 000**	**18 000**

A general volumetric bioprocess scale-up (*Table 2*) was used to model out a hypothetical cost scaling model for the 1L upstream bioreactor work presented in this document, as well as within the assumptions of Table 1. As the volume of the bioreactor increases, there is a significant decrease in the cost of manufacturing per L *(£ 60,000 → £720)*, with a drastic decrease in the cost saving per L *(£ 1,500 → £18)*. However, when total savings per scale are considered, between £ 15,000 and £ 18,000 (*50L → 1000L*) per batch can be achieved for the upstream process with a single day reduction in process duration. On average, ≤ 2.5% cost reduction, is possible per single day reduction in process duration.

Conclusion(s)

The work presented in this report is early data – the first evaluation of AggreGuard™ in a commercially relevant measles vaccine upstream production process. This process is functional but not yet fully optimised for this pilot study of AggreGuard™.

The introduction of AggreGuard™ positively influenced the viral infection kinetics, facilitating peak viral titre to be reached up to two days earlier than with the conventional process. Previously reported benefits of AggreGuard™ (*i.e., ↓cell-to-cell adhesion and ↑cell culture homogeneity*) were observed but not shown in this report, where the positive effect on the overall metabolic state of the Vero cells was deemed more impactful.

Basic assumptions used to estimate the cost of manufacturing highlight the cost reduction impact (***≤ 2.5% per day***) that AggreGuard™ can achieve in an upstream process at various scales, based on the example of duplicate batch processes. The potential benefit of AggreGuard™ increases over an annual production cycle when multiple batches are produced:

- A reduction (***1 – 2 days***) in upstream process time per batch can facilitate the introduction of additional batches during the same annual production window.
- The potential cost reduction (***≤ 2.5% per day***) associated with reduced process time per batch, cumulatively increases over an annual production cycle.

Future work with AggreGuard™ will investigate batch-to-batch consistency as well as interaction with other viral vaccine processes (*i.e. influenza*)

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