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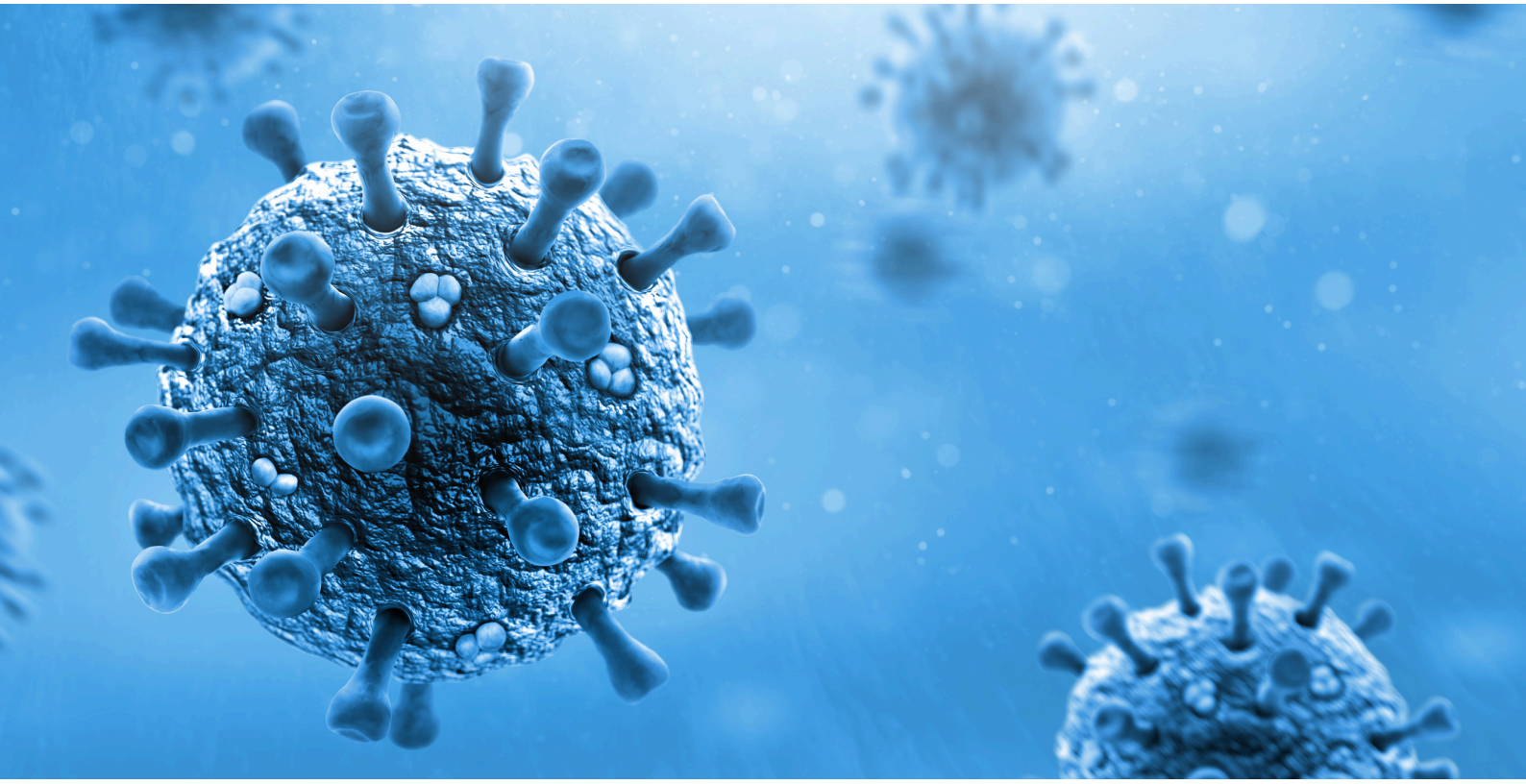

AAV Titre: NHSBT Comparison of Amperia vs Automated ELISA

Faster results with equivalent accuracy and lower sample waste

SCAN TO VIEW
ALL RESOURCES

CASE STUDY

APRIL 2025

Summary

- The Clinical Biotechnology Centre (CBC), Bristol, part of NHS Blood and Transplant, is one of three UK government-selected Innovation Hubs for Gene Therapies, with the aim to accelerate academic-led development of novel gene therapies.
- The team has developed an rAAV2 production platform from 2L to 50L bioreactor scale to support clients inrefining process development (PD) through to GMP compliant production.
- Rapid, accurate quantification of viral titre at every step of the development process is required to check viral particle recovery and quality.
- The team was using a high-throughput automated ELISA system that required samples to be batched toreduce consumable wastage. Titre data was available after the two-week process was completed, impacting real-time optimisation.
- The Amperia system provides a flexible rapid automated platform for titre analysis, matching consumables tonumber of samples run. Titre can be measured cost-effectively at-line, thus picking up errors in the viral manufacturing process in real-time to avoid wastage of expensive consumables and time.
- Comparison of Amperia data with the current automated ELISA system showed equivalent accuracy but the Amperia system offered significant time and cost savings in sample management.
- Using the Amperia system, the team can rapidly switch between assays using different serotypes and respond quickly to data requests for multiple customer projects.
- The team anticipates utilising the Amperia system will increase efficiency and support quick and confidentreporting to clients.

> Speed, accuracy and flexibility are importantfor us when looking for an AAV quantificationsolution, along with cost. Our clients rely on us to meet their process development targets, and having access to accurate titre data in real-time throughout process optimisation will allow us to maximise efficiency and provide client updates quickly and confidently.The Amperia system is very user friendly and compact, with no additional computer equipment to accommodate in the busy lab.

Laura Barry
DEVELOPMENT SCIENTIST,
NHSBT - CBC.

Introduction

- The Clinical Biotechnology Centre (CBC) at NHS Blood andTransplant, Bristol, was set up over 20 years ago to be the preferred provider of quality-assured DNA and protein-based therapeutics for healthcare, academic, and commercial customers seeking to establish the safety and efficacy of innovative cellular and molecular therapies. The NHSBT CBC is one of three government selected Innovation Hubs for Gene Therapies, with the aim of accelerating academic-led development of novel gene therapies throughout the UK.
- The CBC provides services to support the manufacture of Lentiviral Vectors (LVV) and recombinant Adeno-associated Viral Vectors (AAV) to enable early-stage clinical studies.
- It offers both GMP and high-quality non-GMP vector manufacturing to support progress from pre-clinical stages to translate more therapies to clinical stage.
- The team has developed an rAAV2 production platformfrom 2L to 50L bioreactor scale (Figure 1) to support clientsin refining process development (PD) through to GMP-compliant production.
- Rapid, accurate quantification of viral titre at every step of the development process is required to check viral particle recovery and quality to support process optimisation.

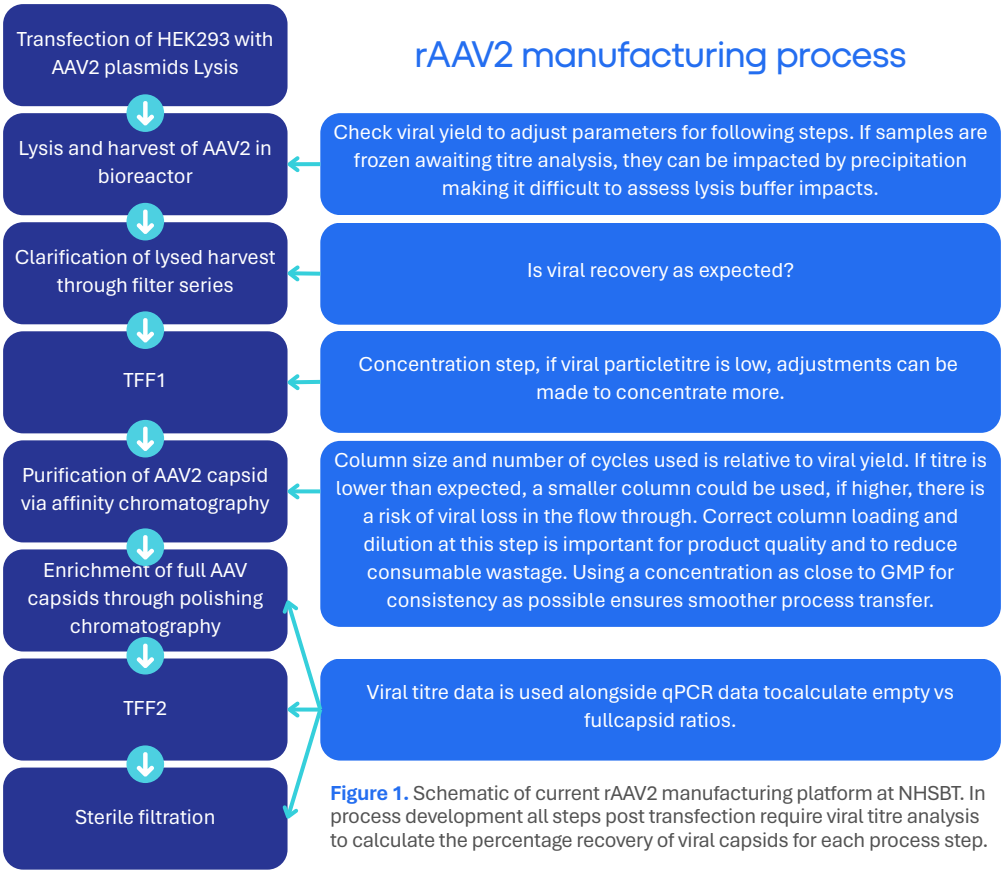

Figure 1. Schematic of current rAAV2 manufacturing platform at NHSBT. In process development all steps post transfection require viral titre analysis to calculate the percentage recovery of viral capsids for each process step.

> There can be a high degree of inherent variability when working with AAV vectors and havingconsistent measurement throughout the processmeans we can more confidently identifythe impacts of process adjustments vs background variation.

Problem: batching samples to save costs

The NHSBT process development team was relying on an automated ELISA system for titre analysis. The system is designed for a high sample throughput, meaning that samples needed to be batched to fill a full cartridge to reduce wastage of expensive consumables.

This, in turn meant that titre analysis was postponed until the two- week process run had been completed. Process checks and adjustments were therefore made after each complete run, rather than with access to accurate data at each step in real-time.

Selecting a solution

The team was looking for a rapid but accurate viral vector quantification solution that would give them the flexibility to cost-effectively run a few samples at a time to obtain titre data at each process step.

The Amperia system provides flexible automated titre analysis, matching consumables to number of samples perrun, avoiding the wastage experienced with pre-loaded assay cartridges. The opportunity to run samples cost-effectively at-line allows errors in the viral manufacturing process to be picked up before potentially wasting expensive resources and operator time.

The team undertook an assessment of the Amperia system to compare performance with the established automated ELISA method.

Study protocol

Samples

Two parallel 3L scale bioreactor rAAV2 manufacturing runs were carried out to compare two sets of AAV2 plasmids. Samples were taken at each stage of the process, and stored at -80°C for batch analysis after completion of the manufacturing process. Harvest samples were sedimented and the supernatant retained for assaying; all other samples were not processed before assaying.

“Flow-through” samples such as TFF permeate and chromatography flow-through were also assayed, to check that there had not been any viral particle break-through before disposal.

Established ELISA process

For the existing automated ELISA system, samples were diluted and applied to pre-calibrated cartridges. Each cartridge comes pre-loaded with reagents for a specific assay. Current suggested sample dilutions are 1 in 2000 for samples from lysis and TFF1 stage, and 1 in 20,000 for all other samples.

The samples were serially diluted in PBS (1 in 10 dilutions) before a final 1 in 2 dilution in sample diluent (provided with the cartridge). Two separate dilution series were made for each sample.

Amperia process

Samples were diluted in dilution buffer to an appropriate working concentration.

Two separate dilution series were made for each sample, making sure that replicates were analysed by a different sensor set.

An initial calibration curve experiment was run using the provided AAV calibrator, and all titre calculations were made using this same calibration curve. A control sample with a concentration of 2.5 x 10 vp/mL was run on each plate in duplicate to compensate for variations in 10 environmental conditions between each run.

Study results

The batched samples were assayed by both the established method and by Amperia on a single run on both systems. Bioreactor 1 (BR1) was terminated post capture chromatography stage due to loss of sample. Bioreactor 2 (BR2) was run until completion (final product). **Figure 2** shows the standard curve generated using the Amperia system demonstrating a high level of precision with low deviation.

Comparison of viral particle titres between the established ELISA method and Amperia (**Table 2**) at each step of the process show high correlation R^2=0.97 (**Figure 3**) between the two methods.

A titre reading was not obtained using the established ELISA method for the capture chromatography step in BR2 as, despite the higher sample dilution (1 in 20000), this was outside the limit of detection (LOD).

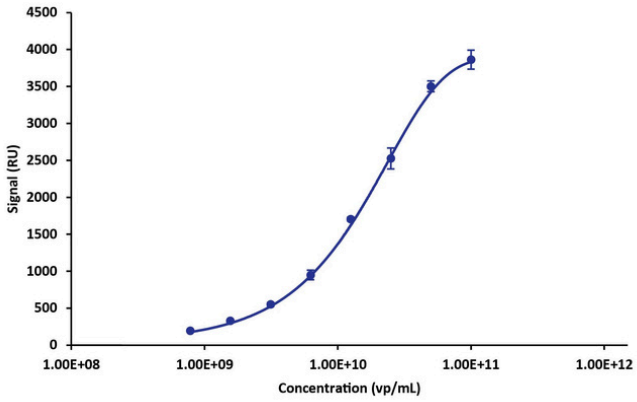

Figure 2. Standard curve generated using serial dilutions of Progen AAV2 standard, used to calculate viral titre for all Amperia assayed samples.

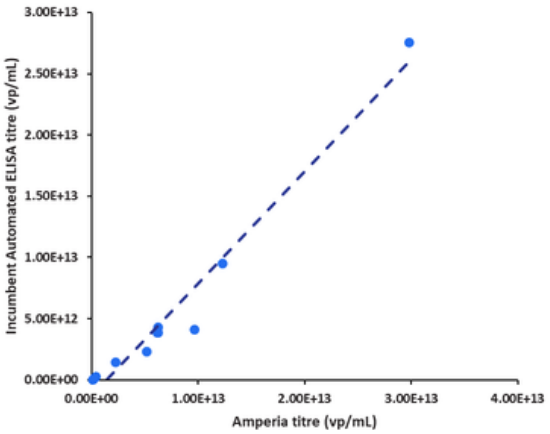

Figure 3. Scatter plot showing the correlation between viral particle titre (vp/mL) assayed by the incumbent automated ELISA instrument and the Amperia instrument. Excellent correlation was observed **R^2=0.97**.

Comparison of final batched viral particle titres between established ELISA method and the Amperia system

Process step	Established ELISA method viral particle titre (vp/mL)	CV (%)	Dilution factor	Amperia viral particle titre (vp/mL)	CV (%)	Dilution factor
BR1-Harvest	2.12E+11	5.07	2000	1.35E+11	2	10
BR2-Harvest	5.14E+12	28.3	2000	2.31E+12	0.3	100
BR1-Clarification	1.04E+11	7.31	2000	6.63E+10	4.4	10
BR2-Clarification	2.22E+12	26.76	2000	1.45E+12	0.8	100
BR1-TFF1	3.72E+11	23.24	2000	2.71E+11	28.1	10
BR2-TFF1	1.23E+13	11.26	2000	9.53E+12	0.7	1000
BR1-Capture chromatography	2.98E+13	11.46	20000	2.76E+13	6.5	1000
BR2-Capture chromatography*	Above LOD	N/A	20000	4.42E+14	6.7	10000
BR2-Polishing-A1*	9.61E+12	3.31	20000	4.12E+12	17.3	100
BR2-Polishing-A2*	6.24E+12	6.05	20000	4.3E+12	13.2	100
BR2-TFF2	6.25E+12	0.8	20000	3.89E+12	12.6	100
BR2-SterileFiltration*	6.15E+12	8.5	20000	3.89E+12	26.3	100

Table 2. Comparison of viral particle titres as assayed by established automated ELISA instrument and Amperia instrument. All samples freeze-thawed and assayed on same plate for each instrument. BR1 - bioreactor 1, BR2, bioreactor 2 respectively. Note the * samples had a reading of above 3500 RU on Amperia instrument which is close to the LOD.

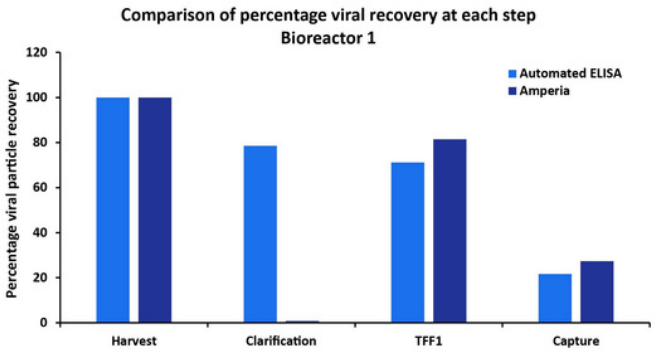

Figure 4a. Comparison of calculated percentage viral particle recovery for bioreactor 1 (BR1) based on established automated ELISA and Amperia titre data at harvest, clarification, TFF1 and chromatography steps (point BR1 was terminated due to loss of sample).

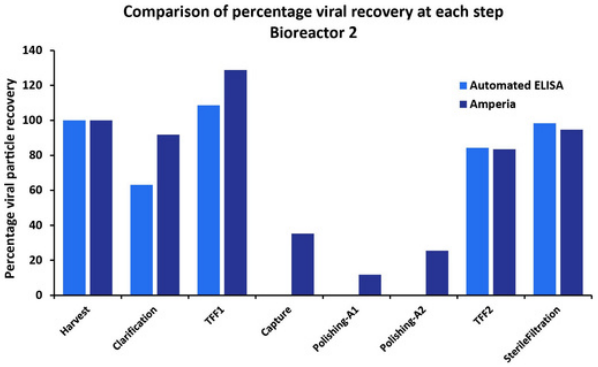

Figure 4b. Comparison of calculated percentage viral particle recovery for bioreactor 2 (BR2) based on established automated ELISA and Amperia titre data from harvest through to sterile filtration steps. **Note:** capture chromatography titre data was outside of the LOD for the automated ELISA system and so calculations for percentage recovery at Capture, Polishing-A1 and Polishing-A2 are not available.

The calculated percentage viral particle recovery between process steps of the viral vector production platform was compared between the existing automated ELISA instrument and Amperia (**Figures 4a** & **4b**). All samples were freeze/thawed and assayed on same plate for each instrument.

Recovery rates predicted by both methods show close alignment. Data was not available for the automated ELISA protocol for BR2 Capture, Polishing-A1, and Polishing-A2 steps due to the limit of detection.

Discussion

Comparison of the established automated ELISA method to the Amperia system against the team's key priorities of speed, accuracy, flexibility, and cost revealed some important benefits of the Amperia system.

Accuracy

Table 2 shows that the total viral titres were very similar with both systems, as was the range of CVs. When the correlation coefficient between viral particle titre (vp/mL) assayed by the incumbent automated ELISA instrument andthe Amperia instrument was calculated, excellent correlation was observed R^2= 0.97 (**Figure 3**).

Viral particle recovery rates calculated based on titre data from both systems showed close alignment, however the Amperia system was able to provide data at all process steps using more concentrated samples.

While the analysis was applied to batched samples for direct comparison, the rapid and flexible throughput of the Amperia system removes the need to batch and freeze/thaw samples for titre measurements in the future.

In the steps prior to chromatography, it is important to process the samples as soon as possible. If samples need to be frozen and stored for later analysis, precipitates can affect the accuracy of the titre measurement.

The ability to accurately check titre at each unit operation helps to establish robustness and consistency in the process which supports with technology transfer to GMP, where reproducibility and confidence in yield at each step is critical.

Costs

Reagent costs per sample for the Amperia system are 70% of equivalent costs of the incumbent ELISA method. However, this does not consider that a full plate must be run on the current automated ELISA instrument, whereas single samples can be run on the Amperia. If only running one sample, the current automated ELISA method is 100 times more expensive per run than the Amperia system.

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Time

The limit of detection and requirement for the final sample to be diluted 1 in 2 for the established automated ELISA method requires at least a 1 in 2000 to 1 in 20000 dilution, and one sample was still above the limit of detection. In comparison, the Amperia system works with dilutions of a factor of 10 less compared with the ELISA method, as the instruments dynamic range aligns with the sample matrices. Reducing the requirement for additional dilution steps translates to a significant time saving when preparing samples.

Preparing samples for automated ELISA adds an additional 45 minutes to the sample preparation for each extra dilution step (10x) when running a full plate. The Amperia system's one step dilution would save considerable time when running larger numbers of samples.

The automated ELISA instrument does have a large dynamic range of 5 logs, but this is at the lower end of viral particle detection and does not address the issue of having to make multiple dilutions for harvest samples.

Productivity

Having access to accurate titre data at any point in the process will allow the team to pinpoint issues quickly and avoid progressing through process steps where yield is not meeting requirements.

The team believes this efficiency will help to increase their overall productivity and ability to support clients with project data.

As Amperia assays utilise standard 96-well plates, with the flexibility to cost-effectively run a few samples quickly, it is also easy for the team to quickly switch between different serotypes. When working on client projects, time is of the essence and being able to provide feedback on projects with precise real-time measurements is extremely valuable.

In addition to measuring titre after each process step, it is also important to check permeates and waste for viral particles before disposal. Immediate testing can reduce unnecessary storage in the laboratory.

Conclusion

The team concluded that the Amperia system provided equivalent accuracy to the incumbent automated ELISA system but greater agility and simplicity in practice to meet their throughput and project needs. They selected the Amperia system for process development to cost-effectively access at-line titre data and drive efficient process optimisation.

Acknowledgements

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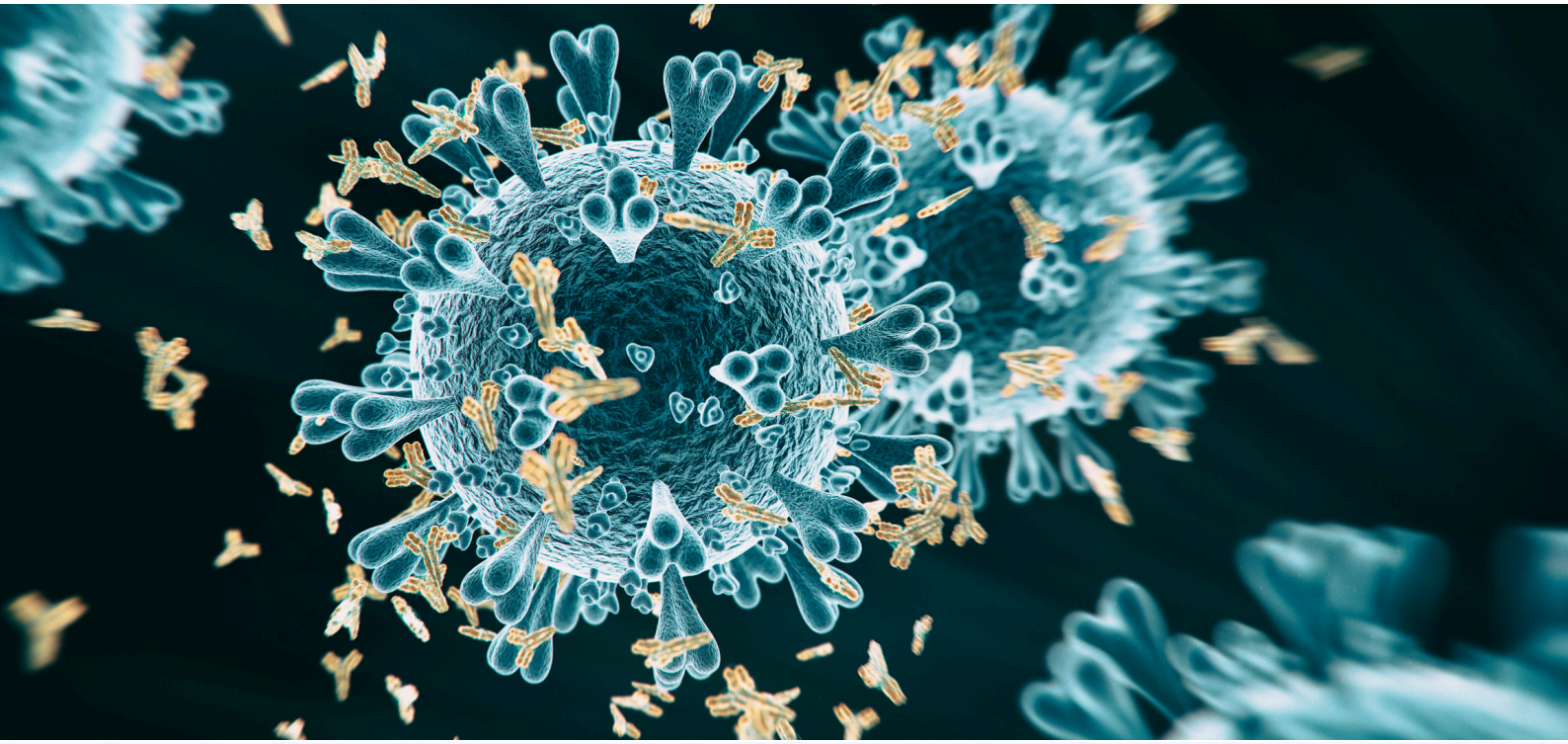

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