The Economics of Synthetic mRNA Capping Strategies

Abstract & Executive Summary

mRNA vaccines and therapeutics are expanding rapidly, in part fueled by the success of COVID-19 vaccines. Manufacturing mRNA requires significant expertise, equipment, and experience. An essential part of any mRNA compound is the 5' cap structure. The 5' cap is critical to the stability, expression, and immunogenicity of an mRNA. *In-vitro* transcribed mRNA is not capped by default, so capping must be built into the manufacturing process.

Currently, there are three major options for capping mRNAs *in vitro*: Enzymatic capping, ARCA (Anti-Reverse Cap Analog) and CleanCap® capping technology. To assess the complete cost of manufacturing an mRNA therapeutic, it is important to consider the difference in price, time, complexity, and availability for each of these methods.

To better understand the complete manufacturing costs, customer experiences, and customer needs with regards to mRNA capping manufacturing, TriLink BioTechnologies commissioned a third-party study. This study, conducted by a third party consulting firm, analyzed the manufacturing costs of the three methods of mRNA capping. The analysis included conversations with 30 subject matter experts, outside of TriLink, who are developing mRNA therapeutics from many different companies across the US and the EU. In this technical note, we present the qualitative findings of this analysis and qualitative quotations from customer experiences to estimate the relative cost of each of these capping methods.

Introduction & Review of Currently Available Technologies

Manufacturing mRNA for vaccine and therapeutic applications requires significant expertise, equipment, and experience. Capping of synthetic mRNA must be built into the manufacturing process. The inherent nature and manufacturing requirements of the three available technologies for capping synthesized mRNAs can have a varied impact on the overall economics of the mRNA manufacturing requirements.

Enzymatic capping is the historical option. The process and methods for enzymatic capping require two bioreactor reactions, the first to synthesize mRNA and the second to cap the mRNA. Thus, the capping is done post-transcriptionally. The often-used Vaccinia virus capping enzyme produces a Cap0 structure, which is not the natural Cap structure in eukaryotes. Therefore, enzymatic capping is often paired with a 2'O-Methyltransferase reaction to produce the natural Cap1 structure. A major drawback to this technology is low yield, typically reaching expected recovery yield values in the range of 50-70% according to the third-party study. The lower recovery yield is due to the requirement for multiple bioreactors and subsequent purification steps. Yet, this is a popular option due to the readily available commercial reagents and enzymes required for this process.

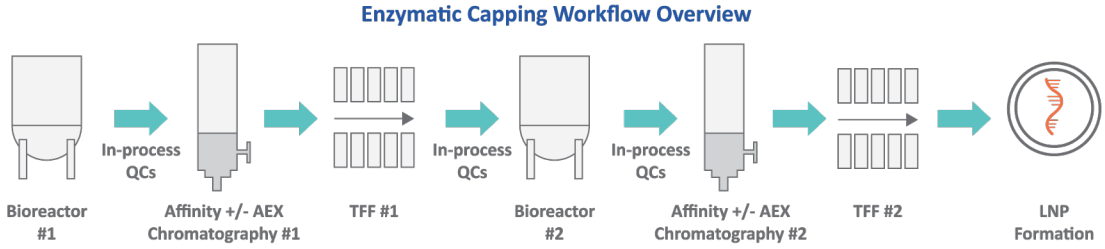

Figure 1. Overview of the manufacturing process for an mRNA with the enzymatic capping method.

ARCA was one of the first co-transcriptional capping methods developed for manufacturing mRNAs. Although it is a co-transcriptional method, it produces a Cap0 structure similar to enzymatic strategies, and thus also requires two bioreactor reactions. In this case, the second reaction is a 2'O-Methyltransferase reaction to replace the Cap0 with the naturally occurring Cap1 structure. The recovery yield with ARCA may be higher than enzymatic capping but varies depending on downstream purification steps. The third party study indicates expected recovery yields with ARCA are between 50-80%. Like enzymatic capping, the reagents for ARCA are available from multiple commercial sources.

ARCA Capping Workflow Overview

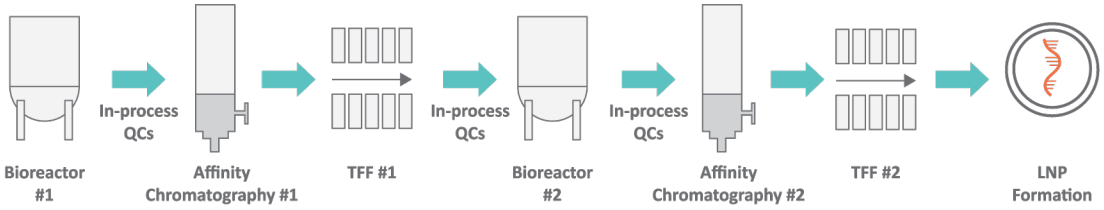

Figure 2. Overview of the manufacturing process for an mRNA with the ARCA capping method.

"I'm impressed by the [crude] transcription yield, which is 2-3 times higher [with CleanCap®] than ARCA. Especially when you scale it up, that's something significant. With this high yield, you can save 20-30% of the budget."

— EU Biopharma Expert

The final mRNA capping strategy available is the CleanCap® capping technology. The CleanCap® technology was developed by TriLink BioTechnologies and was the first mRNA capping solution to produce a Cap1 structure during a single co-transcriptional reaction, thus requiring only one bioreactor and purification step. This simplified manufacturing process contributes to an expected recovery yield ranging from 80-95% from the crude yield, which is notably higher than the other two capping strategies, according to the third-party study. The patented CleanCap® co-transcriptional technology is only available through TriLink BioTechnologies.

CleanCap® Technology Capping Workflow Overview

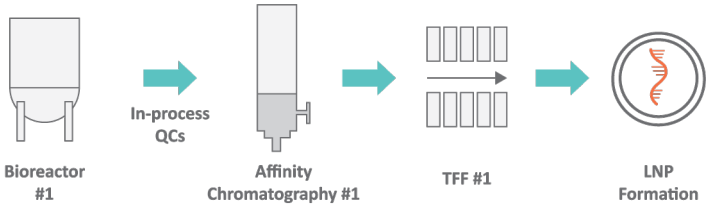

Figure 3. Overview of the manufacturing process for an mRNA with the CleanCap® capping method.

There are many steps to producing a successful mRNA vaccine and/or therapy, but manufacturing a safe, effective product is a vital component to a program's success. The cost of manufacturing an *in vitro* transcribed mRNA can be substantial depending on client needs, but always requires specific personnel and equipment. With the rapid growth of mRNA-based therapeutics in drug development pipelines, the competition and need within this space is growing. It is becoming critical for a program's success to employ streamlined manufacturing processes. Here, we outline the major costs to consider for mRNA capping manufacturing, how they vary across the capping technologies, and, based on the third party study, highlight some subject matter expert opinions on the process.

Major Economic Considerations for mRNA Capping Manufacturing

Recovery rate of in vitro synthesis

In vitro synthesis of mRNA can be a challenging biochemical reaction because of mRNA's inherent instability. The complexity of the reaction, combined with the need for pure product during pharmaceutical development, necessitates stringent purification and QC testing. Depending on the technology, equipment, downstream purification processes and user expertise, the expected overall yield ranges from 50% with enzymatic to 95% (the higher end of the range being associated with the use of CleanCap® capping technology, as seen in the third-party study).

"With fewer steps and less processing time with CleanCap®, there is the benefit of an almost full recovery rate. One purification step is all we need."
— US Biopharma Expert

The recovery rate directly influences the overall cost of product manufacturing: a higher recovery yield per reaction means less input is needed to produce the same amount of product, or, vice versa, a low recovery yield requires more input reagents for the same amount of final product. **Figure 4** shows the direct reagent costs for the GMP manufacturing of 1 gram of mRNA product with each of the three outlined mRNA capping methods. This figure demonstrates how enzymatic capping and ARCA require additional reagent costs for the same amount of product due to their reduced yields per reaction. Overall, based on the findings of the third-party study, it is estimated both enzymatic capping and ARCA technologies lead to higher total reagent cost per quantity of produced mRNA: an estimate of $248 for enzymatic, $221 for ARCA and $215 for CleanCap® technology. This data demonstrates how influential the recovery rate is on the total reaction reagent costs.

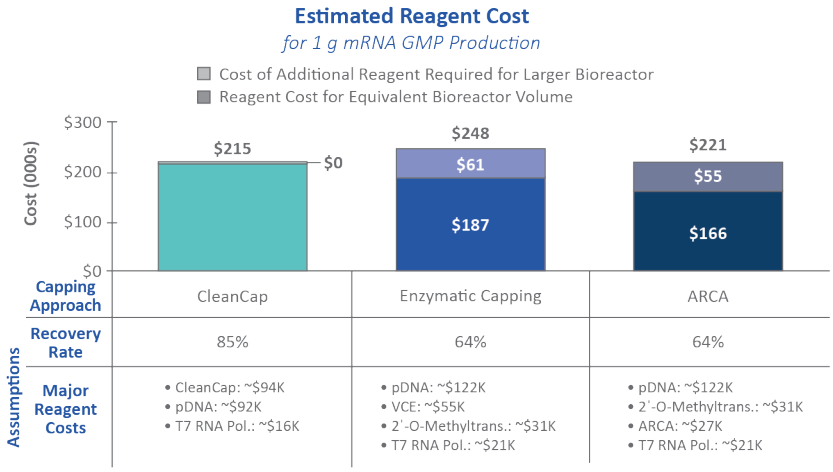

Figure 4. Estimated recovery rate and reagent cost comparison for the three mRNA capping methods to produce 1 g of GMP mRNA product.

Throughout the manufacturing process, every bioreactor reaction has required subsequent purification and QC steps, each with their own costs. The third party study estimates enzymatic reactions cost more, not simply due to their required reagents, but also due to repeating costs from necessary purification and QC follow-up steps. **Figure 5** shows estimated purification costs across the three capping methods for both non-GMP and GMP manufacturing of 1 gram batch of mRNA product. Quite simply, the results from the study indicate that the second necessary bioreactor reaction for enzymatic and ARCA capping typically lead to doubled purification costs compared to capping with CleanCap® technology.

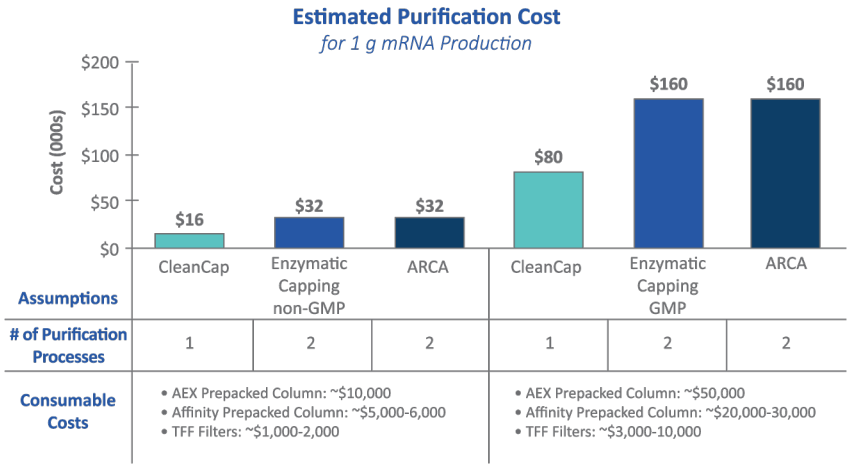

Figure 5. Estimated purification cost comparison for the three mRNA capping methods to produce 1 g of either non-GMP or GMP mRNA product.

Time

Time is a significant cost for pharmaceutical development. Time saved during pharmaceutical development, including for mRNA vaccines and therapeutics, can directly lead to cost savings in two major ways. Reduced process time can increase the velocity of development and scale up, speeding up time to market for a program. The faster a pharmaceutical product reaches commercialization, the larger potential impact it can have on enhancing or saving patient lives. There is an opportunity cost in choosing complex and multi-step manufacturing methods, and a simpler manufacturing process can accelerate time to market and reduce costs associated with equipment and process errors.

One of the most straightforward ways to compare time savings for mRNA capping technologies is by examining the number of days it takes to produce one batch of mRNA product. Again, the number of bioreactor reactions needed with a manufacturing project greatly influences the time it takes to complete a round of synthesis. More bioreactor steps require more time. For GMP production of a single batch of mRNA, the estimated time for enzymatic capping was eleven days, for ARCA it was ten days and for CleanCap® it was six days (**Figure 6**). These additional manufacturing days can be further compounded if there is a deviation or rework required in the manufacturing process, which is more likely to occur with multiple steps and multiple bioreactor reactions. In this side-by-side comparison in GMP mRNA production, CleanCap® capping technology produced usable product nearly a week sooner than either of the other two technologies.

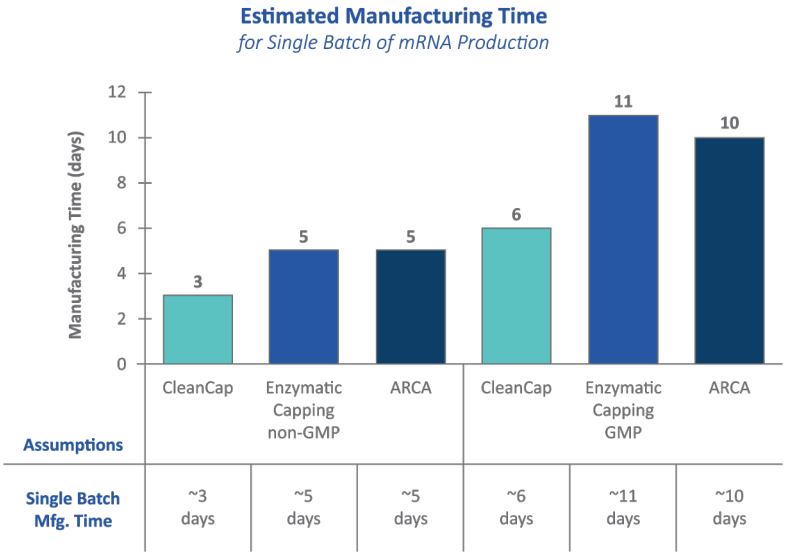

Figure 6. Comparison of estimated calculated Manufacturing Time with each mRNA capping technology for a single batch of mRNA in non-GMP and GMP conditions.

"Learning from the COVID-19 vaccines, both enzymatic and co-transcription can be scaled up just fine. With CleanCap, we are eliminating the 10-25% risk of some process failure that needs process optimization during scale-up in enzymatic."

— EU Biopharma Expert

A more holistic evaluation of time savings for mRNA capping strategies is to consider the time required for process development scale up during clinical development. Process optimization is required as reaction volumes change, and technical issues can arise with yield or quality as a biochemical reaction is scaled up. From a risk assessment standpoint, adding process steps expands the complexity of a process Failure Mode and Effect Analysis failure, which also slows the development process. Here again, the simplicity of a one-pot reaction provides a cost saving benefit to using CleanCap® capping technology. On average, process development time utilizing CleanCap® co-transcriptional technology is estimated to be about one-half of that required for enzymatic or ARCA capping (**Figure 7**). Two months of time savings in process development could have significant impact for earlier to market profit savings.

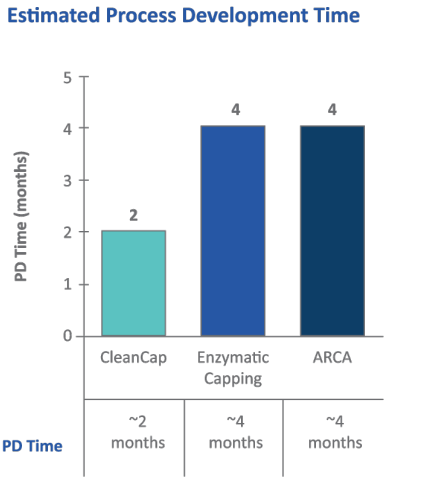

Figure 7. Comparison of estimated calculated Process Development Time for each mRNA capping technology.

The additional investment in skilled Full Time Employees (FTE) to run multi-step processes must also be factored in overall manufacturing cost calculations. Improved simplicity and time savings for the entire manufacturing process reduces the necessary labor and associated costs of FTEs to support the process. The simplicity of the manufacturing process when using CleanCap® dramatically reduces the necessary labor costs as compared to the enzymatic and ARCA approaches (**Figure 8**). It is estimated for either non-GMP or GMP production, the use of CleanCap® capping procedures typically leads to ~70-75% lower overall labor costs than enzymatic or ARCA preparation, and requires an estimated 40% fewer FTEs and half the number of hours to complete the process (**Figure 8**).

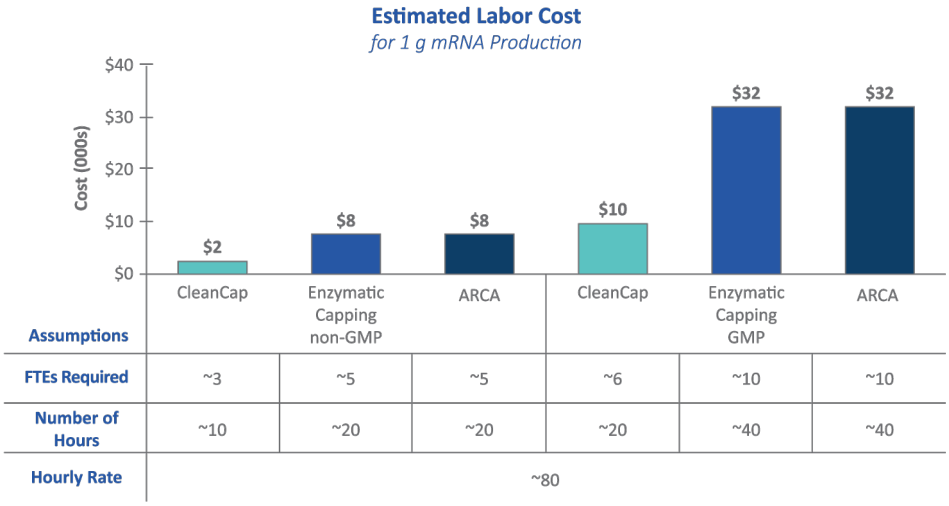

Figure 8. Estimated labor cost comparison for the three mRNA capping methods to produce 1 g of either non-GMP or GMP mRNA product.

"Compared to CleanCap, the FTEs required for the purification double in enzymatic capping. It takes more time for them to prepare and set up the column and TFF."

— US SMID Biopharma Expert

Overall, our research through the third-party study indicates an expected reduction in manufacturing time by using the CleanCap® reagent technology for *in vitro* mRNA synthesis and capping. This has the potential to lead to higher profitability and a reduced time to market for new therapeutic programs.

Cost-effectiveness

Cost-effectiveness describes the total cost of a particular mRNA capping process from start to finish in the manufacturing process. All manufacturing facets influence the cost and economics of mRNA capping methods and, thus, impact the overall cost-effectiveness of each strategy.

As **Figure 9** shows, the expected higher yields, simplified reaction setup, reduced purification needs, and anticipated reduced labor costs all contribute to the CleanCap® co-transcriptional technology being the most cost-effective mRNA capping strategy. The overall cost of the CleanCap® technology is estimated to be 30% less than enzymatic capping and 20% less than ARCA. For 1 gram of GMP grade mRNA batch, this equates to approximately ~$135,000 saved with CleanCap® capping reagent compared to enzymatic capping and ~$110,000 compared to ARCA capping. These estimates do not quantify the additional benefits associated with speed to market opportunity costs, which further establish CleanCap® capping technology as an ideal *in vitro* mRNA capping method.

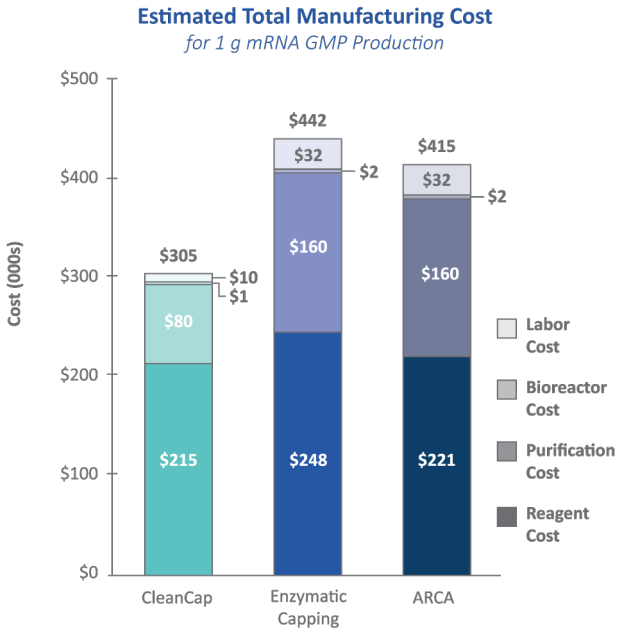

Figure 9. Comparison of Estimated Total Manufacturing Costs with each mRNA capping technology for 1 gram of GMP grade mRNA.

"The benefit of high yield is going to be more apparent when we scale up. With this high yield, we can save 20-30% of overall manufacturing cost."

— EU SMID Biopharma Expert

The long-term success of a program will require manufacturing and production scale up as a pharmaceutical product enters and succeeds through the clinical phase. It is not uncommon to require a nearly 10-fold increase in pharmaceutical product output requirements during scale up. All the costs discussed thus far contribute to, and are magnified, as this large-scale up process takes place. CleanCap® co-transcriptional technology offers considerable cost savings during these final stages of development. As **Figure 10** shows, the costs are non-linear in the range of 1 gram to 40 gram mRNA scale up. At the greatest amount of production as predicted for Phase III studies, ARCA and enzymatic capping are estimated to be 30% and 40% more expensive than CleanCap® capping technology, respectively.

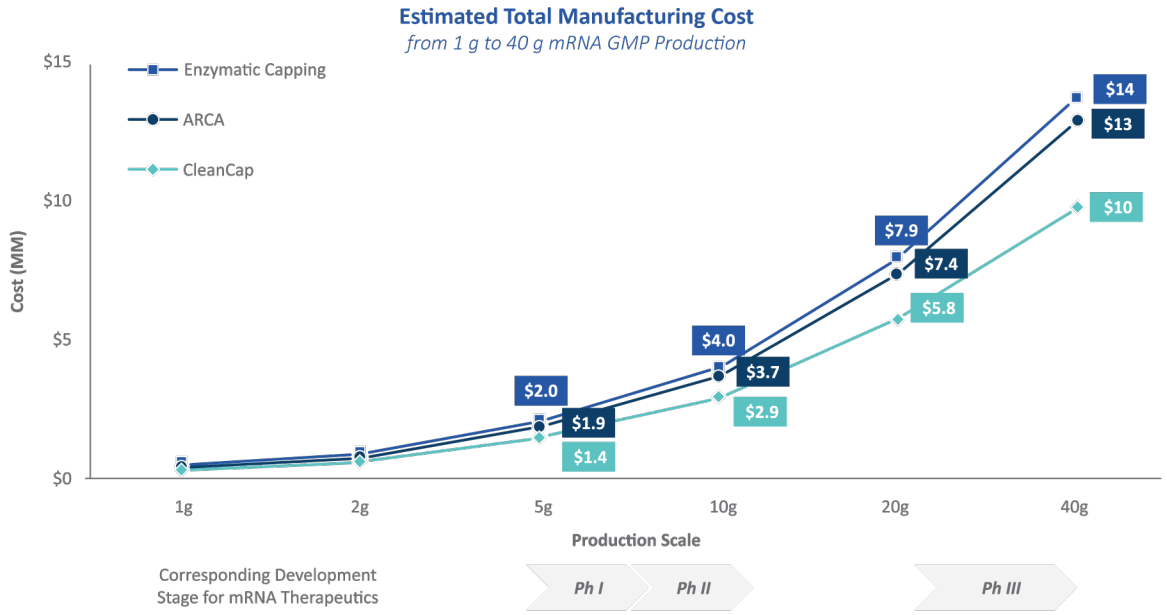

Figure 10. Comparison of Estimated Total Manufacturing Cost with each mRNA capping technology as production scales from 1 gram to 40 grams of GMP grade mRNA.

"With the existence of data from different approaches, biological efficiency, reduced immunogenicity. We are confident that CleanCap will have a higher probability of technical regulatory success. It goes into internal NPV model."
– EU Large Biopharma Expert.

Conclusion

After conducting a comprehensive qualitative and quantitative assessment, we present here an analysis of the comparative estimated manufacturing costs of the mRNA capping strategies. In assessing complexity and performance, the simplicity of the CleanCap® technology has [consistently] demonstrated a high capping efficiency, batch-to-batch consistency, a high yield, low immunogenicity, and ability for simplified scale up. In terms of process development time, comparisons showed the CleanCap® capping solution saves an estimated week of manufacturing time on a batch-by-batch basis and reduces process development time by an estimated 50%, compared to ARCA and enzymatic capping. Finally, reviewing cost-effectiveness, the use of the CleanCap® capping solution demonstrated overall comprehensive manufacturing costs that are estimated to be 20-40% lower than other methods, dependent on scale. The economic data are clear: for in vitro mRNA capping, CleanCap® offers the greatest benefit for its estimated cost.

Data and customer insights conducted by a third party consulting firm.