Controlling host cell DNA in viral vaccine manufacturing

An integrated evaluation of EndoCleave in a Vero cell–based measles virus process, assessing endonuclease performance within the full context of viral vaccine bioprocessing.

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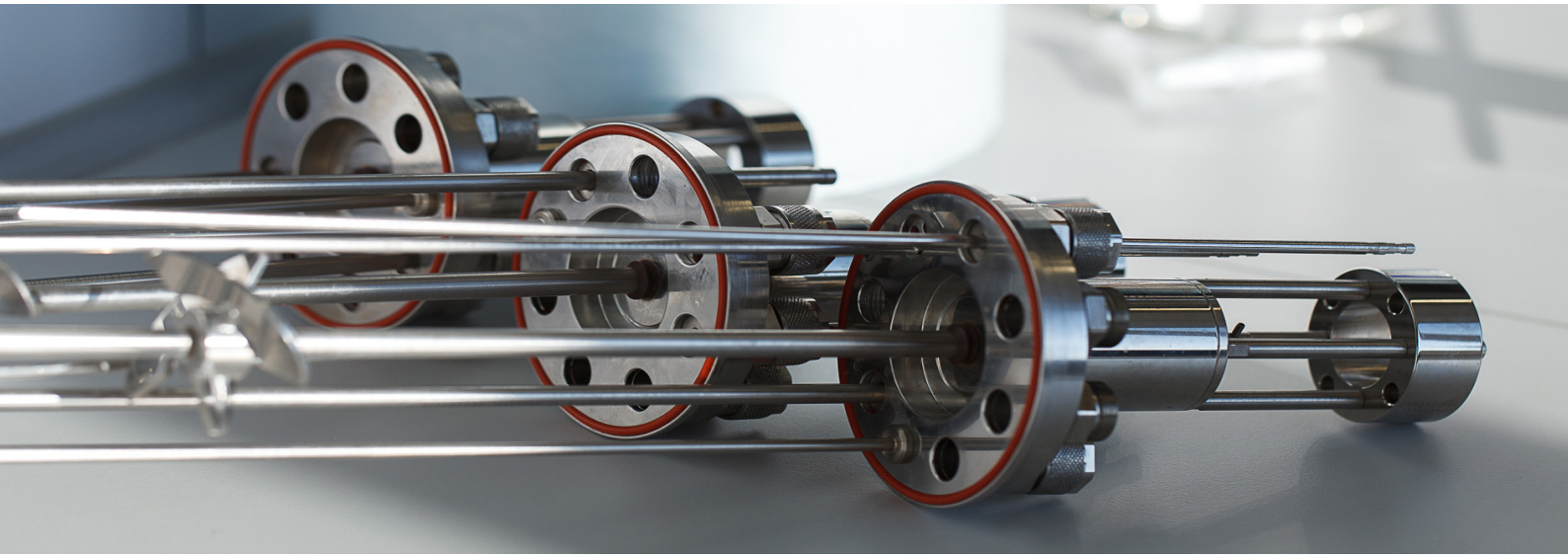


ABSTRACT

Residual host cell DNA is a critical process-related impurity in viral vaccine and viral vector manufacturing. Endonuclease treatment is widely used to digest this DNA and can substantially reduce its levels. However, the residual DNA concentration in the final process intermediate also depends on the degree of product concentration required to reach the target dose.

EndoCleave (from Roche) and a commercially available benchmark endonuclease were evaluated in a Vero cell-based measles virus manufacturing process. A design-of-experiments study first assessed the effects of enzyme concentration, incubation time, temperature and added $MgCl_2$ for both enzymes. They were then evaluated in a process sequence comprising endonuclease treatment, clarification, tenfold concentration by tangential flow filtration (TFF) and diafiltration.

Meeting the final host cell DNA specification requires more than efficient enzymatic digestion. Endonuclease performance must be assessed alongside upstream product titer, the TFF concentration factor and downstream impurity clearance, since these collectively determine the residual DNA level per dose.

The two endonucleases performed comparably, each reducing amplifiable Vero host cell DNA by more than 99%, while DNA-fragment size analysis demonstrated a pronounced shift from higher-molecular-weight DNA toward predominantly sub-200-bp fragments. No consistent loss of infectious virus titer attributable to treatment was observed.

1. Introduction

Host cell DNA (hcDNA) is a process-related impurity that must be adequately reduced and controlled during the manufacture of viral vaccines and viral vectors. Cytopathic effects, cell detachment and cell lysis can release substantial amounts of chromosomal DNA into the harvest, increasing the residual DNA burden that must be addressed during downstream processing.

In many biopharmaceutical processes, chromatography contributes significantly to hcDNA clearance. For large and structurally complex viral products, however, chromatographic purification may compromise product recovery[1]. In such cases, a greater clearance burden is placed on the remaining downstream unit operations, particularly clarification, endonuclease treatment, and tangential flow filtration (TFF). Endonucleases support DNA clearance by fragmenting long DNA molecules into smaller species that may be more readily removed during subsequent processing.

Endonuclease-mediated reduction of DNA fragment size may also reduce the potential biological activity of residual cellular DNA. FDA guidance identifies approximately 200 bp as the size below that of a functional gene and recommends consideration of both the amount and size distribution of residual DNA[2].

The final hcDNA concentration is nevertheless determined by the complete manufacturing process, not by endonuclease performance alone. It depends on the initial DNA burden, the extent of enzymatic digestion, downstream clearance and the concentration factor required to achieve the intended product dose. This concentration factor is directly linked to the infectious titer generated upstream. When harvest titers are low, greater downstream concentration is required and the residual DNA remaining after effective nuclease treatment may be concentrated together with the product. Consequently, an elevated hcDNA concentration in the final drug substance does not necessarily indicate inadequate nuclease performance.

In this study, EndoCleave was benchmarked against a commercially available endonuclease under process-relevant conditions.

The objectives were to:

01 Identify the principal process parameters governing DNA reduction by EndoCleave and the commercially available endonuclease.

02 Compare both enzymes within an integrated clarification and TFF process.

03 Assess their effects on total DNA, host cell-specific DNA, DNA-fragment size and infectious virus recovery.

04 Evaluate how upstream titer, target dose, and downstream concentration requirements influence final residual hcDNA levels.

2. Materials and Methods

2.1 Generation of measles virus harvest material

Measles virus-containing harvest material was generated using Vero cells grown on Cytodex 1 microcarriers in four parallel 1 L stirred-tank bioreactors. Cells were cultivated in serum-free VP-SFM supplemented with 4 mM L-glutamine at 36.5°C.

Following cell expansion and medium exchange, the cultures were infected with measles virus at a multiplicity of infection of 0.001 and further cultivated at 32.5°C for virus production.

The cultures were harvested six days after infection and supernatants were pooled to generate a homogeneous batch for endonuclease characterization and downstream processing.

Upstream performance was monitored throughout the seed train and bioreactor process by cell concentration and viability, together with glucose, glutamine, lactate, ammonium and lactate dehydrogenase concentrations measured on a Cedex Bio Analyzer (Roche). Infectious virus titers were determined using a $TCID_{50}$ assay based on cytopathic-effect formation in Vero cells. Total double-stranded DNA was quantified using fluorescence-based Qubit assays.

2.2 Design-of-experiments-based endonuclease characterization

EndoCleave and the commercially available benchmark endonuclease were evaluated independently using clarified material from the same pooled measles virus harvest.

A three-level full factorial design was used to investigate four process parameters:

Table 1: Parameters evaluated in DOE study.

Parameter	Evaluated levels
Endonuclease concentration	25, 50, and 100 U/mL
Incubation time	1, 2, and 3 h
Temperature	25, 30, and 35°C
Added $MgCl_2$ concentration	2, 4, and 6 mM

The design comprised 81 factorial conditions and 54 predefined replicates, controls and center point reactions, resulting in 135 reactions per enzyme.

Total double-stranded DNA was quantified before and after treatment using the Qubit 1X dsDNA Assay. Normalized DNA reduction was used as the response variable. Statistical evaluation was performed independently for each enzyme using regression modeling and analysis of variance, with $p < 0.05$ applied as the significance threshold. Main-effects plots, contour plots, and response surfaces were generated to visualize the influence of the investigated parameters.

2.3 Integrated downstream process evaluation

Following the design-of-experiments study, EndoCleave and the benchmark endonuclease were evaluated under fixed process conditions of 100 U/mL, 30°C, and 3 h incubation time.

For each enzyme, two process configurations were investigated:

1. Endonuclease treatment before clarification.
2. Clarification followed by endonuclease treatment.

Clarification was performed using depth filters with a nominal pore size of 3 µm.

Following endonuclease treatment and clarification, the material was processed by tangential flow filtration using a Hydrosart membrane with a nominal molecular-weight cut-off of 300 kDa. The TFF process comprised a tenfold volumetric concentration step, designated CF10, followed by ten diavolumes of diafiltration, designated DV10.

Processing was performed under controlled transmembrane pressure conditions. Diafiltration was conducted using a Tris-based buffer containing sucrose, arginine, and histidine.

Each downstream process configuration was evaluated in a single experimental run. The results therefore describe observed process trends and do not establish statistical equivalence between enzymes or process configurations.

Samples collected throughout the downstream process were analyzed for infectious measles virus titer using a $TCID_{50}$ assay and for total double-stranded DNA using the Qubit 1X dsDNA Assay.

Residual Vero host cell DNA was quantified at selected process stages (harvest, after endonuclease treatment, and following concentration and diafiltration) using the extraction-based resDNASEQ™ Quantitative Vero DNA qPCR Kit.

For clarity, results obtained using the Qubit 1X dsDNA Assay are referred to throughout this application note as total DNA, whereas results obtained using the Vero-specific qPCR assay are referred to as host cell DNA (hcDNA). Total DNA represents fluorescence-detectable double-stranded DNA, while hcDNA represents extractable Vero DNA containing an intact amplifiable qPCR target. The fluorescence-based total DNA assay and the host cell-specific qPCR assay were used as complementary analytical methods. The Qubit assay enabled comparative assessment of multiple process conditions, whereas qPCR was used to determine residual amplifiable Vero host cell DNA at selected downstream stages.

DNA-fragment size distributions were determined in untreated harvest material and following treatment with either EndoCleave or the benchmark endonuclease using a fragment analyzer (Agilent).

3. Results and Discussion

3.1 DoE identified comparable parameter effects

Statistical evaluation of the design-of-experiments data identified incubation time, enzyme concentration and temperature as significant parameters affecting total DNA reduction for both EndoCleave and the benchmark endonuclease. Variation in added $MgCl_2$ did not produce a measurable effect for either enzyme. Additionally, no statistically significant interaction effects were identified.

The responses of both enzymes were adequately described by regression models incorporating the significant main effects and a quadratic enzyme-concentration term. Incubation time was the dominant process parameter with consistent total DNA reduction between one and three hours for both enzymes. Increasing enzyme concentration further improved total DNA reduction, although the incremental benefit decreased at higher concentrations. Temperature had a moderate positive effect within the investigated range of 25–35°C.

The regression models described the experimental data well, with an R^2 of 0.93 for EndoCleave and 0.86 for the benchmark endonuclease. Both models predicted a maximum total DNA reduction of 65–70% within the evaluated design space. EndoCleave and the benchmark endonuclease therefore showed comparable qualitative behavior in clarified measles virus harvest. Incubation time was the strongest driver for both enzymes, followed by enzyme concentration and temperature.

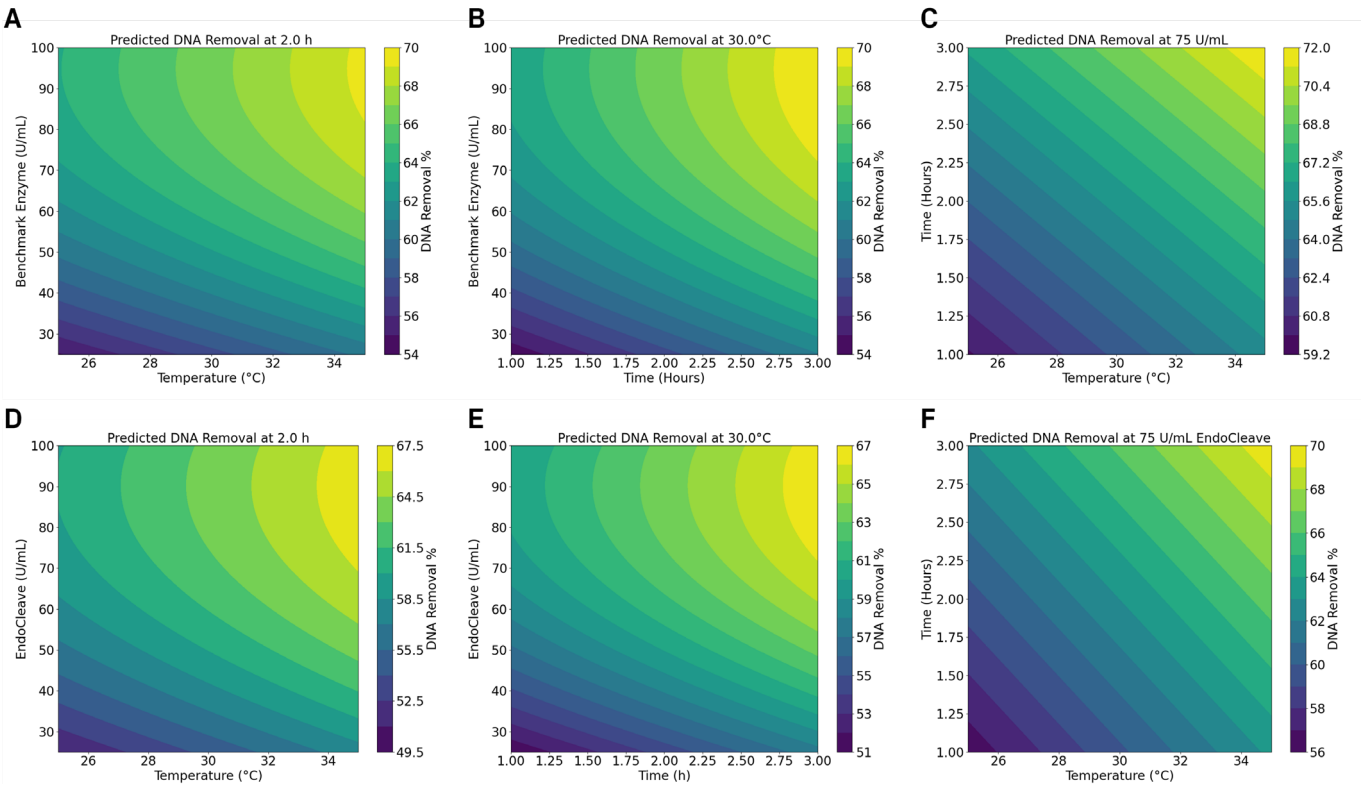


Figure 1: Results from the DOE experimentation. Predicted total DNA reduction for EndoCleave (A–C) and the benchmark endonuclease (D–F) as a function of temperature, incubation time and enzyme concentration. Both enzymes showed comparable response patterns, with incubation time having the strongest effect.

The evaluated parameter ranges were selected to reflect practical process constraints. Higher temperatures were excluded because of the limited thermal stability of measles virus, while longer incubation times were considered undesirable due to the associated increase in processing time. Higher enzyme concentrations were also not extended further, as they provided only limited additional total DNA reduction.

Conditions of 100 U/mL, 30°C, and 3 h were therefore selected for the integrated downstream evaluation as a practical balance between total DNA reduction, product stability and process time.

3.2 Integrated evaluation confirmed efficient hcDNA removal

EndoCleave and the benchmark endonuclease were evaluated with treatment performed before or after clarification. No substantial change in infectious virus titer was observed for either enzyme or treatment position. Virus titer increased tenfold during TFF concentration, as expected from the volume reduction, and remained stable during diafiltration.

Both endonucleases reduced total DNA by 65–70%. The order of clarification and endonuclease treatment affected individual intermediate values but did not produce a consistent difference in overall process performance.

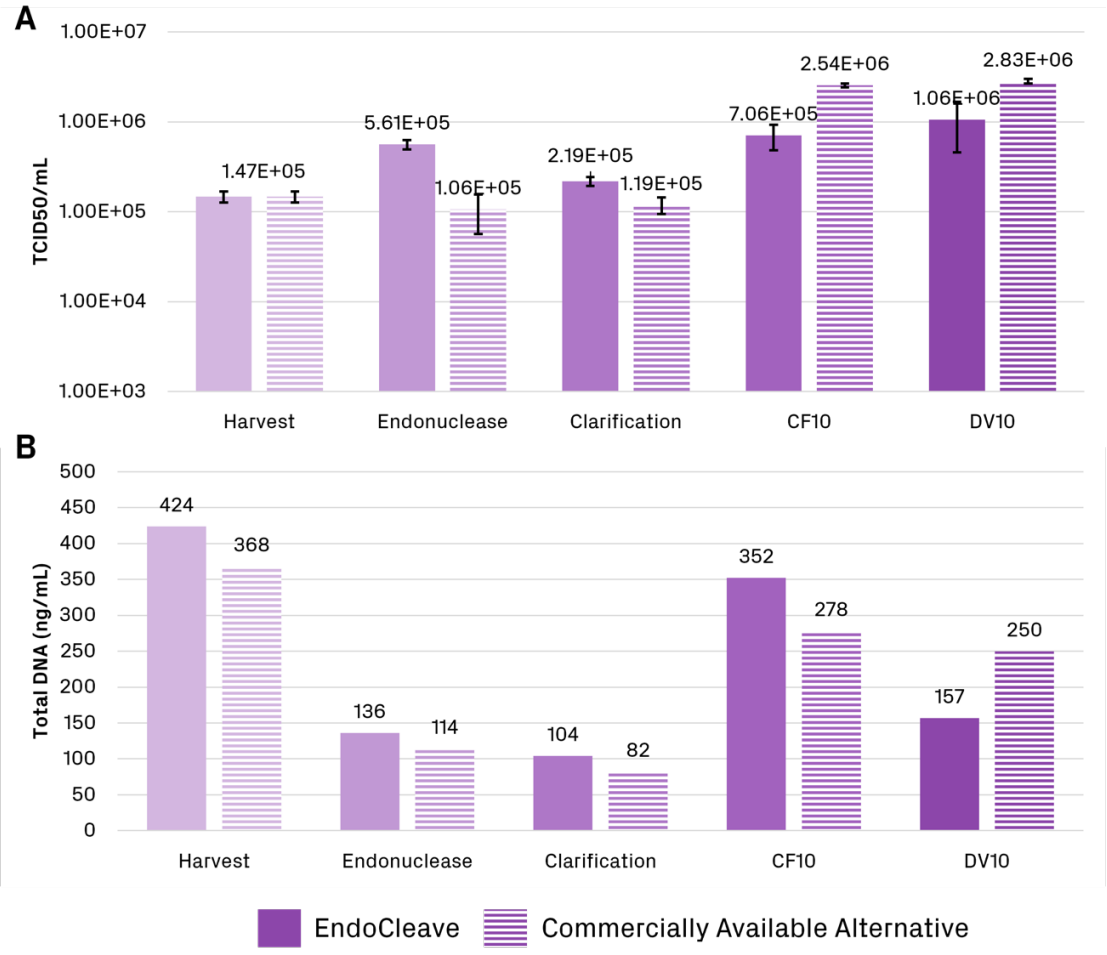


Figure 2: Endonuclease treatment-before-clarification process stream. (A) Infectious virus titer, (B) fluorescence-based total DNA. Error bars represent technical assay replicates n=3. CF10 – Concentration Factor 10; DV10 – Diafiltered/Buffer Exchange Drug Substance.

For graphical presentation (Figure 2), the endonuclease treatment-before-clarification configuration was selected as a representative process stream for comparison. In this stream, total DNA decreased from 424 ng/mL in the harvest to 136 ng/mL for EndoCleave treatment (67% reduction) and from 368 ng/mL in the harvest to 114 ng/mL for the benchmarked endonuclease treatment (69% reduction). As expected, Total DNA levels subsequently increased during ten-fold concentration (CF10).

3.3 Endonuclease treatment shifts DNA toward fragments below 200 bp

DNA-fragment size analysis was performed to assess the physical fragmentation of DNA following endonuclease treatment. Untreated material showed a broad fragment-size distribution dominated by higher-molecular-weight DNA, with the main signal extending into the kilobase range. In contrast, treatment with either EndoCleave or the benchmark endonuclease resulted in a pronounced shift toward smaller DNA fragments. For both enzymes, the predominant DNA signal following treatment was located below 200 bp, with lower-intensity signal extending into larger fragment sizes.

The fragment-size profiles therefore confirm extensive DNA fragmentation by both endonucleases under the evaluated process conditions. EndoCleave and the benchmark endonuclease showed comparable qualitative shifts in DNA-fragment size, with the predominant signal following treatment occurring below 200 bp. This is consistent with the fragment-size range identified in FDA guidance as being below the size of a functional gene[2].

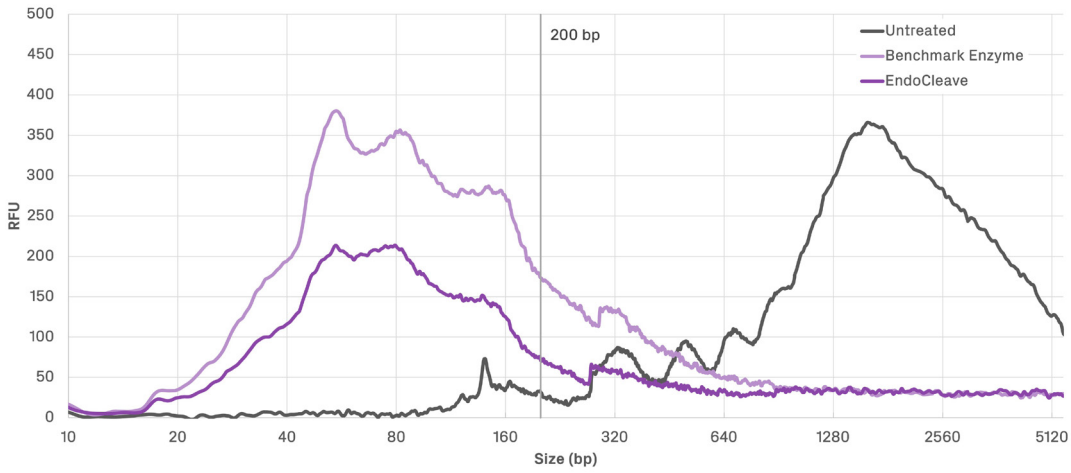


Figure 3: DNA fragment-size distributions before and after endonuclease treatment. Electropherograms of untreated material and material treated with EndoCleave or the benchmark endonuclease are shown as fluorescence signal (RFU) as a function of DNA fragment size. The vertical line indicates 200 bp.

3.4 Host cell-specific DNA clearance and co-concentration during TFF

To complement the fluorescence-based total-DNA assay with a host-cell-specific measurement, residual Vero hcDNA was quantified by qPCR at selected process stages. As shown in Figure 4, Vero hcDNA decreased from 2,086 ng/mL in the harvest to 5.8 ng/mL following EndoCleave treatment, corresponding to a reduction of more than 99%. The concentration subsequently increased 10.4-fold to 60.6 ng/mL in the DV10 intermediate, closely matching the nominal tenfold TFF concentration factor. This indicates that the small residual amplifiable hcDNA that was retained was co-concentrated proportionally with the viral product, consistent with the applied concentration factor. Comparable overall trends were observed for the benchmarked endonuclease and when EndoCleave was applied after clarification.

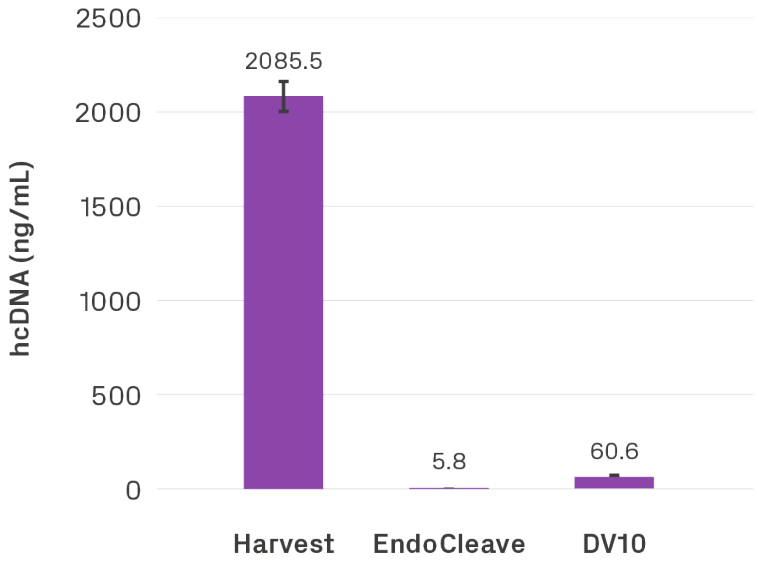


Figure 4: Residual Vero host cell DNA (hcDNA) concentration measured by qPCR in the initial harvest, following EndoCleave treatment, and in the final diafiltered material (DV10) in the treatment-before-clarification process stream.

3.5 Beyond digestion, upstream productivity governs hcDNA compliance

The TFF concentration factor is dependent on the planned dose and is closely linked to the product titer achieved during USP. A high upstream titer requires less volume reduction to reach the target drug-substance concentration and consequently limits enrichment of the residual hcDNA fraction.

As shown in the present study, the increase in hcDNA concentration between initial endonuclease treatment and the final DV10 intermediate demonstrates why upstream productivity must be considered when defining the overall hcDNA-control strategy. Once qPCR-detectable hcDNA reduction already exceeds 99%, further nuclease intensification may provide limited additional benefit with respect to the final hcDNA concentration, particularly where downstream co-concentration becomes the dominant factor. Achieving compliance with the final hcDNA target may therefore require higher upstream productivity, a lower downstream concentration factor, or additional clearance during DSP.

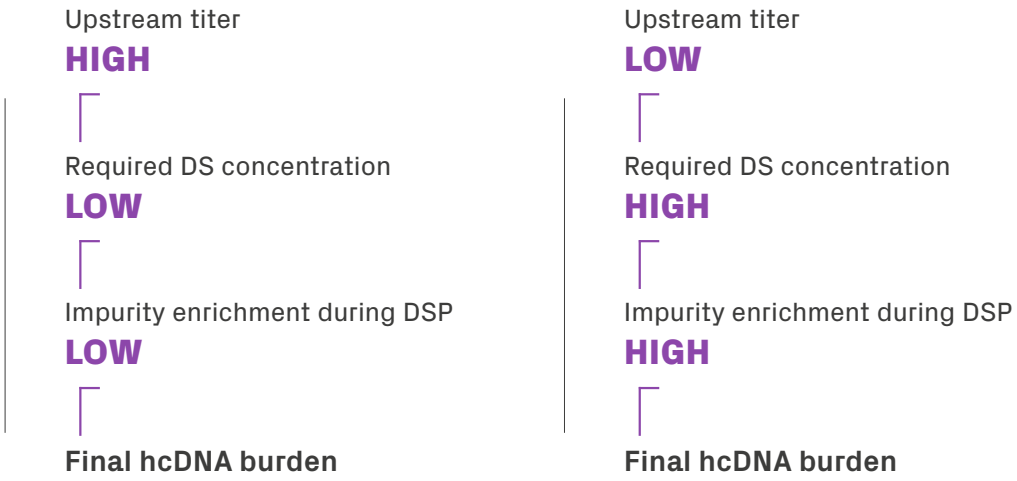


Figure 5: Conceptual relationship between upstream product titer and residual host cell DNA in the final process intermediate. Processes with lower upstream titers may require greater downstream concentration to achieve the target product dose. Residual DNA retained by the downstream process is concentrated together with the product, potentially offsetting the high relative reduction achieved during enzymatic treatment.

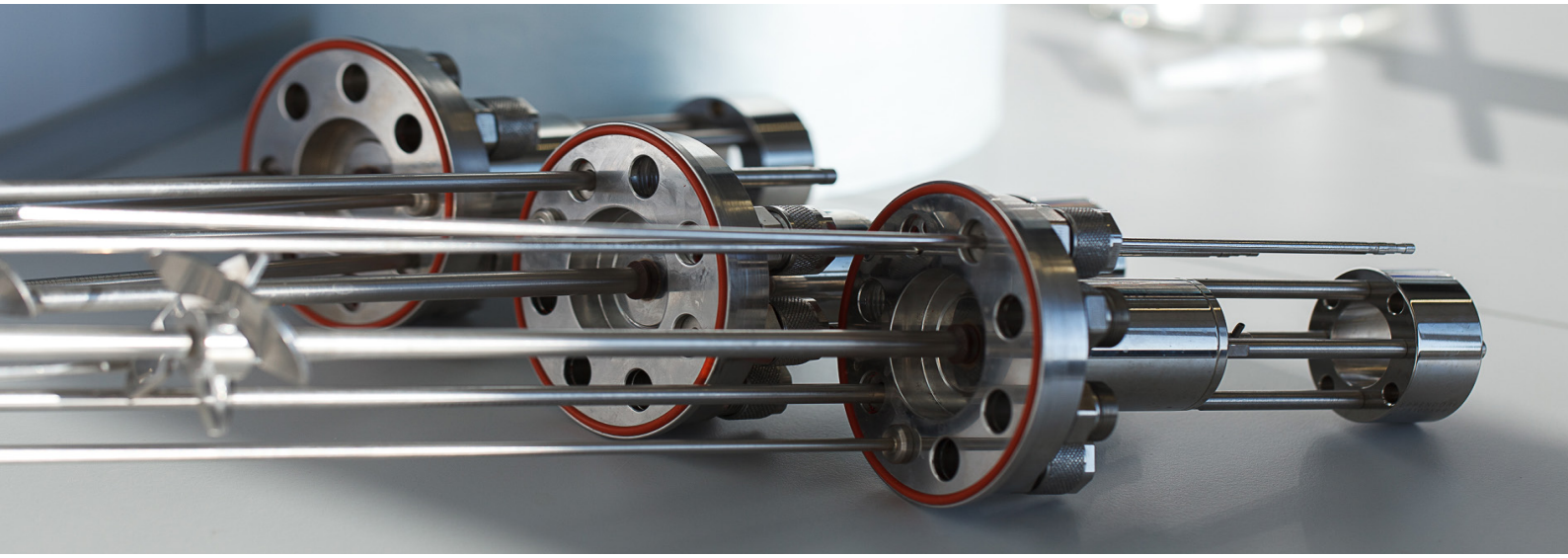
4. Conclusion

EndoCleave and a commercially available benchmark endonuclease showed comparable performance in a Vero cell-based measles virus process. Incubation time was the dominant parameter for both enzymes, followed by enzyme concentration and temperature. Under the selected conditions of 100 U/mL, 30 °C and 3 h, both enzymes reduced total DNA by 65–70% and qPCR-detectable Vero hcDNA by more than 99%. DNA-fragment size analysis additionally demonstrated extensive fragmentation of higher-molecular-weight DNA, with the predominant DNA signal following treatment occurring below 200 bp for both endonucleases. This is consistent with the objective of reducing residual DNA to small fragments with reduced potential biological activity. No consistent reduction in infectious virus titer attributable to nuclease treatment was observed.

Residual hcDNA nevertheless increased proportionally to the TFF concentration, from 5.8 ng/mL after EndoCleave treatment to 60.6 ng/mL in the DV10 intermediate. Thus a high percentage reduction during digestion therefore does not automatically translate into a low absolute hcDNA concentration in the final process intermediate. Once qPCR-detectable hcDNA reduction already exceeds 99%, further nuclease intensification may provide limited additional benefit with respect to the final hcDNA concentration.

Compliance with the final hcDNA specification is more effectively addressed through higher upstream titer, a lower downstream concentration factor, or additional clearance during DSP. Endonuclease performance and hcDNA control should thus be defined at the level of the complete manufacturing process rather than as an isolated unit operation.

Within this integrated process, EndoCleave delivered DNA digestion performance comparable to the established benchmark endonuclease, combining substantial reduction of amplifiable Vero hcDNA with extensive fragmentation of residual DNA toward predominantly sub-200-bp species.



References

1. Konstantinidis, S. et al. Purification processes of live virus vaccine candidates expressed in adherent Vero cell lines via multimodal chromatography in flowthrough mode. Biotechnol. Bioeng. 121, 2482–2499 (2024).

2. U.S. Food and Drug Administration (FDA) & Center for Biologics Evaluation and Research (CBER). Guidance for Industry- Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. (2010).