

## CASY<sup>VIVO</sup> Cell Counter & Analyzer - Powering Breakthroughs in Cell Research

### Cholesterol's Role in Cultured Red Blood Cell Quality

Claessen et al. (2024) Production and stability of cultured red blood cells depends on the concentration of cholesterol in culture medium. Scientific Reports (2024) 14:15592. DOI10.1038/s41598-024-66440-z

Cell Culture; Red Blood Cells, cultured RBC	
Index	CC4
Standardization	X
Counting	X
Viability	
Volume	X

#### The Challenge:

To investigate how cholesterol concentration and timing of supplementation affect the expansion, enucleation, quality (volume/stability), and recovery of cultured red blood cells (cRBC) for transfusion purposes.

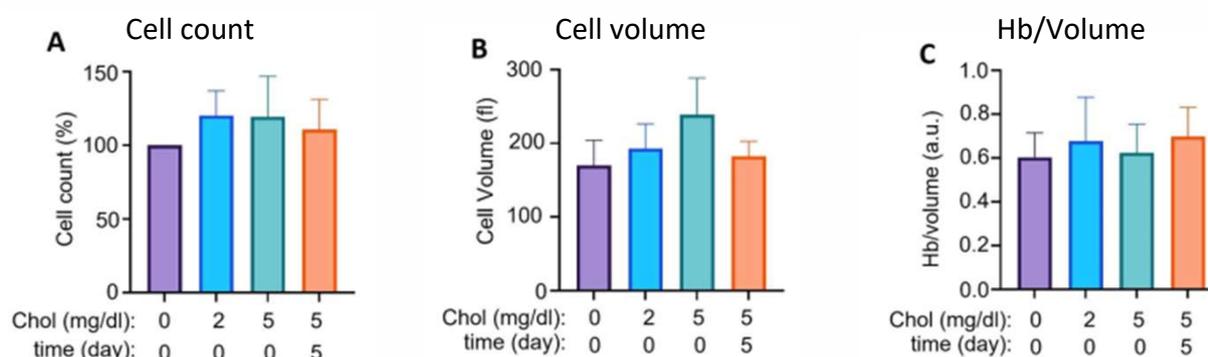
#### CASY's Contribution:

CASY was used to monitor cell density during the expansion phase (days 7 onwards), ensuring cultures were maintained between 0.7 and 2.0 x 10<sup>6</sup> cells/mL. It also measured cell count and cell volume (femtoliter, fL) at the end of differentiation (day 11) for normalization and volumetric analysis<sup>33</sup>

#### Key Benefits to Researchers:

- Cell Expansion Monitoring:** CASY confirmed that cholesterol supplementation did not alter the cumulative cell numbers during the initial 24-day expansion phase.
- Cell Volume/Size Analysis:** CASY provided the data showing that cRBC mean volume increased with increasing cholesterol concentration, but only when cholesterol was added at the start of differentiation.
- Normalization of Hemoglobin:** By measuring cell volume, CASY provided the essential factor to demonstrate that while hemoglobin (Hb) per cell increased (due to size increase), the Hb concentration per cell volume remained constant across all conditions.

#### Effect of Cholesterol on Erythroblast Growth and Volume



**Figure 2. (A–B)** Proliferation and volume kinetics. Cell counts **(A)** were **normalized** to non-supplemented controls, while cell volume **(B)** was tracked in femtoliters (fL) to monitor morphological stability. **(C)** Hemoglobin levels (a.u.) corrected for cell volume to provide a standardized measure of protein density. Cultures were supplemented with 2 or 5 mg/dL cholesterol at day 0 or day 5 of induction. Data represent mean ± SD (n = 3); \*p < 0.05 vs. control.