



# HyClone™ CDM4CHO powdered medium stability investigation

**As shipping is often performed at ambient temperatures, we have investigated the effects of temperature on CDM4CHO cell culture medium. To simulate shipping conditions, powder medium was stored at -20°C, 25°C, and 37°C for up to four weeks before hydration and evaluated for cell culture performance.**

## Introduction

Shipping at ambient temperature can mean large variations in the thermal environment, and trans-oceanic shipping is more likely to experience temperature extremes. While most shipments last for only a few days, shipments including stopovers can take several days. This study was performed to investigate the stability of HyClone CDM4CHO powdered cell culture medium in temperatures outside of the recommended storage temperature of 2°C to 8°C.

## Methods

Aliquots from three lots of CDM4CHO powdered medium (SH30556) were stored in three non-humidified incubators, each maintained at different temperatures (-20°C, 25°C, and 37°C). Temperature monitoring data loggers, set for 30 min readings, were included with the samples. Two bottles of CDM4CHO liquid medium (SH30558) stored at 4°C were used as controls.

Samples were removed from the incubators at weekly intervals, and stored until hydration at 4°C. Upon hydration, sodium bicarbonate and poloxamer 188 were added as per hydration instructions. At time of use, the hydrated medium was supplemented with 4 mM L-glutamine.

For each test condition, duplicate 125 mL shaker flasks were seeded at  $2.5 \times 10^5$  cells/mL in 50 mL medium and incubated at 37°C, in 5% CO<sub>2</sub> at approximately 125 RPM agitation.

Beginning on day two, daily cell counts were obtained using a Vi-CELL™ counter (Beckman Coulter) until cell viability dropped below 50%.

Cultures were sampled for nutrient and metabolite analyses at culture initiation, mid-culture, and culture termination. Samples were centrifuged at low speed (~ 600 × g) for 5 min, followed by freezing of the supernatant for later analysis using the Bioprofile Flex™ instrument (Nova Biomedical).

Two runs of 40 mL/flask each were performed separately to ensure proper handling of each run. Samples from weeks 1 and 2 were examined in the first run; samples from weeks 3 and 4 were examined in the second run.

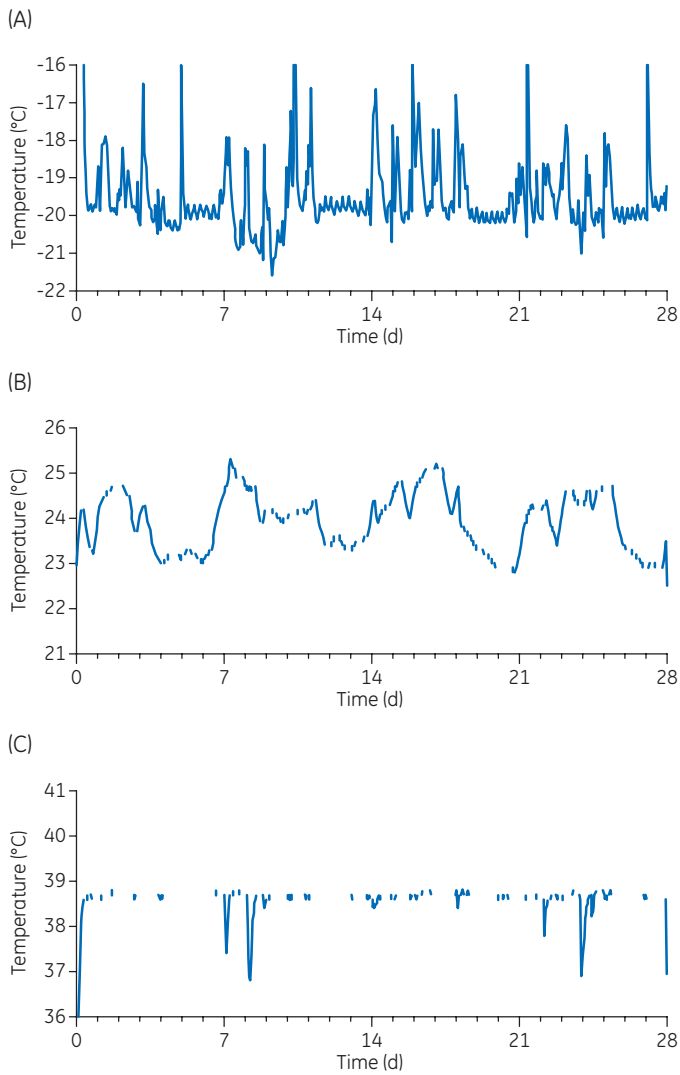
## Results

With increased incubation time, powdered medium samples stored at 37°C congealed into near-tablet form, and darkened in color. Samples stored at 25°C also compacted over time, but to a much lesser extent. Powdered medium stored at -20°C remained free-flowing for the duration of the experiment. Pelletized powdered medium took much longer to dissolve, but all samples did go into solution with no visual precipitate.

Figure 1 documents the temperature exposure history of each sample, with vertical lines signifying one week intervals. Recorded mean temperatures for the 25° and 37°C treatments were closer to 24°C and 38.5°C, respectively.

Prolonged exposure of CDM4CHO powdered medium to temperatures in the range of -20°C to 25°C had little, if any, impact on cell culture performance. Medium stored at 37°C, showed an increase in brown discoloration (Fig 2). Powdered medium discoloration is likely due to the Maillard reaction (non-enzymatic browning), a chemical reaction between amino acids and sugars. Medium stored at 37°C also showed a pronounced decrease in culture performance over time.

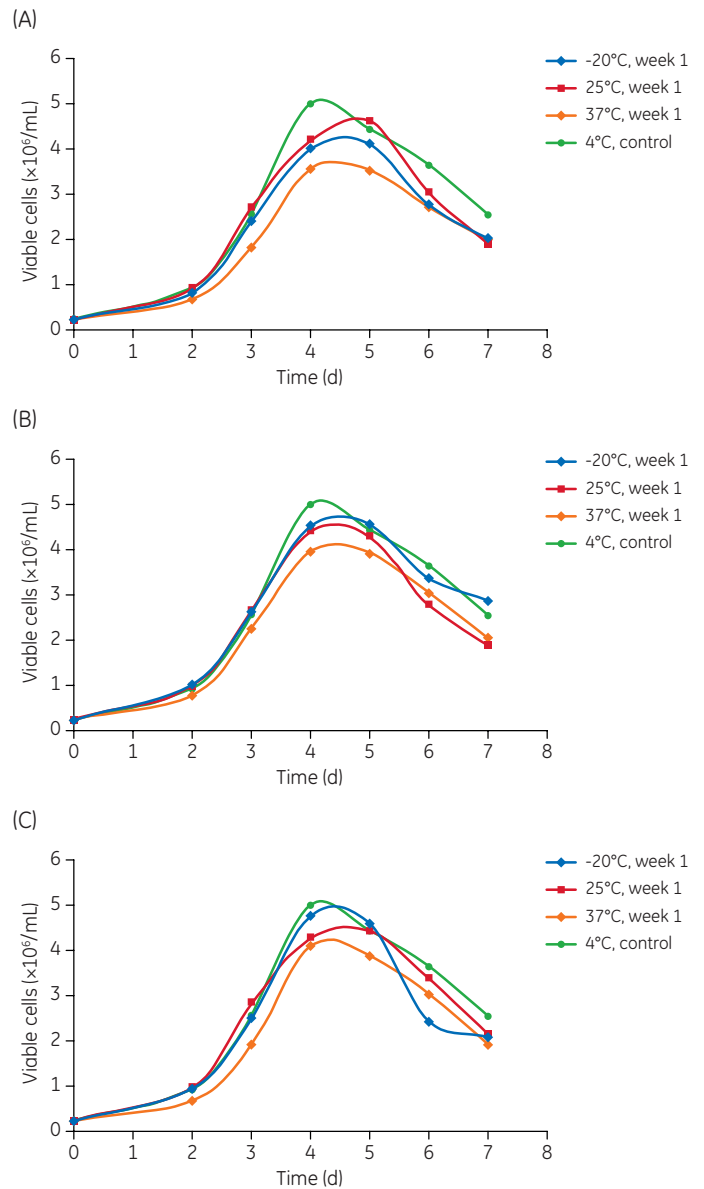
Figures 3 to 6 are standard growth curve comparisons of the three lots of powdered medium evaluated at one week sampling intervals. Data points represent the mean viable cell count of duplicate flasks. Note that only the 37°C treatment performed significantly different from controls, with performance deteriorating with increased exposure time.



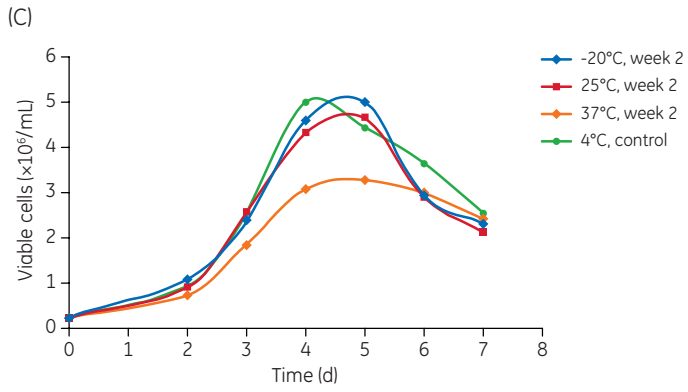
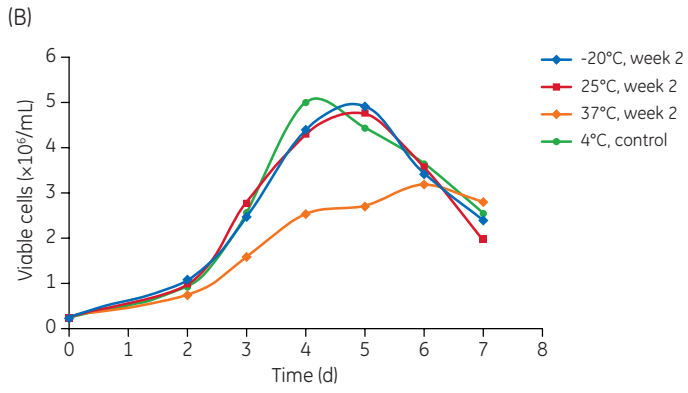
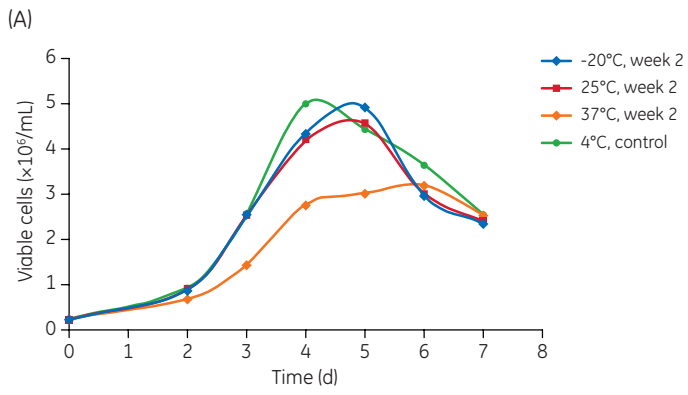
**Fig 1.** Monitored temperature within the three incubators used: (A) -20°C, (B) 25°C, and (C) 37°C.



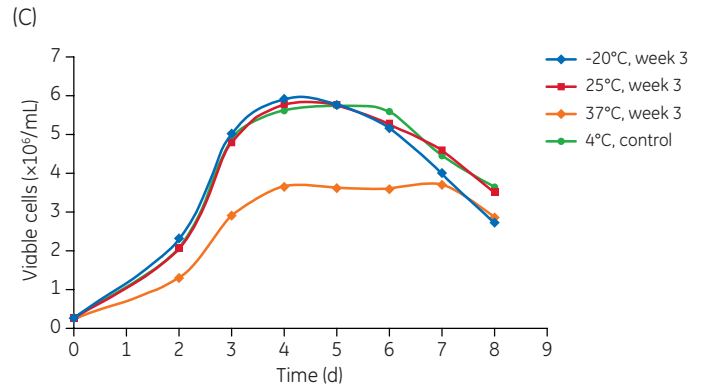
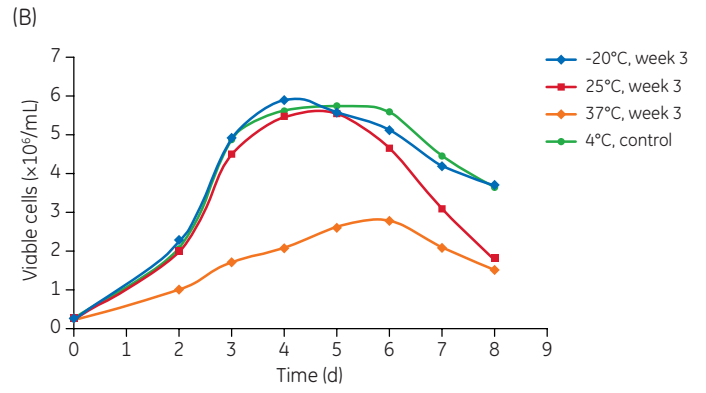
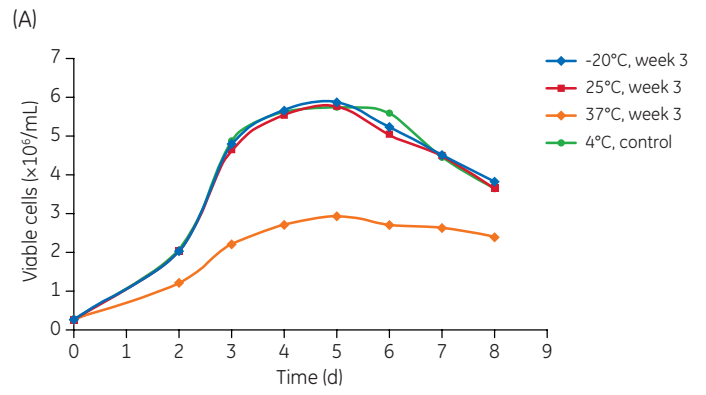
**Fig 2.** Increasing color development over time when CDM4CHO medium was incubated at 37°C.



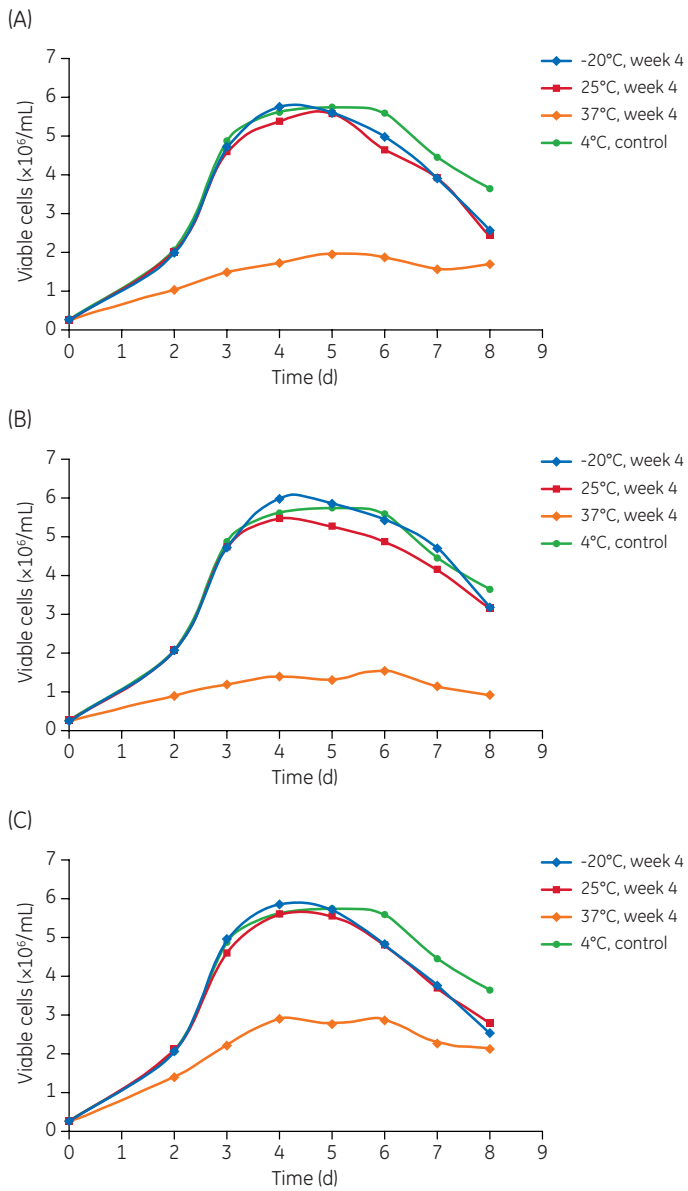
**Fig 3.** Growth curves of week 1 sample cultures in three CDM4CHO medium lots: (A) Lot -082, (B) Lot -684, and (C) Lot -741. Points are mean values of duplicate.



**Fig 4.** Growth curves of week 2 sample cultures in three CDM4CHO medium lots: (A) Lot -082, (B) Lot -684, and (C) Lot -741. Points are mean values of duplicate.

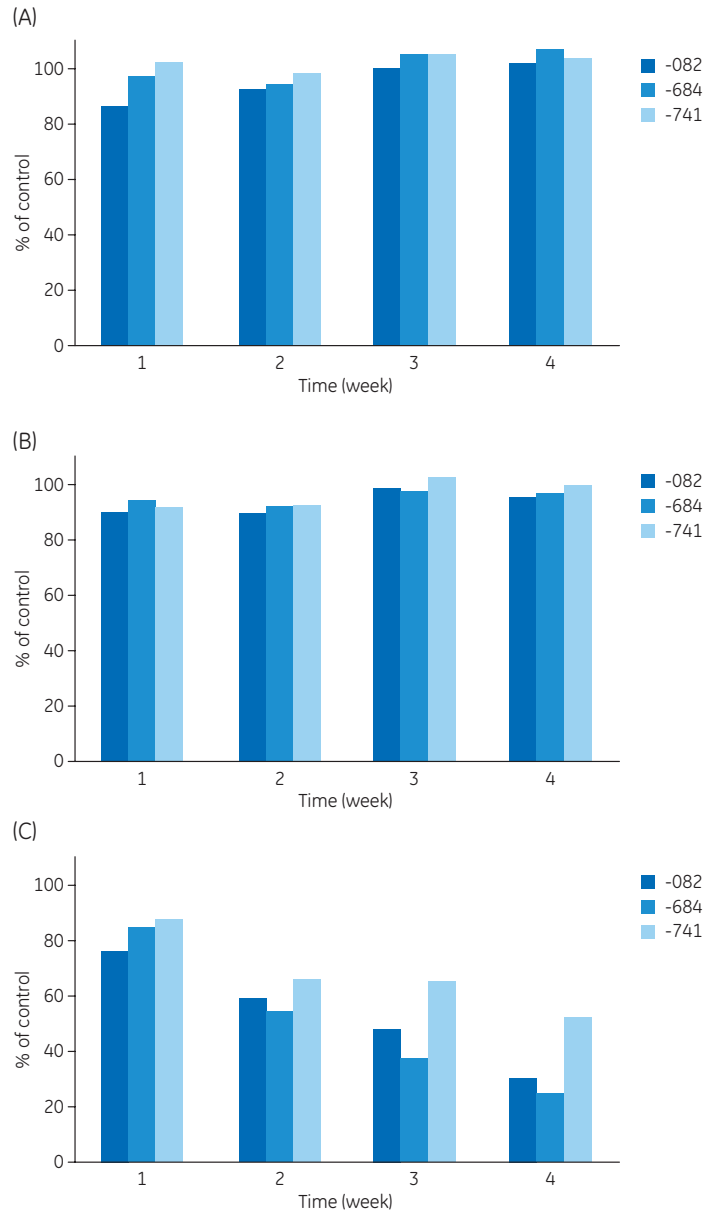


**Fig 5.** Growth curves of week 3 sample cultures in three CDM4CHO medium lots: (A) Lot -082, (B) Lot -684, and (C) Lot -741. Points are mean values of duplicate.

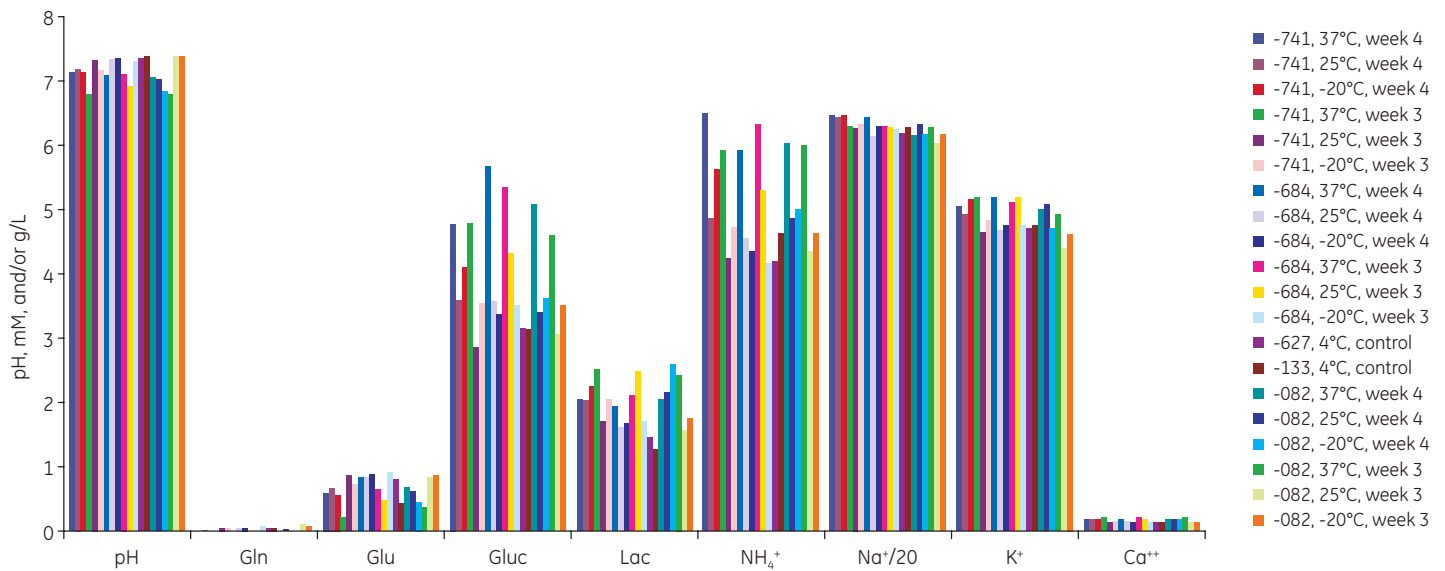


**Fig 6.** Growth curves of week 4 sample cultures in three CDM4CHO medium lots: (A) Lot -082, (B) Lot -684, and (C) Lot -741. Points are mean values of duplicate.

Culture performance at peak viable cell density is shown in Figure 7, expressed as percent of control culture. Peak cell densities were either on day 4 or day 5; graphs based on day 5 data were nearly identical to those in Figure 7 (data not shown).



**Fig 7.** Summary of culture performance of three CDM4CHO medium lots on day 4, expressed as percent of control. (A) -20°C, (B) 25°C, and (C) 37°C.



**Fig 8.** Nutrient and metabolite analysis results from final sampling (one of six samplings).

Figure 8 displays nutrient and metabolite analyses on the final harvest of weeks 3 and 4 sample runs. The 37°C samples showed higher glucose and pH, lower lactate, and elevated ammonium content, when compared with the other treatments. These findings are consistent with the significantly fewer cells growing in these flasks.

## Conclusion

CDM4CHO powdered medium exposed to prolonged incubation at -20°C and 25°C exhibited little or no alteration in cell culture performance upon hydration compared with liquid control medium stored at 4°C. However, powder medium stored at 37°C, showed an increase in brown discoloration and a pronounced decrease in culture performance over time. From these results, it is concluded that shipment of CDM4CHO powdered medium would not be deleterious to its culture performance characteristics, as long as temperatures do not exceed 25°C.





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