Virus production in suspension-adapted avian cells using a chemically defined medium

This application note describes the performance of HyClone™ CDM4Avian cell culture medium that was developed to improve production processes for viral vaccines using Valneva's EB66® cell line derived from duck embryonic stem cells. Productivity of EB66 cells was assessed using representatives of both secreted and non-secreted viruses. The results show high cell density, viability, and productivity from T-flasks to bioreactor scale.

Introduction

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, allowing for much easier and more efficient scale-up, with virus production levels comparable to attachment cells (Fig 1).

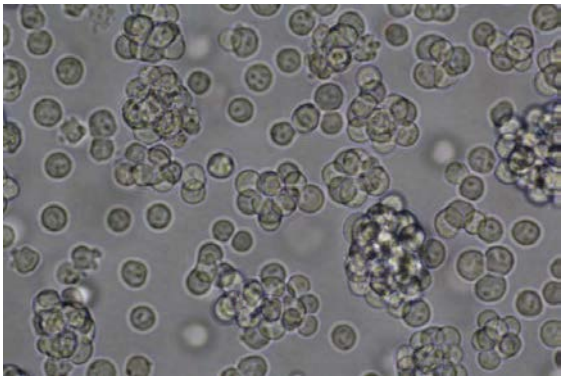

Fig 1. Typical morphology and loose aggregate structure characteristic of EB66 cells. Cellular aggregates facilitate infection of non-secreted, cell-to-cell transmitted viruses.

As with many viral cell culture-based production processes, viral production in EB66 cells is biphasic. In contrast to a monophasic process, which uses one single medium that supports both cell expansion and virus production, a biphasic process requires separate media for the cell proliferation phase and the virus production phase (Fig 2). Hence, a biphasic 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.

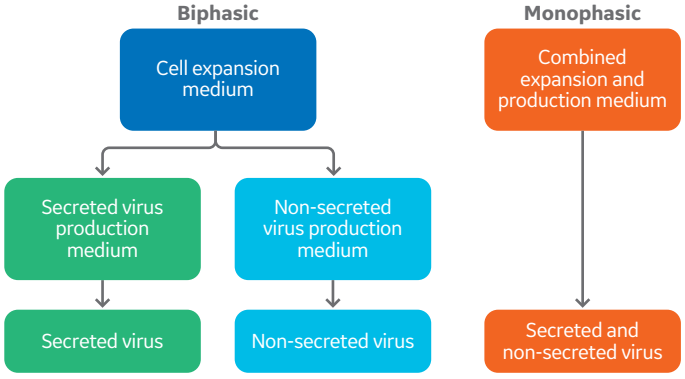

Fig 2. Difference between a biphasic process, requiring two or more media and multiple additives, and the simpler monophasic approach, requiring fewer additives.

Here, we describe the performance of CDM4Avian, a chemically defined and animal-derived component-free medium for cultivation and virus production in avian cell lines such as the EB66 cell line. CDM4Avian was developed to be used in a monophasic process, while supporting the production of a broad spectrum of different viruses. Evaluated medium performance characteristics were:

- Support of both cell expansion and virus production.
- Maintenance of cell line stability for multiple passages.
- Support of high cell density and viability.
- Maintenance of short, passage-consistent population doubling times (< 20 h).
- Support of process scaling by yielding similar growth and virus production from T-flasks to bioreactor scale.
- Support of high productivity of a variety of virus types.
- Support of using cryopreservation and cryorecovery methods of both GE and Valneva.

Materials and methods

EB66 cells (Valneva SE) were grown in shaken T-flask cultures using CDM4Avian as culture medium. Cell counts and viability were determined with a Vi-CELL™ XR counter (Beckman Coulter). EB66 cells were assessed for viral production with representatives of both secreted and non-secreted viruses, namely paramyxovirus, poxvirus, alphavirus, and two strains of orthomyxovirus. Viral titer was determined by standard tissue culture infective dose 50% ($TCID_{50}$) methods.

Scale-up to bioreactor level was also evaluated. Bioreactors evaluated included the single-use Xcellerex™ XDR-10 bioreactor (10 L), and WAVE Bioreactor™ 20 (10 L) systems as well as Applikon™ 3 L glass, 7 L glass, and 30 L stainless steel systems.

Cryopreservation and cryorecovery studies compared methods recommended by GE and Valneva, differing primarily in the presence or absence of conditioned medium. GE recommends a 90:10 ratio of fresh medium to DMSO. The Valneva recommended method is similar, but considered proprietary and is not detailed here.

Results

The results show that CDM4Avian meets the evaluated performance characteristics. Figure 3 shows aggregates of EB66 cells grown in CDM4Avian and infected with modified vaccinia Ankara (MVA) virus expressing green fluorescent protein (GFP). Ultraviolet (UV) illumination of the cells reveals fluorescence evident of viral infection.

Direct adaptation of EB66 cells to CDM4Avian medium is shown in Figure 4. Equivalent productivity ten passages apart demonstrates stable performance. Cell surface marker analysis also confirms no detectable drift over this time period (data not shown).

Process scaling with CDM4Avian, from T-flasks to bioreactor scale, is demonstrated in Figure 5. Growth in the XDR-10 bioreactor system was slightly slower, but all three cultures yielded similar peak viable cell densities. Figure 6 shows pre-infection growth and two-day post-infection secreted virus production in three different bioreactor scales.

Lot-to-lot consistency of the medium was demonstrated by comparing three different lots of CDM4Avian medium for growth kinetics as shown in Figure 7.

A comparison between virus production in a biphasic process, using a serum-free medium and non-chemically defined feeds, and in a monophasic process, using CDM4Avian medium, is shown in Figure 8. Interestingly, by using CDM4Avian medium in a non-optimized monophasic process, similar or even higher virus titers were obtained. For example, cell productivity of orthomyxovirus strain B is significantly improved with CDM4Avian, with a hemagglutinin concentration four times the level obtained in the reference biphasic process.

Comparison of cell growth and population doubling times during a three-passage cryorecovery assessment is shown in Figure 9. No significant difference was observed between the two cryopreservation methods tested.

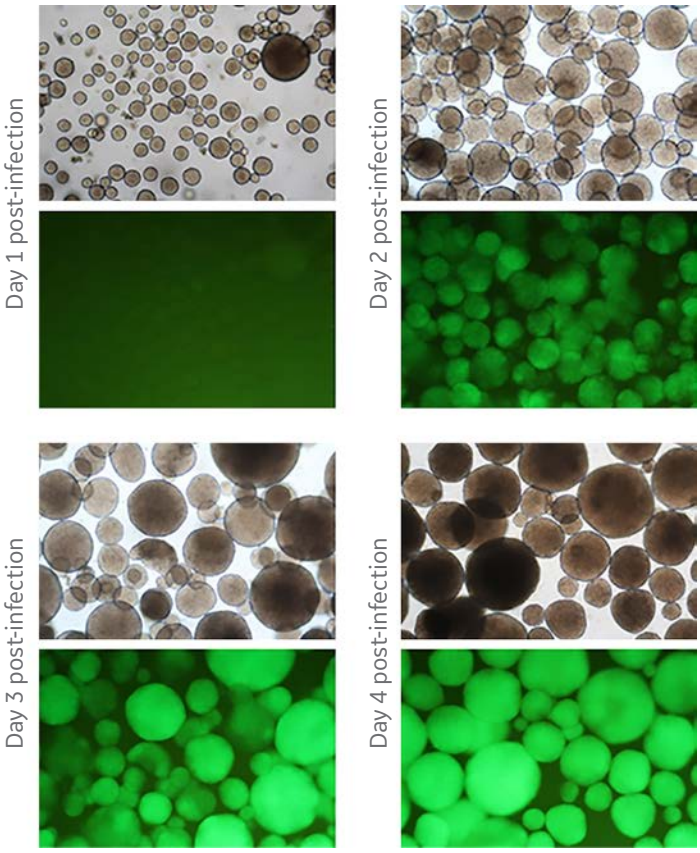

Fig 3. Use of an MVA-GFP construct demonstrates viral infection and proliferation in EB66 cell aggregates. Top row is visible light illumination; bottom row is UV illumination with fluorescence evident from infected cells.

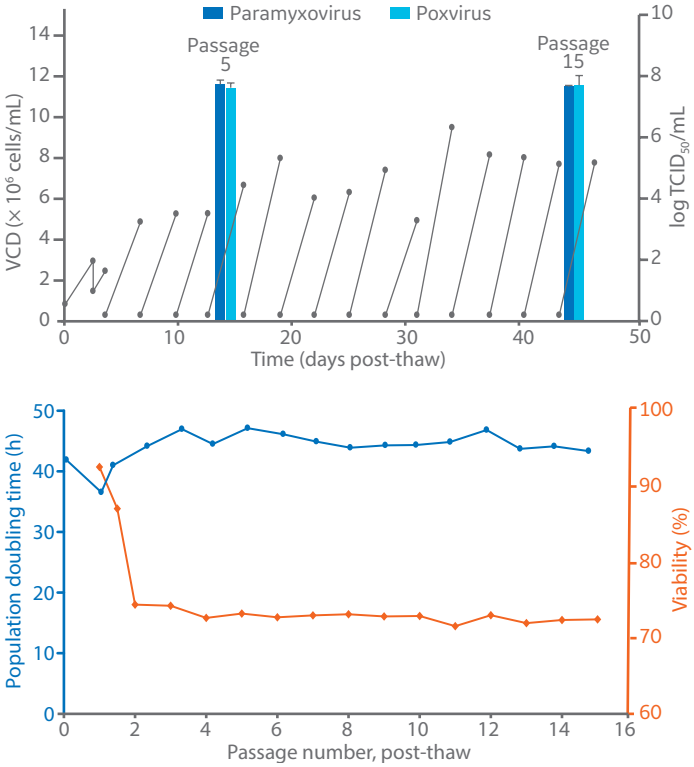

Fig 4. Passage study with viable cell density (VCD), viability, population doubling time, and viral titer for both paramyxovirus and poxvirus production. Both graphs are from the same fifteen-passage study.

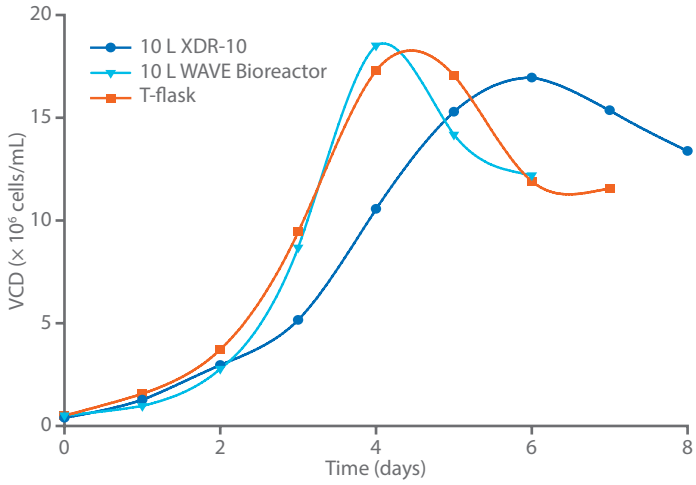

Fig 5. Growth-only bioreactor and T-flask cultures.

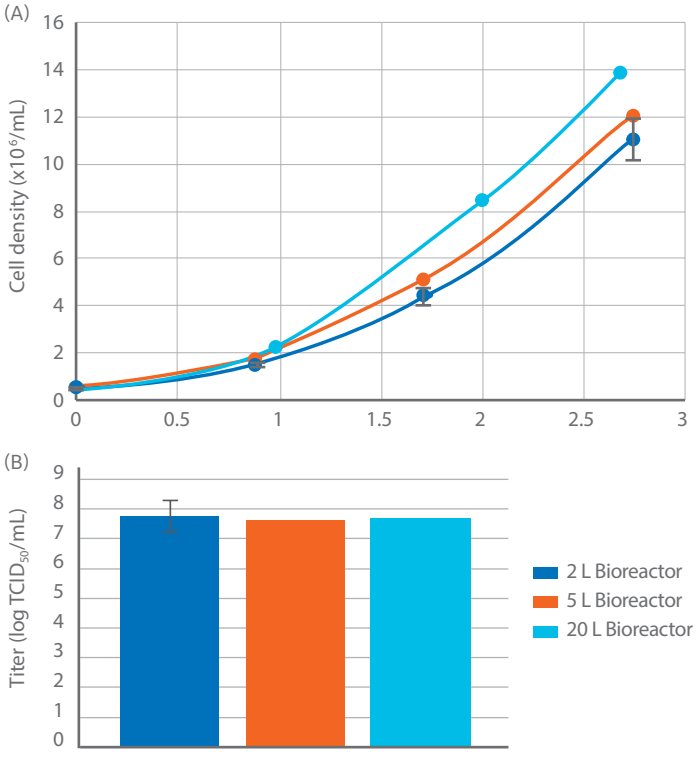

Fig 6. (A) Pre-infection growth and (B) two-day post-infection secreted virus production in three different bioreactor scales. The 2 L bioreactor data displayed is mean ± SD, n = 8.

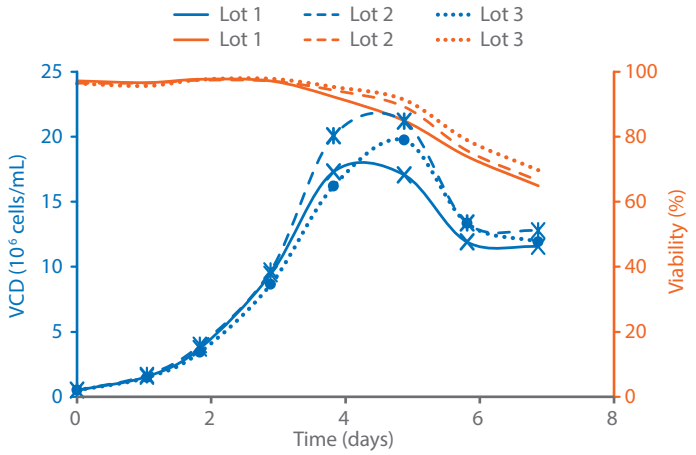

Fig 7. Comparison of three different lots of CDM4Avian in growth-only T-flask cultures.

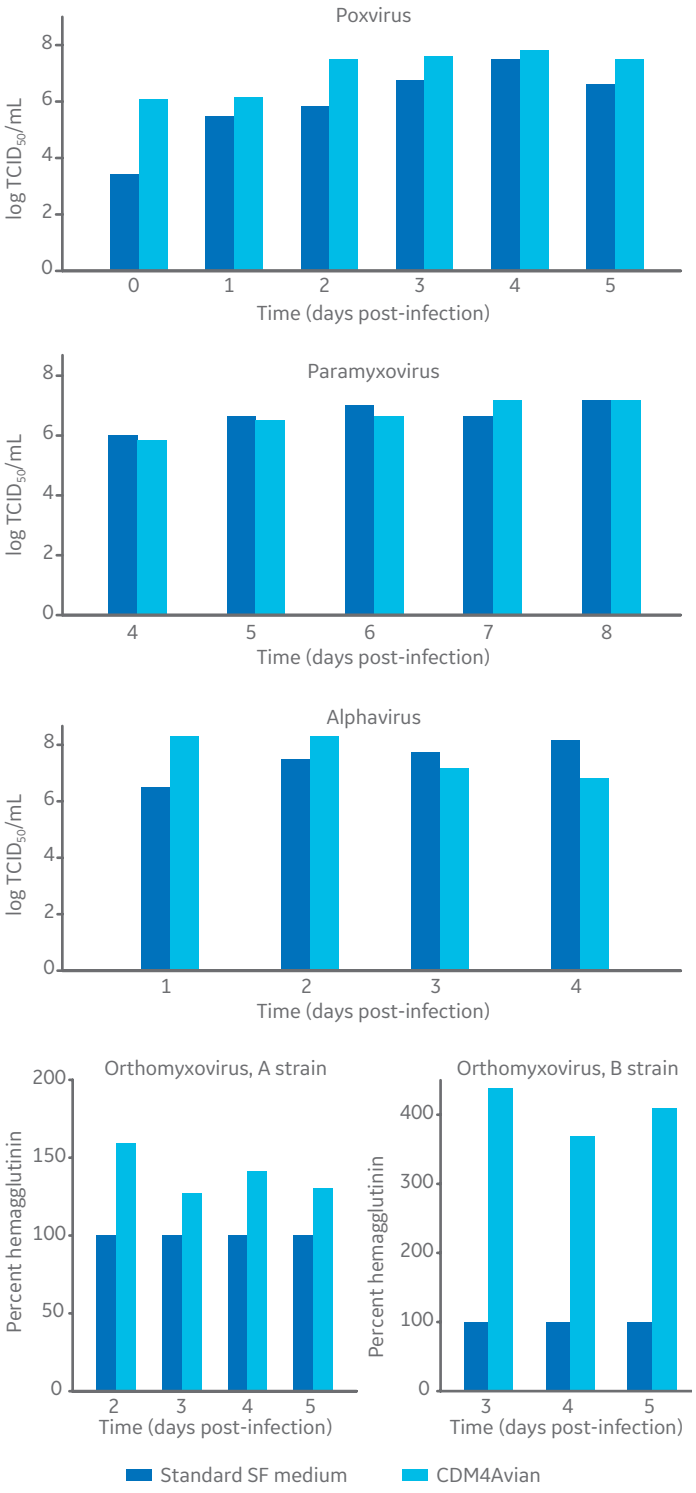

Fig 8. Kinetics and titers of various virus types produced with the CDM4Avian monophasic medium approach. The reference treatment corresponds to an optimized biphasic process, which uses serum-free (SF) medium and non-chemically defined feeds.

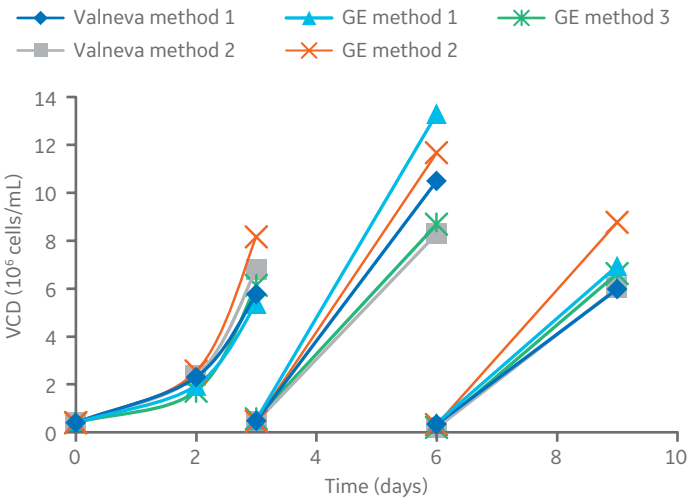

Fig 9. Comparison of cell growth and population doubling times during a three-passage cryorecovery assessment.

Discussion

This work demonstrates the performance of CDM4Avian medium in the production of both secreted and non-secreted viruses from EB66 cells. The medium supported high cell growth and productivity, with similar results in different vessel formats and bioreactor scales. Using non-optimized monophasic production processes, virus titers in CDM4Avian were comparable with the standard biphasic production process, where serum-free medium was used. EB66 cells banked in other serum-free media quickly recovered from cryopreservation and readily adapted to CDM4Avian. The stability of the EB66 cell line was exceptional in CDM4Avian, with cell phenotype, aggregate size, densities, population doubling times, and embryonic cell surface markers remaining consistent after thirty passages, while virus productivity remained constant for at least fifteen passages. Working cell banks made with CDM4Avian medium performed well, exhibiting rapid recovery from cryopreservation. The CDM4Avian formulation with a monophasic approach have been investigated using several other viruses, with encouraging results. Overall, CDM4Avian medium is the first chemically defined medium for EB66 cells that fulfills the critical requirements needed to grow viruses in a modern cell culture system.

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