Whitepaper

APPLICATION OF CYTOSTOR™ FOR PRESERVATION OF VIRUSES & VIRAL VECTORS

CYTOSTOR™

ATELERIX
SAVE YOUR CELLS

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1. Introduction

Viruses, viral vectors, and messenger RNA lipid nanoparticles (mRNA-LNPs) play a critical role in biomedical research, vaccine development, and global healthcare. However, their safe storage and transportation present numerous logistical, biological, and regulatory challenges. Among these, temperature sensitivity is one of the most critical factors, directly influencing the viability and integrity of viral samples and virus-based products such as vectors and vaccines. Viruses are composed of nucleic acids encased in a protein coat, and in some cases, a lipid envelope. These structures are susceptible to damage by heat, freezing, or fluctuations in environmental conditions. Many viruses are quickly inactivated by heat, since it denatures proteins, disrupts the lipid bilayer (of enveloped viruses), and causes degradation of viral RNA or DNA. For this reason, the standard method currently used is to keep viral samples frozen through transport on dry ice or liquid nitrogen. This is costly, restrictive due to the hazardous nature of coolants, has a heavy carbon footprint, and has a high risk of sample wastage due to samples thawing (e.g. if they are held at customs).

Currently, cold-chain distribution accounts for 3% of global gas emissions, and it is estimated that up to 99% of the total carbon footprint of COVID-19 vaccinations, a mRNA-LNP vaccine, was associated with transport/distribution (3). In the UK, of the £2.9 billion spent on COVID-19 vaccines, £2.2 billion was allocated to distribution, and up to 30% of doses were wasted (4). Replacing frozen storage and distribution with hypothermic technology will reduce carbon footprint, reduce cost, and increase accessibility. The World Health Organisation (WHO) estimates that ~50% of global vaccines are wasted, including 25-30% in Africa alone (UNICEF) (5). Hypothermic distribution will overcome existing restrictions to rural areas, such as densely populated areas, including India and Indonesia. It is estimated that removing ultracold-chain logistics could dramatically expand access in 20-30% more countries and serve hundreds of millions more people (6, 7).

In addition to this, freeze-thaw cycles can damage viral envelopes and proteins. It is documented that freezing of lentivirus vectors leads to poor functionality in cells, since lentivirus titer may drop by 50% with just one freeze-thaw cycle, which requires the addition of extra products. Stabilisation solutions have therefore been developed to avoid freeze-thaw-associated damage, including cryoprotectants such as LentiGuard. Such solutions are effective at improving the viral functionality of lentivirus against freeze-thaw cycles, but still necessitate freezing, thereby incurring high cost (1).

Lyophilisation, or 'freeze-drying', is a freeze and then dehydration process which allows vaccines to be stored and shipped at room temperature. This preserves the structure and bioactivity of vaccines and extends shelf life at room temperature for months-years once lyophilised. Lyophilisation, however, is expensive, and the process itself is energy-intensive and time-consuming. For live viral vectors, the majority of viral viability is already altered during freeze-dried storage; therefore, determining viral-based vaccine stability with functionality after freezing can be a challenge (2). Clinical determinations of the highest viral dose that is safe and the lowest dose that is efficacious must be established to elucidate the "stability window", and the extent of viral degradation that is acceptable across the shelf life of the vaccine product. Storing at room temperature would allow the removal of freeze-thaw cycles, and in turn improve viral viability and predicted dose, reflected in functionality tests.

1. Introduction

This whitepaper documents how Atelerix's hydrogel technology, CytoStor™, can enable hypothermic viral and vaccine stabilisation for cryo-free logistics, providing case examples specifically of SARS-CoV-2, Adenovirus, Lentivirus, Lentiviral Vectors, and LNP-mRNA viral vectors. Overcoming the requirement for ultra-cold chain distribution through Atelerix's innovation would provide huge benefits to the supply chain of live free viruses for multiple applications. CytoStor™-stabilised viral samples would enhance population monitoring and surveillance of new or emerging pathogenic viral species and variants, allowing for rapid and effective deployment of viral samples for epidemiological studies and public health interventions.

Overcoming complex logistics also facilitates wider geographical distribution of viral samples between research institutions, contract research organisations (CROs), biopharma, and biotech companies. Reliable shipping of viral material is crucial for the development of diagnostic assays and proficiency testing worldwide. Such improvements in the supply chain of live free viruses would enhance accessibility of models for drug discovery and precision medicine. For viral vectors such as lentiviral vectors, hypothermic stabilisation using Atelerix's technology will be beneficial to the cell and gene therapy sector, by enabling secure distribution to regions with limited cold chain infrastructure, such as remote laboratories or clinical sites. In response to global outbreaks, Atelerix's technology will facilitate stockpiling and rapid deployment of viral vectors or vaccines in response to epidemics or pandemics, with a broader distribution.

2. Methods

2.1 Preservation of Free Viruses

Preservation of free viruses including coronavirus, adenovirus, and lentivirus, were conducted in collaboration with Perfectus Biomed. Preservation of Lentiviral Vectors and mRNA-LNPs was conducted by Atelerix.

Preservation of Coronavirus 229E and its host cells: Free CoV 229E virus was stored at room temperature in CytoStor™ or viral transport medium (VTM) for up to 2 weeks before isolating the virus and assessing functional viability by cytopathic effect. Human Airway Epithelial Cells (HAECs) were stored for up to 2 weeks before assessing cell viability. HAECs inoculated with the CoV 229E virus were stored for up to 2 weeks before assessing viral functional viability by cytopathic effect.

Preservation of Adenovirus: Adenovirus and Phi6 were stored for 12 weeks at room temperature before examining the cytopathic effect (Adenovirus) and plaque assay (Phi6).

Preservation of Lentivirus: Lentivirus (Visna virus) was stored for 2 weeks at room temperature before examining cytopathic effect.

2.2 Preservation of Lentiviral Vectors

Lentiviral vectors encoding Green Fluorescent Protein (GFP) were stored for 14 days at 2-8°C using CytoStor™ or medium alone. The viral vector was a Lentiviral control particle with CMV promoter-driven expression of eGFP and SV40-puro, ultracentrifugation purified (pLenti-SV40-PURO-CMV-GFP). HEK293 cells were seeded at 7.5×10^4 cells per well in black-walled 96-well plates 24 hours prior to transfection. For CytoStor™-encapsulated virus (MOI 5), 60 µL of Gel A was mixed with 238.2 µL

medium and 1.8 µL lentivirus, then added to 200 µL GelBASE beads and incubated for 1 hour to allow gelation. Virus was recovered by adding 100 µL of 0.2 M sodium citrate dissolution buffer to the base of the gel, incubating for 10 minutes with intermittent inversion, and gently pipetting to ensure complete dissolution. Medium was added to bring the total volume to 2 mL, and 200 µL of the virus-containing solution was applied per well. After 7- or 14-day storage and release, Lentiviral vector (MOI 5) was transduced into HEK293 cells and plates were incubated at 37°C for 24–72 hours. GFP expression was monitored via fluorescence microscopy and quantified using a plate reader. Cell number was controlled using Alamar Blue assay, measured using appropriate filters on the same plate reader.

2.3 Preservation of mRNA-LNPs

mRNA-LNPs encoding GFP were stored using CytoStor™ or medium alone for either i) 7 and 30 days at 2-8°C before release, or ii) cryopreserved at -80°C with subsequent transfer to -180°C liquid nitrogen for 2-3 days followed by thawing and storage for up to 14 days at 2-8°C and subsequent release.

HEK293 cells were seeded at 7.5 × 10^4 cells per well in black-walled 96-well plates 24 hours prior to transfection. On the day of the experiment, LNP-formulated mRNA was thawed at 4°C for 20–30 minutes, mixed by gentle inversion, and kept on ice. For non-stored LNP-mRNA, 10-25 µL was diluted in 990–975 µL of culture medium, and 100 µL was applied per well following medium aspiration. For CytoStor™-encapsulated LNP-mRNA, 60 µL Gel A was mixed with 230–215 µL of medium and 10-25 µL (2 µg–5 µg) of LNP-mRNA. This mixture was combined with 200 µL of GelBASE beads and incubated for 1 hour to allow gelation. To dissolve the gel, 100 µL of 0.2 M sodium citrate dissolution buffer was added to the base of the tube and incubated for 10 minutes with occasional inversion. If necessary, remaining beads were gently dissolved by pipetting. Medium was then added to bring the total volume to 2 mL, and 200 µL was applied per well. Plates were incubated at 37°C for 24–72 hours. GFP expression was monitored by fluorescence microscopy and quantified using a plate reader. Alamar Blue assay was used to normalise for cell number, with fluorescence measured using appropriate settings.

3. Results

3.1 Preservation of Free Viruses: CoV-229E, CoV229e infected host cells, Adenovirus and Phi7 Ebola Surrogate

Atelerix's product, CytoStor™, can conserve viral envelope integrity and is highly effective at extending the functional viability (cytopathic effect) of free viruses at room temperature for at least 2 weeks. When preserving Coronavirus CoV 229E, CytoStor provides a 5-fold shelf-life extension compared to VTM, up to and beyond 2 weeks (Fig. 1ai), alongside a considerable protection of human airway epithelium host cells (Fig. 1aii). Virus stored in Virocult® viral transport medium (a commercial alternative used for viral swabs) fell below the limit of detection at 5 days (Fig. 2a) In combination, no reduction in the cytopathic effect of Coronavirus within infected host cells was achieved (Fig. 1aiii), demonstrating no decline in either viral functional activity or RNA stability (quantity and quality) after room temperature storage. CoV 229E-infected host cells stored in VTM fell rapidly to below the limits of detection within 3 days.

The results demonstrate that CytoStor™ preserves both free coronavirus and infected host cells at room temperature without loss of viral infectivity or RNA integrity. In contrast, samples stored in standard VTM exhibited rapid decline in detectable viral activity within three days. This enhanced stability enables reliable downstream analyses, including functional assays and molecular diagnostics, even after extended transport or storage. Importantly, the ability to maintain viable virus and infected cells supports accurate detection and monitoring of circulating viral strains, facilitating robust population-level surveillance and genomic studies, particularly in remote or resource-limited settings.

Atelerix's CytoStor™ preserves both free coronavirus and infected host cells at room temperature without compromising viral infectivity or RNA integrity, in stark contrast to standard viral transport medium (VTM), where viral activity declines rapidly within three days. This capability enables reliable transport and analysis of clinical samples, supporting accurate molecular diagnostics, functional assays, and downstream research. Critically, maintaining viable virus and infected cells enhances population-level surveillance and genomic monitoring, allowing public health authorities to track emerging variants, assess transmissibility, and implement timely intervention, even in remote or resource-limited regions.

Adenovirus is a non-enveloped viral vector that is known for its inherent resilience to environmental stressors such as pH, desiccation, and temperature change. Many non-enveloped viruses are naturally associated with gastrointestinal infections, reflecting their ability to withstand harsh external conditions. Studies conducted in collaboration with Perfectus Biomed demonstrated that CytoStor™ stabilises Adenovirus for 2 weeks (Fig. 1bi). Further work demonstrated that Adenovirus (Fig. 1bi) and the Ebola surrogate Phi6 bacteriophage activity (Fig. 1bii) can be stabilised for 12 weeks at room temperature. Notably, enveloped viruses carry a lipid membrane that is known to be much more vulnerable to destabilisation, and therefore the ability of CytoStor™ to stabilise Phi6, an enveloped surrogate for Ebola virus, highlights how Atelerix's hydrogel technology not only preserves the natural durability of non-enveloped vaccine vectors like Adenovirus, but also provides essential protection for more fragile lipid-enveloped viruses and vaccines.

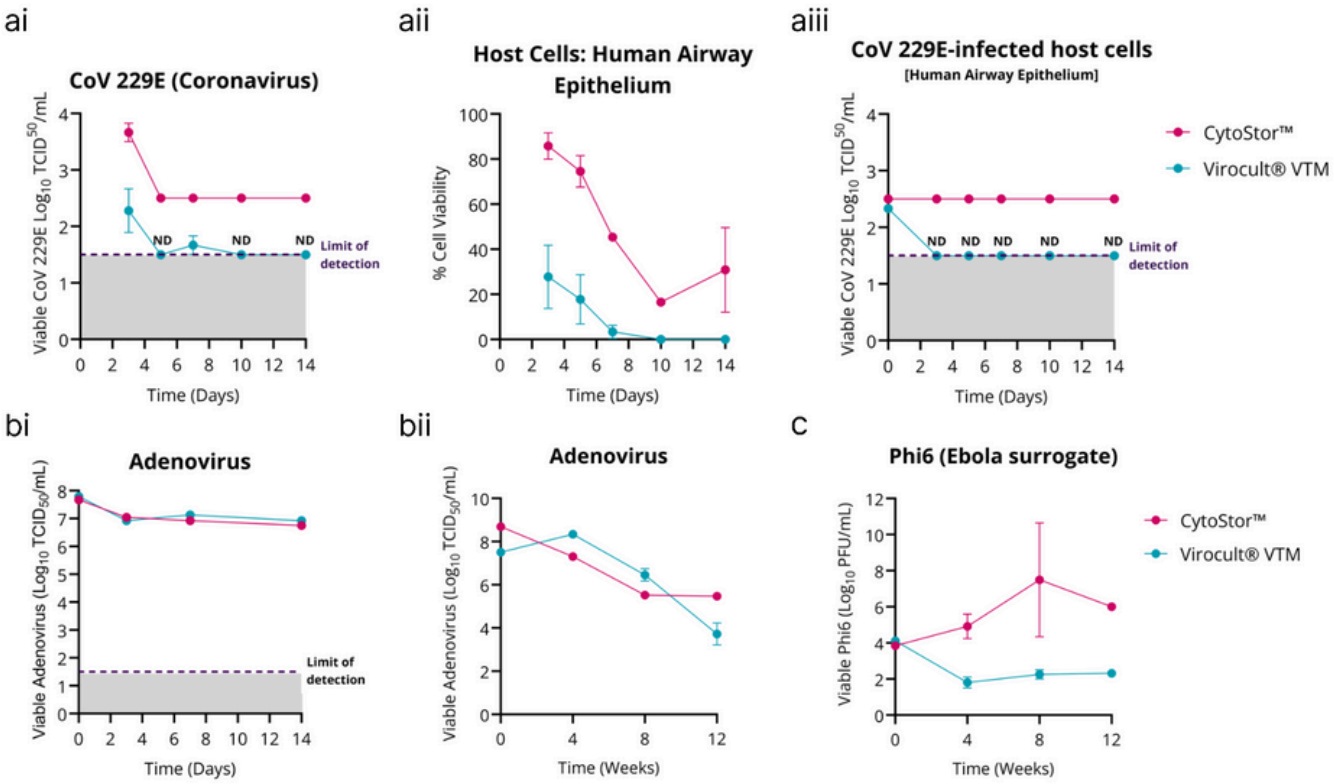

Figure 1. Atelerix's technology is highly effective at extending the functional viability (cytopathic effect) of free viruses. *ai: Cov229E, aii: human airway epithelium host cells and aiii: CoV229E infected host cells were stored for 2 weeks at room temperature. Cytopathic effect (ai and aiii) and cell viability (aii) were measured post-storage. bi: Adenovirus was stored for 2 weeks at room temperature before examining cytopathic effect. bii: Adenovirus and biii: Phi6 were stored for 12 weeks at room temperature before examining cytopathic effect (Adenovirus) and plaque assay. c: Phi6 were stored for 12 weeks at room temperature before examining cytopathic effect through plaque assay.*

3. Preservation of Lentivirus & Lentiviral Vector

Lentiviral vectors are a useful tool for delivering genetic material into mammalian cells and are becoming increasingly used in cell and gene therapy and as vaccines. However, these vectors are not stable when stored at ambient temperatures, and a significant proportion of viral titre can be lost upon freeze-thaw. Evaluation of Atelerix's hypothermic preservation technology for stabilising lentivirus firstly demonstrated that CytoStor™ can successfully preserve free lentivirus (visna virus) for at least 2 weeks, providing a 5-fold extension from buffer alone (PBS) (Fig. 2ai). In buffer alone, the level of viable lentivirus drops below the limits of detection within 3 days, highlighting the protective function of CytoStor™. Furthermore, lentiviral vectors encoding GFP can be preserved for up to 7 days at 2-8°C with no decrease in transduction efficiency (Fig. 2aii). In contrast, lentiviral vectors stored in culture medium only (Medium Ctl.) experience a rapid fall in transduction efficiency. After 2 weeks in CytoStor™, lentiviral vector transduction efficiency is continually maintained, with a 5-fold improvement from the medium only control.

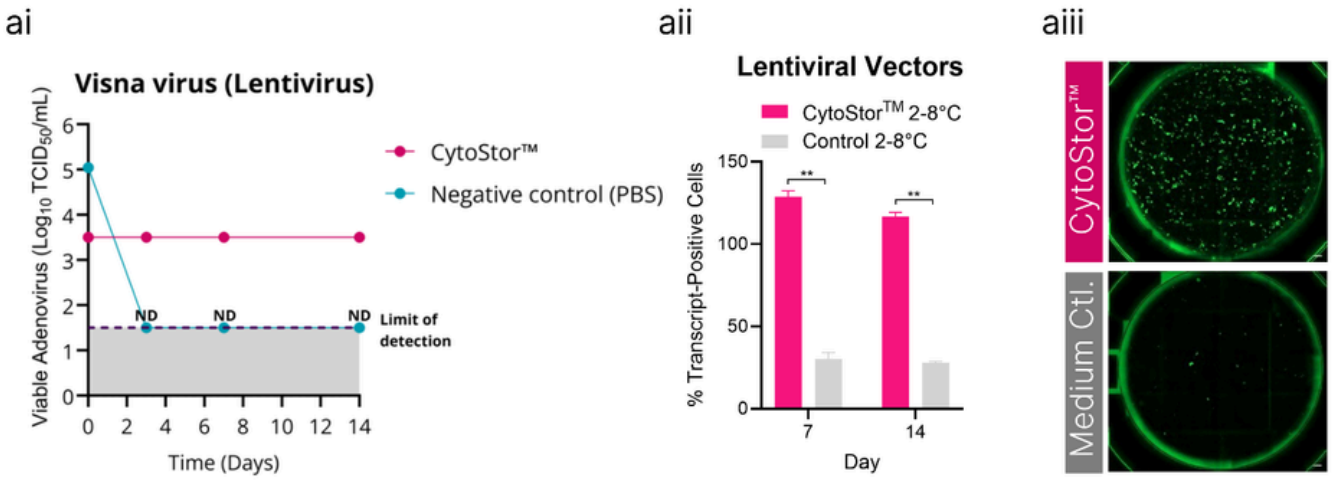

Figure 2. Atelerix's CytoStor™ is effective at preserving free Lentivirus & Lentiviral vectors.
ai: Lentivirus (visna virus) was stored for 2 weeks at room temperature before examining cytopathic effect. aii: Viral vectors encoding GFP were stored for 7- and 14- days at 2-8°C using CytoStor™ or medium alone. Lentiviral vector encoding GFP was transduced into HEK293 cells. GFP was visualised by fluorescent imaging and the number of positive cells calculated. aiii: representative images of GFP positive cells after 7 days of storage of lentiviral vector in CytoStor™ or medium-only control.

3.3 Preservation of mRNA-LNPs

In addition to free viruses and lentiviral vectors, Atelerix has evaluated the use of CytoStor™ to enable hypothermic storage and distribution of mRNA-LNPs, to overcome the logistical challenges of mRNA-LNP distribution. mRNA-LNPs are nanoscale delivery vehicles designed to protect and transport synthetic mRNA into host cells. The mRNA encodes a target antigen (such as the SARS-CoV-2 spike protein), while the lipid nanoparticle serves several key functions: shielding the mRNA from nuclease degradation, facilitating cellular uptake via endocytosis, and promoting endosomal escape for cytoplasmic mRNA release. This platform enables *in situ* translation of antigens, initiating a robust adaptive immune response. mRNA-LNPs represent a major advancement in vaccinology and gene therapy due to their non-viral, scalable, and rapidly adaptable nature (8-10). They are the core technology behind the first approved mRNA vaccines, such as Pfizer–BioNTech's BNT162b2 (Comirnaty) and Moderna's mRNA-1273 (Spikevax) (11).

However, mRNA-LNP formulations are highly sensitive to temperature fluctuations; both the integrity of the mRNA and the physical stability of the lipid nanoparticles can be compromised by heat or freeze-thaw cycles, necessitating stringent cold chain requirements (-20°C to -80°C) e.g. Pfizer–BioNTech's BNT162b2 (Comirnaty) requires -90°C to -60°C, and Moderna's mRNA-1273 (Spikevax) requires storage/transport at -25°C to -15°C, to maintain efficacy. This thermal sensitivity presents a key challenge in the global distribution and accessibility of mRNA-based therapeutics. Notably, no direct competitive technologies exist that integrate frozen storage with ambient shipping whilst maintaining the viability and function of biological samples.

Atelerix's proof of concept data has shown that CytoStor™ can significantly retain the potency of mRNA-LNPs for up to 30 days at 2-8°C (Fig. 3ai). In buffer only, the potency of mRNA-LNPs drops to ~50% by day 7, highlighting the protective function of CytoStor™. Secondly, integration of CytoStor™-encapsulated mRNA-LNPs with cryopreservation has shown that mRNA-LNP efficacy is also retained after freeze-thaw and subsequent hypothermic storage at 2-8°C for up to 14 days (Fig. 3aii). These findings demonstrate that CytoStor™ can dramatically extend the stability of mRNA-LNPs under standard refrigeration, reducing reliance on costly ultra-cold chain logistics. By preserving potency during both hypothermic storage and freeze-thaw cycles, CytoStor™ provides a practical "pick and ship" solution for mRNA-LNPs: doses can be stored frozen until needed, then simply thawed and shipped at 2-8°C using standard refrigerated transport. This removes the need for complex ultra-cold chain systems or dry ice, greatly simplifying logistics. Such flexibility lowers costs, reduces handling risks, and makes mRNA vaccines far easier to distribute at scale, even to regions without advanced cold-chain infrastructure, thereby expanding global access and accelerating deployment of mRNA-based vaccines

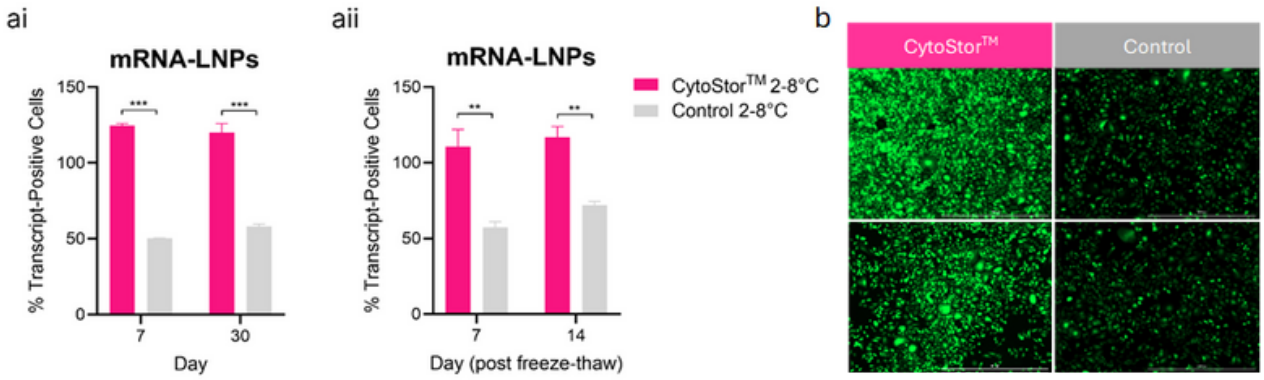

Figure 3. Atelerix's CytoStor™ is effective at stabilising mRNA-LNPs. *ai: mRNA-LNPs encoding GFP were stored using CytoStor™ or medium alone (Control) at 2-8°C. mRNA-LNPs were transduced into HEK293 cells. GFP was visualised by fluorescent imaging and number of positive cells calculated. mRNA-LNPs were stored for 7 and 30 days at 2-8°C before examining transduction efficiency. aii: mRNA-LNPs were encapsulated in CytoStor™, frozen at -80°C, thawed, and stored for up to 14 days at 2-8°C. Data are presented as mean ± SEM. Statistical comparisons between CytoStor™ and Control groups were performed using a two-tailed Welch's t-test. Significance is indicated as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). b. representative images of GFP positive cells after 30 days storage at 2-8°C in CytoStor™ or buffer-only (Control).*

By stabilising and extending the shelf life of free viruses and two critical vaccine platforms (LNP-encapsulated RNA vaccines and lentiviral vector vaccines) at 2–8°C or room temperature, Atelerix's technology has the potential to significantly reduce or eliminate the need for ultra-low temperature storage and transportation. This would enable broader distribution by increasing the number of facilities capable of storing and handling these products, eliminate the need for expensive dry ice shipments, reduce the rate of shipping failures, and extend delivery windows to end users. In turn, these improvements would enhance global distribution and healthcare access to regions lacking ultra-cold infrastructure.

4. Conclusion

The data presented demonstrates the ability of CytoStor™ to preserve the viability and functional integrity of viral products without the need for cryopreservation. By enabling the safe, room temperature or hypothermic storage of free viruses, lentiviral vectors, and mRNA-LNPs, CytoStor™ addresses critical challenges in vaccine distribution, including high costs, carbon emissions, and limited accessibility in low-resource settings. Atelerix's CytoStor™ technology may ultimately offer a practical and scalable alternative to energy-intensive cold-chain logistics, supporting wider global access to viruses and viral vectors, enhancing vaccine equity, and strengthening supply chains across biomedical and public health sectors. See Table 1, for a full list of applications of Atelerix's technology described in this whitepaper and the associated beneficial impacts.

Table 1. Hypothermic stabilisation of Viral Products & Vectors by CytoStor™ and the associated impact on research, drug discovery, cell and gene therapies, and global access to therapeutics.

Application / Area	Experimental Outcomes	Impact on Supply-Chain and Global Access
Free viruses	CytoStor™ preserves the viability and functional integrity of free viruses at room temperature for weeks-months without the need for cryopreservation.	Enhanced stability enables reliable transport, functional assays, molecular diagnostics, and downstream research, while supporting accurate population-level surveillance and genomic monitoring, critical for tracking emerging variants and informing public health responses, even in remote or resource-limited settings.
Lentiviral Vectors	CytoStor™ stabilises lentiviral vectors, maintaining infectivity and functionality during storage and transport at 2–8°C.	Removes ultra-cold logistics requirements for transporting lentiviral vectors, reducing manufacturing and shipping costs, improving yield consistency, and streamlining the production of cell therapies, namely CAR-T production.
mRNA-LNPs (messenger RNA lipid nanoparticles)	CytoStor™ preserves the potency and functional integrity of mRNA-LNPs during hypothermic storage (2–8°C) for up to 30 days and maintains efficacy through freeze-thaw cycles followed by refrigerated storage for up to 14 days.	Hypothermic stabilisation of mRNA-LNPs, especially after freeze-thaw, enables practical "pick and ship" handling, reducing reliance on ultra-cold chain logistics, thereby simplifying vaccine and gene therapy distribution, lowering cold-chain complexity and carbon emissions, and enabling broader global access in low-resource settings.
Cross-platform benefits	Demonstrated ability to stabilise multiple viral and nucleic-acid-based platforms under non-cryogenic conditions. Atelerix's technology not only maintains the inherent durability of non-enveloped vaccine vectors but also provides critical protection to lipid-enveloped viruses.	Offers a practical, scalable alternative to energy-intensive cold-chain logistics, strengthening biomedical supply chains, enhancing vaccine equity, and reducing overall production costs. Enabling centralised manufacturing and reducing the need for costly, decentralised cold chain infrastructure. Broad utility across a wide range of vaccine platforms and viral therapeutics.

5. Acknowledgements

We would like to sincerely thank Perfectus Biomed for their invaluable collaboration and expertise in supporting key elements of this proof-of-concept work. Their contributions were instrumental in generating the foundational data for the preservation of free viruses, helping to advance the development and validation of this innovative technology.

PERFECTUS BIOMED GROUP

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