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# Development of chemically-defined medium for virus vaccine production in a duck suspension cell line

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## Introduction

A project was initiated to improve the production process for viral vaccines through the use of the EB66<sup>®</sup> cell line derived from duck embryonic stem cells and proprietary to Valneva. The primary goal of this project was to develop a chemically-defined medium that could be used in a simplified process while supporting the growth and production of a broad spectrum of different viruses.

As with many viral cell culture-based production processes, viral production in EB66 cells requires separate media for the cell proliferation phase and the virus production phase (Fig 1). Hence, the process includes two different basal medium formulations, with several separate additives or feeds required. While these processes are serum-free, they are not considered chemically-defined.

Many cell lines employed in vaccine production are obligate attachment cells, requiring non-defined additives or even serum to maintain successful culture conditions. However, EB66 cells grow in serum-free suspension culture at high cell density (Fig 2), allowing for much easier and more efficient scale-up, as well as virus production levels comparable to attachment cells.

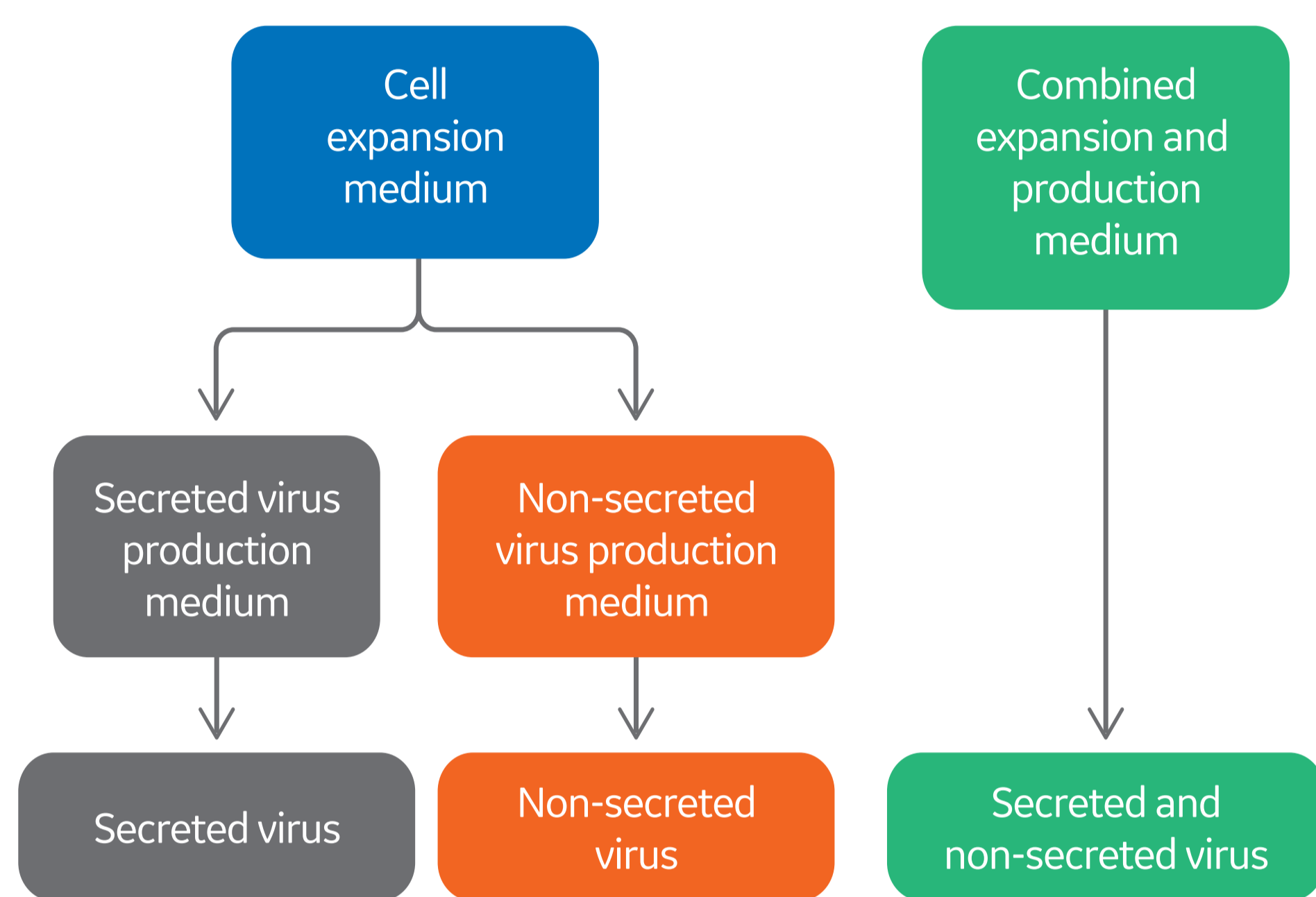


Fig 1. Difference between multiple media approach, requiring two or more media and multiple additives, and the simpler, single medium approach.

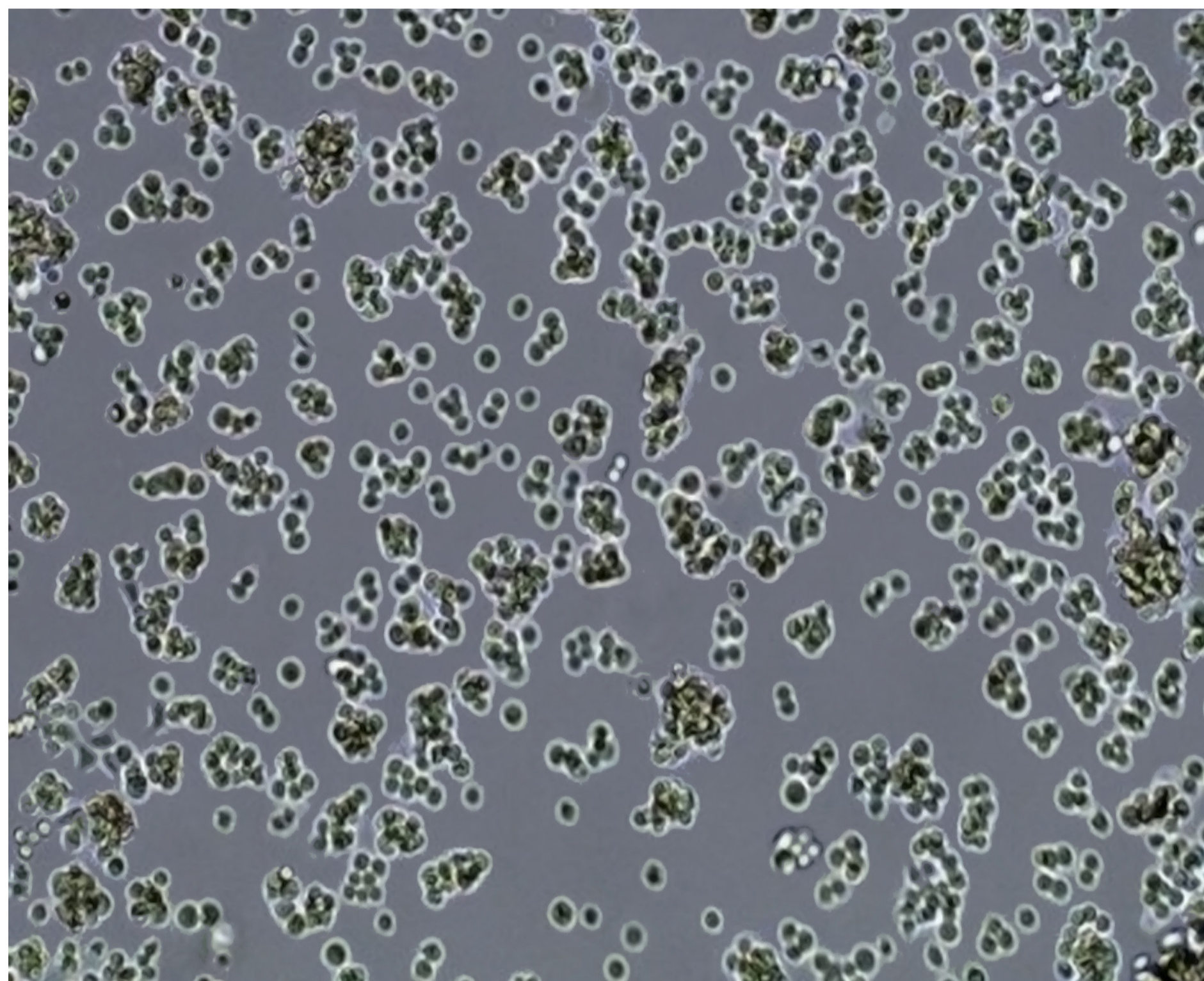


Fig 2. Typical morphology and loose aggregate structure characteristic of EB66 cells. Cellular aggregates facilitate infection of non-secreted, cell-to-cell transmitted viruses.

## Materials and methods

An initial growth-only screen of 20 different medium formulations, mixtures, and/or fortifications of existing media was performed to identify the most promising formulations to support good cell growth, viability, consistent morphology, and level of cellular aggregation. Cell counts and viability were determined with a Vi-CELL<sup>™</sup> XR counter (Beckman Coulter).

Screened formulations were assessed for viral production with representatives of secreted and non-secreted viruses, namely paramyxovirus and poxvirus. Viral titer was determined via standard TCID<sub>50</sub> methods. Scale-up to bioreactor level was also evaluated, prior to selection of a final formulation.

Bioreactors evaluated included the single-use Xcellerex<sup>™</sup> XDR-10 bioreactor, Wave Bioreactor<sup>™</sup> 20, Applikon<sup>®</sup> 3 L glass, 7 L glass, and 30 L Pilot stainless systems.

In addition to the two viruses already mentioned, several other viral types were assessed, including a representative of alphavirus and two strains of orthomyxovirus.

## Results

Figure 3 depicts direct adaptation of cells to the HyClone<sup>™</sup> CDM4Avian medium and productivity stability, with equivalent performance ten passages apart. Cell surface marker analysis also confirms no drift detectable over this time period (data not shown).

Figure 4 demonstrates scalability of the CDM4Avian medium formulation, from shake flask to bioreactor. Growth in the XDR-10 bioreactor system was slightly slower, but all three cultures yielded similar peak viable cell densities.

Figure 5 demonstrates scalability and repeatability of growth and virus production in three different sized bioreactors.

Figure 6 compares growth and virus production in the multiple media process vs the single medium process. Interestingly, by using CDM4Avian medium in a non-optimized single medium process, similar or higher virus titers were obtained. For example, virus productivity of a B strain of orthomyxovirus is significantly improved with the CDM4Avian medium, with a hemagglutinin concentration four times the level of that obtained in the reference process.

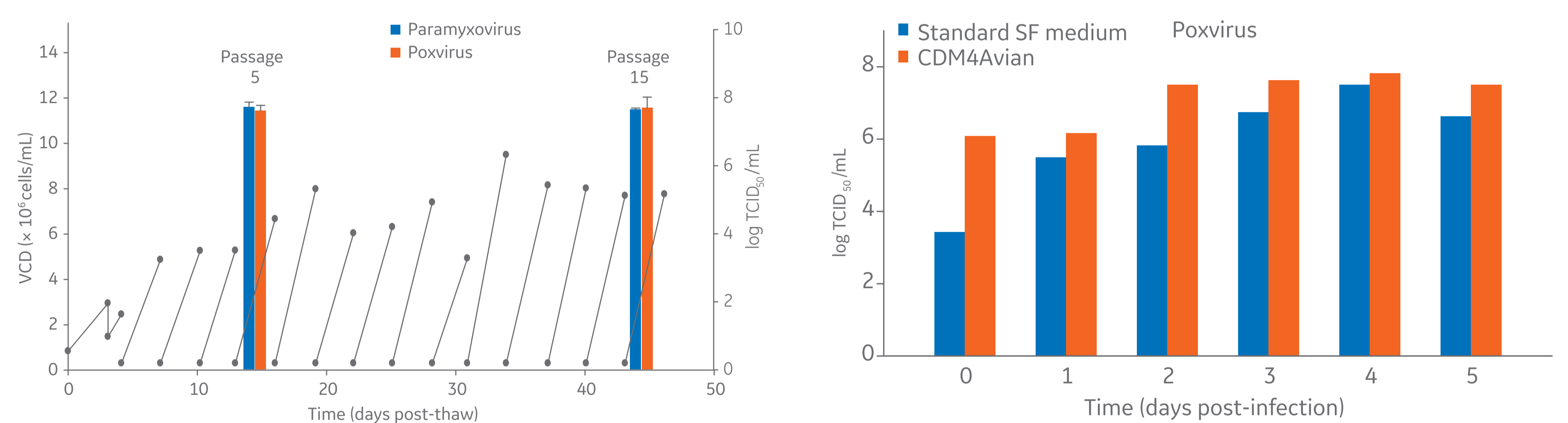


Fig 3. Passage study with viable cell density (VCD) and viral titer for both paramyxovirus and poxvirus production ten passages apart.

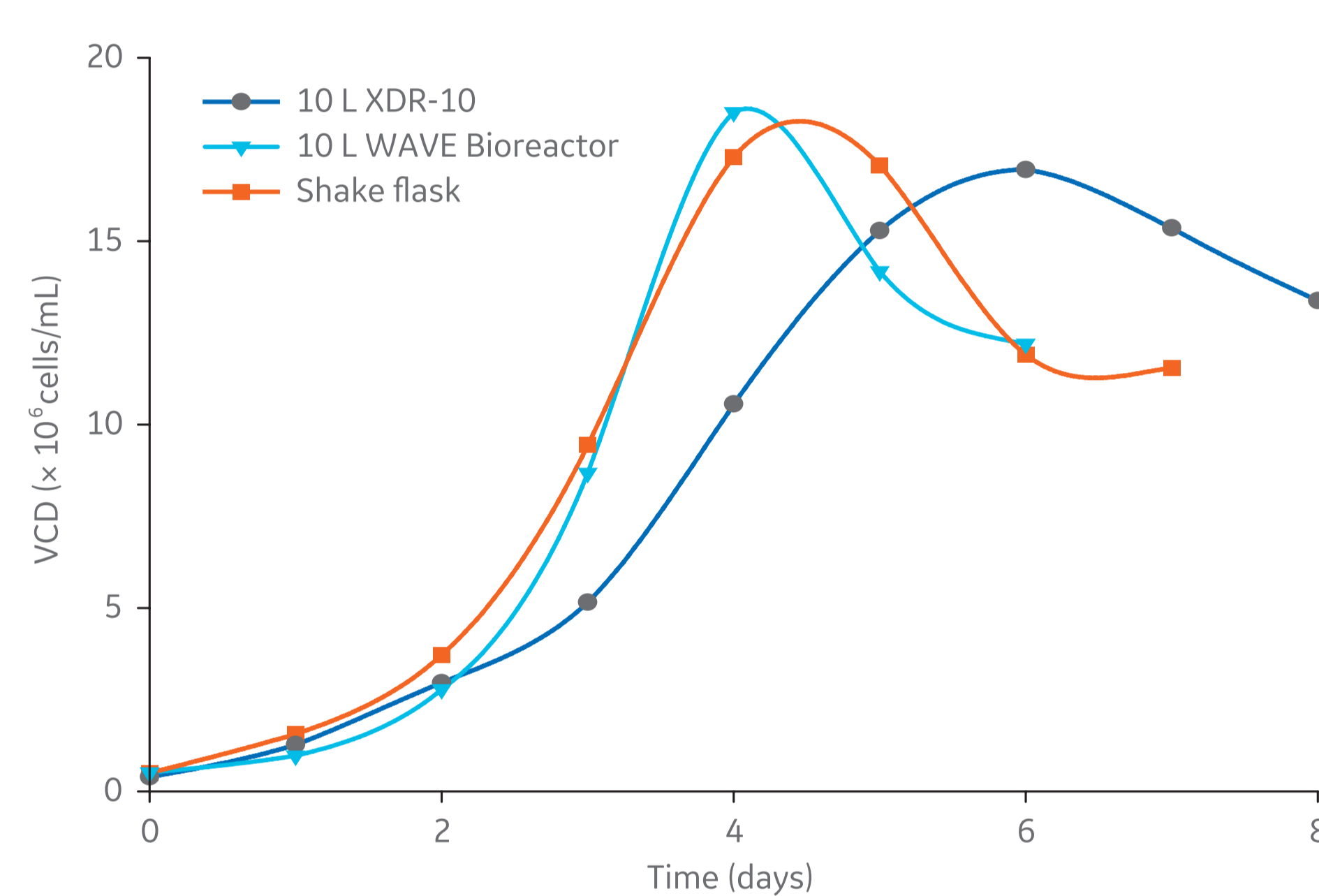


Fig 4. Growth-only bioreactor and shake flask cultures.

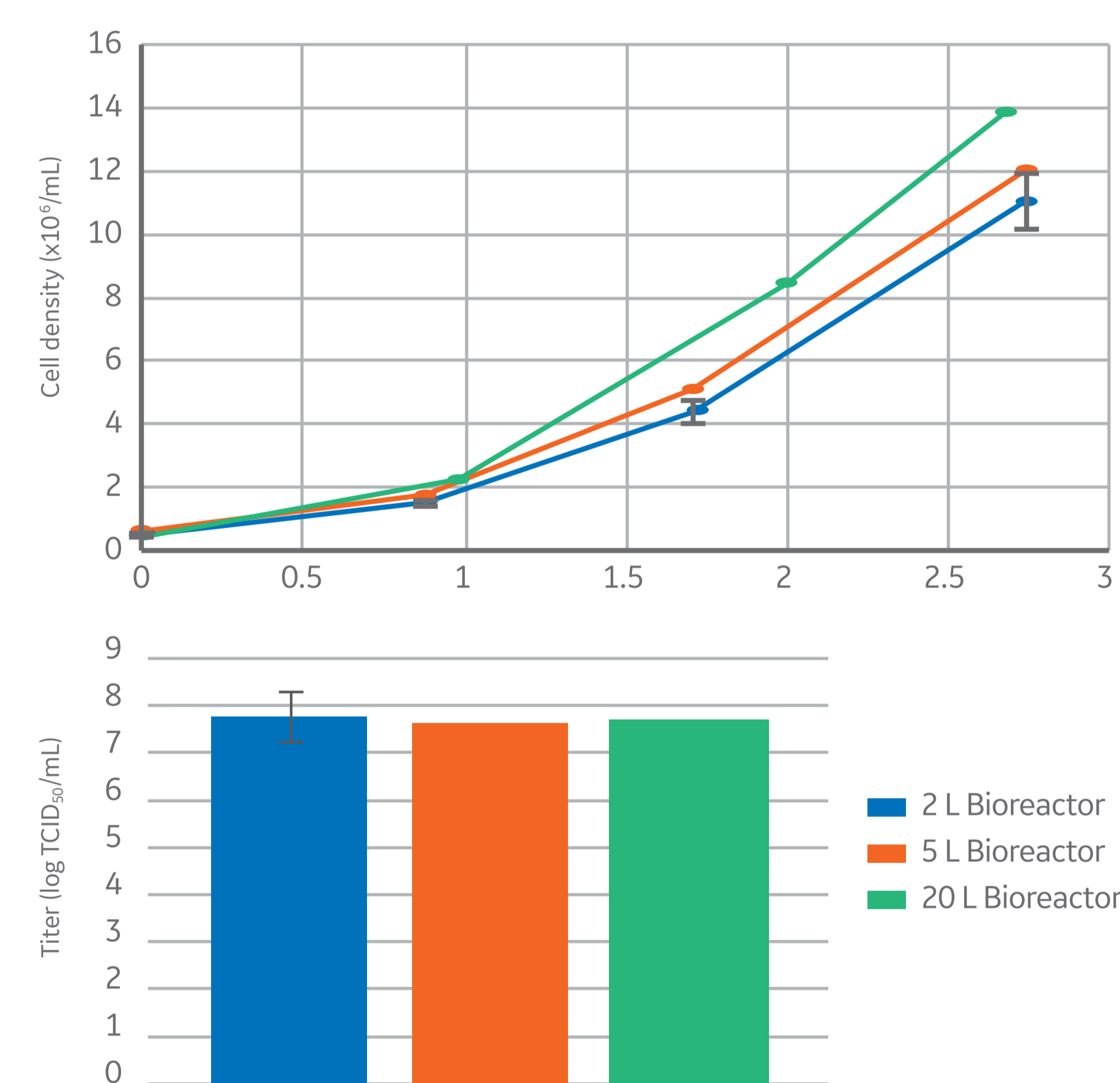


Fig 5. Pre-infection growth and two-day post-infection secreted virus production in three different scales of bioreactors. 2 L Bioreactor data displayed is mean ± SD, n=8.

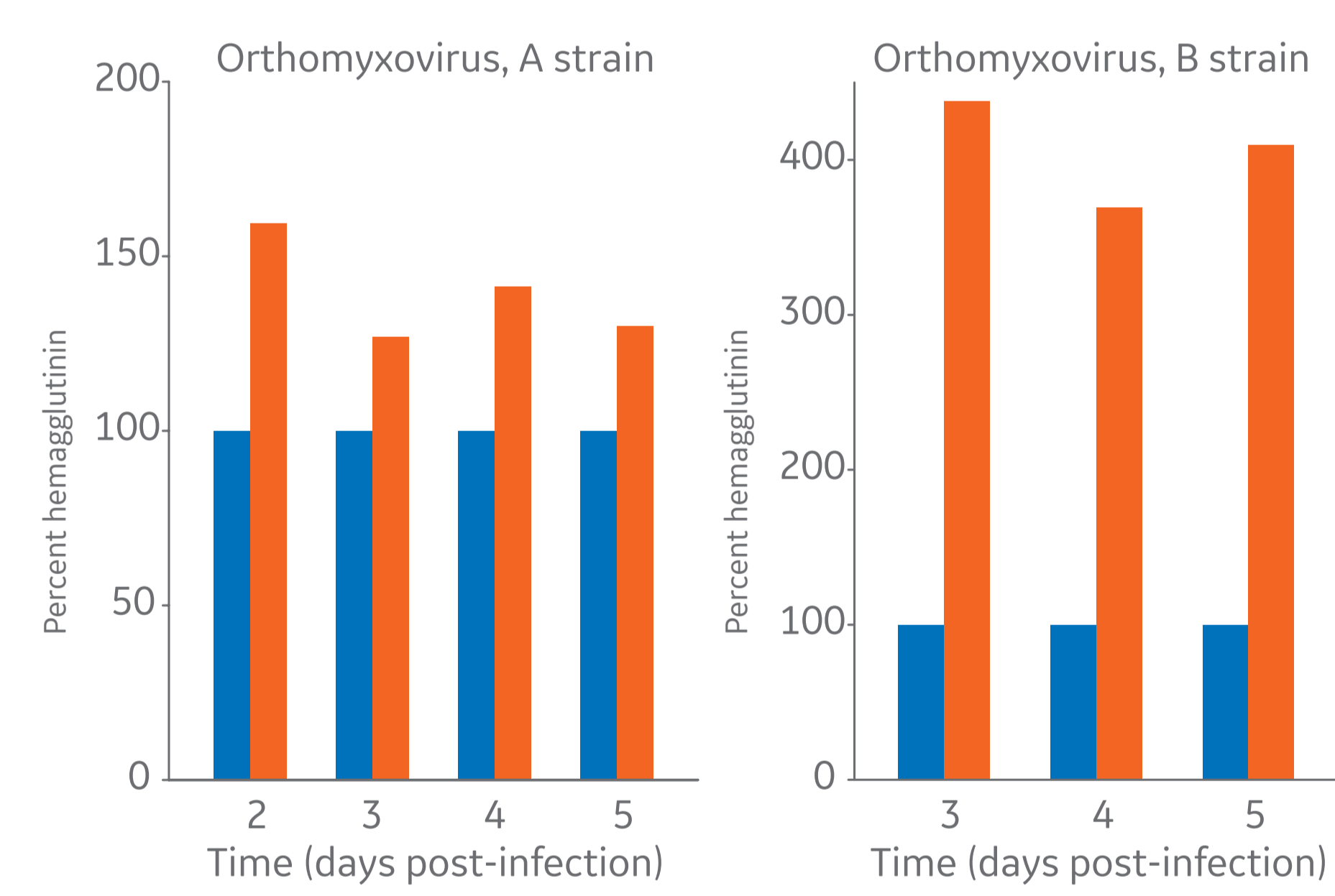
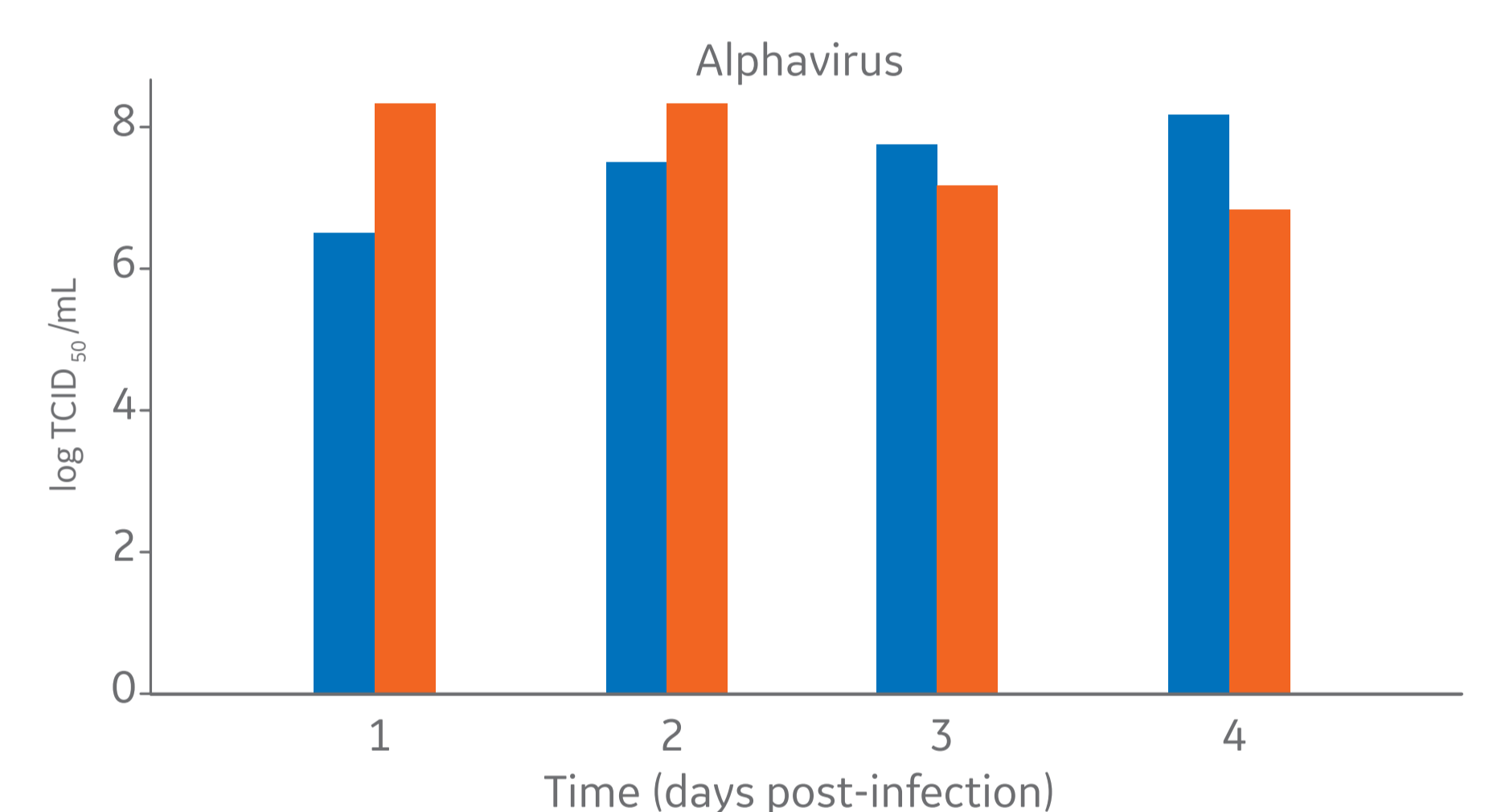
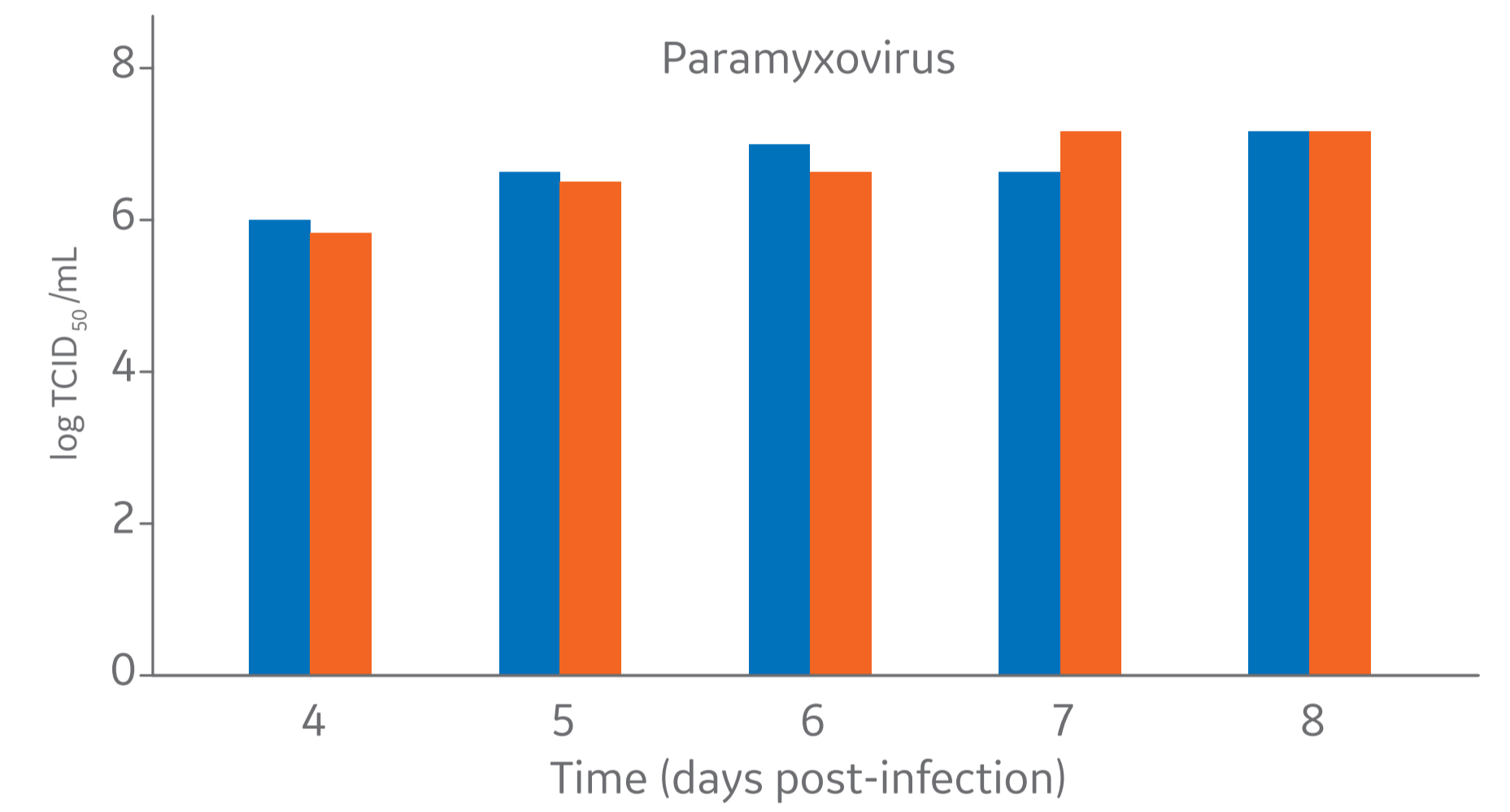
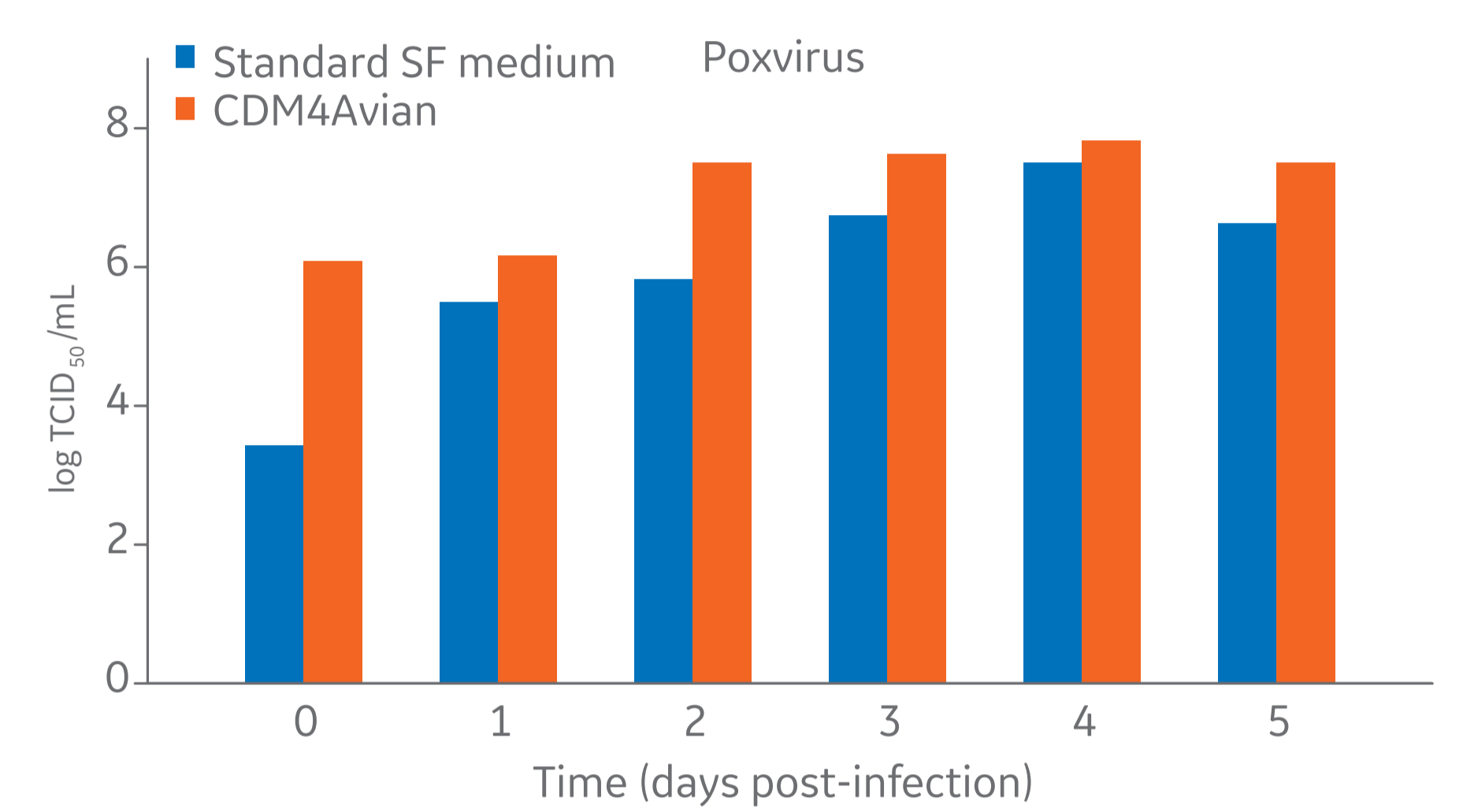


Fig 6. Kinetics and titer of various viral types produced with the chemically-defined single medium approach. The reference treatment corresponds to an optimized multiple media process, which uses serum-free (SF) media and non-chemically-defined feeds.

## Discussion and conclusions

Using a non-optimized single medium production process, viral titer was comparable to the multiple media production process, where serum-free media are used. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to the new formulation. Stability of the EB66 cell line was exceptional in this medium, with phenotype, aggregate size, cell densities, population doubling times, and embryonic cell surface markers remaining consistent after thirty passages (viral productivity remained constant for at least fifteen passages). Working cell banks made with HyClone CDM4Avian medium have worked well, exhibiting rapid recovery from cryopreservation. The CDM4Avian formulation and the single medium approach are currently being investigated with several other viruses with encouraging results. Overall, CDM4Avian medium is the first chemically-defined medium for EB66 cells that fulfills all critical requirements needed to grow viruses in a modern cell culture system.