Please cite this article as: Engstrom, I. A. *et al.* (2021). Packed Cell Volume Is an Overestimate in Common Cancer Cell Lines. *Bio-protocol* 11(21): e4216. DOI: 10.21769/BioProtoc.4216.

bio-101 www.bio-protocol.org/e4216 Bio-101: e4216. DOI:10.21769/BioProtoc.4216

Packed Cell Volume Is an Overestimate in Common Cancer Cell Lines

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[Abstract] Originally developed to determine the volume of red blood cells in blood, packed cell volume (PCV) measurements have more recently been used also to determine the volume of cells in tissue culture experiments. For blood, PCV is proportional to cell volume as determined using the Coulter principle, which relates particle volume to the change in electrical impedance as the particle passes through an aperture. However, this assumption has not been tested for the diverse cell lines used in cell culture. We compared PCV with Coulter counter-based cell volume measurements for five cell lines and found that standard PCV protocols comparatively overestimate cell volume compared to Coulter-based measurements. We hypothesize that this discrepancy is related to incomplete cell packing and demonstrate that it can be mitigated in some cells using fast and prolonged centrifugation.

Keywords: Packed Cell Volume, Metabolomics, Absolute quantification, Metabolism, Cancer Metabolism

[Background] Accurate measurements of cell volume are essential for several biological research applications, particularly for normalizing material loading when calculating the absolute concentration of intracellular molecules. Systemic errors in cell volume measurements could therefore skew intracellular molarity calculations and substantially alter interpretations. For example, intracellular concentrations of central carbon metabolites are often near the Km of the enzymes that consume them (Park *et al.*, 2016), meaning that changes in steady-state metabolite levels can proportionally change metabolic fluxes across many metabolic reactions. Thus, systemic errors in cell volume calculations skew metabolite concentration calculations and thereby alter interpretations of metabolic state based on calculated molarities. Accurate cell volume measurements are therefore critical for quantitative measurements of cell content and biological interpretation.

Packed cell volume (PCV) is a common and convenient technique used to measure the total cell volume of a cell suspension. These measurements typically use a microcentrifuge tube with a narrow, calibrated capillary at the bottom. Upon centrifugation, cells pellet in the capillary, and PCV is determined by the height of the cell pellet from the bottom of the tube. This technique has been well validated as an accurate method to measure the volume of red blood cells from blood, but whether it is accurate for cell volume measurements for other cell types has not been widely determined. While red blood cells are smooth and regularly shaped, other cell types have differences in expression of extracellular facing molecules and distinct biophysical properties that may alter packing efficiency.

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To evaluate the accuracy of PCV-based measurements in common cell culture cell lines, we compare standard and modified PCV measurement protocols with Coulter counter-based impedance measurements. The Coulter counter is widely accepted as a gold standard method to measure particle volume but is less accessible than PCV since it requires specialized equipment. Direct comparison of these protocols here indicates that standard PCV protocols can lead to an overestimate of cell volume in all tested cell lines compared with Coulter Counter measurements, which can be partially or fully corrected by fast and prolonged centrifugation depending on cell line.

Materials and Reagents

1. 15 ml conical tube (Fisher Scientific, Fisherbrand, catalog number: 055395)
2. NIST latex particles (Beckman Coulter, Beckman Coulter, catalog number: 6602798)
3. 10 cm tissue culture dishes (Fisher Scientific, Fisherbrand, catalog number: FB012924)
4. Tissue culture flask (Fisher Scientific, Thermo Scientific, catalog number: 169900)
5. Isoton II (Beckman Coulter, catalog number: 8546719)
6. Accuvette (Beckman Coulter, catalog number: A35471)
7. PCV tube (TPP, catalog number: 87005)
8. 143B, H1299, A549, TF-1, and Jurkat cells (ATCC, catalog numbers: CRL-8303, CRL-5803, CCL-185, CRL-2003, TIB-152)
9. Fetal Bovine Serum (Sigma-Aldrich, Millipore Sigma, catalog number: F0392)
10. Trypsin (Gibco, catalog number: 25200056)
11. DMEM (Mediatech, Corning, catalog number: 50003PC)
12. RPMI-1640 (Mediatech, Corning, catalog number: 50020PC)
13. FBS (Gibco, catalog number: 26140079)
14. Penicillin-Streptomycin Solution (Mediatech, Corning, catalog number: 30002CI)
15. Dulbecco's Phosphate Buffered Saline (Sigma-Aldrich, Millipore Sigma, catalog number: D8537)
16. Granulocyte-macrophage colony-stimulating factor (GMCSF) (Shenandoah Biotechnology Inc., catalog number: 100-08-100ug)

Equipment

1. Multisizer 4 Particle Analyzer (Beckman Coulter, catalog number: A51387AB)
2. Benchtop Centrifuge (Fisher Scientific, model: Sorvall Legend X1R, catalog number: 75004261)
3. Swinging Bucket Rotor (Fisher Scientific, model: TX-400, catalog number: 75003181)
4. Microcentrifuge (Fisher Scientific, Fisherbrand, model: accuSpin Micro 17, catalog number: 75002461)

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Software

1. Multisizer 4 software (Beckman Coulter, https://www.beckman.com/)
2. Python (Python Software Foundation, https://www.python.org/)
3. Matplotlib (The Matplotlib Development Team, https://matplotlib.org/)
4. Seaborn (Michael Waskom, https://seaborn.pydata.org/)

Procedure

A. Culture and collect adherent or suspension cells
   1. Adherent cell lines:
      a. Culture to desired confluence in standard media conditions.
         143B, H1299, and A549 cells were cultured in DMEM with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin on 10 cm tissue culture dishes to approximately 90% confluence.
      b. Aspirate media.
      c. Wash with 2-5 ml PBS, aspirate.
      d. Add 5.0 ml (0.25%) Trypsin and incubate in a tissue culture incubator at 37°C until detached from plate.
      e. Transfer cells in Trypsin to 15 ml conical tube.
      f. Rest on ice throughout cell volume measurements.
   2. Suspension cell lines:
      a. Culture to desired cell concentration in standard media conditions.
         TF1 and Jurkat cells were cultured in RPMI-1640. TF1 cells were supplemented with 2 ng/ml granulocyte-macrophage colony-stimulating factor (GMCSF).
      b. Transfer cells in media to 15 ml conical tube.
      c. Rest on ice throughout cell volume measurements.

B. Measure cell volume and concentration on Multisizer 4 Particle Analyzer
   1. Calibrate aperture prior to measurements using NIST traceable nominal 20 µm latex particles.
   2. Prepare plastic accuvette with cells.
      a. Mix cells to homogeneity and dilute cell mixture 200 fold in Isoton II in a plastic accuvette to a total volume of 10 ml.
      b. Measure 500 µl of diluted cell solution three times and record average cell count and total cell volume of detected cells.
         Use the total cell volume per milliliter to calculate the volume of trypsinized or suspension cell solution that produces approximately 2-2.5 µl of cells. The PCV tubes can only quantify volumes between 1 and 5 µl, so calculating an intermediate volume between 2 and 2.5 µl helps prevent any issues measuring over packed or under packed cells.

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C. Measure PCV and compare to Multisizer 4 Particle Analyzer volume measurements

1. Add 2-2.5 µl cell volume of suspension or trypsinized adherent cells to PCV tubes.
   a. Remove conical tubes containing cells from ice and gently pipette up and down to create a homogenous solution.
   b. Add calculated volume to produce 2-2.5 µl of cells to PCV tubes.
      If the concentration of cells is too low, you may need to concentrate the cell solution by centrifuging for 5 min at 300 × *g*, aspirating, and resuspending in a smaller volume of cell culture media to be within the volume allowance of the PCV tubes.
2. Centrifuge cell solutions in PCV tubes and record PCV measurements
   Centrifuge cells with the following parameters and record PCV after each centrifugation step.
   a. 2,000 × *g* for 1 min.
      Note: Manufacturer protocol calls for 2,500 × g for 1 min.
      (Optional) Spin cells at the most horizontal angle possible so that the top line of the cells in the PCV tube is easier to read. To achieve this, perform steps C2a-C2c with a TX-400 Rotor in the Sorvall Legend X1R centrifuge. Place PCV tubes into 15 ml conical tubes and transfer to the centrifuge (Figure 1).

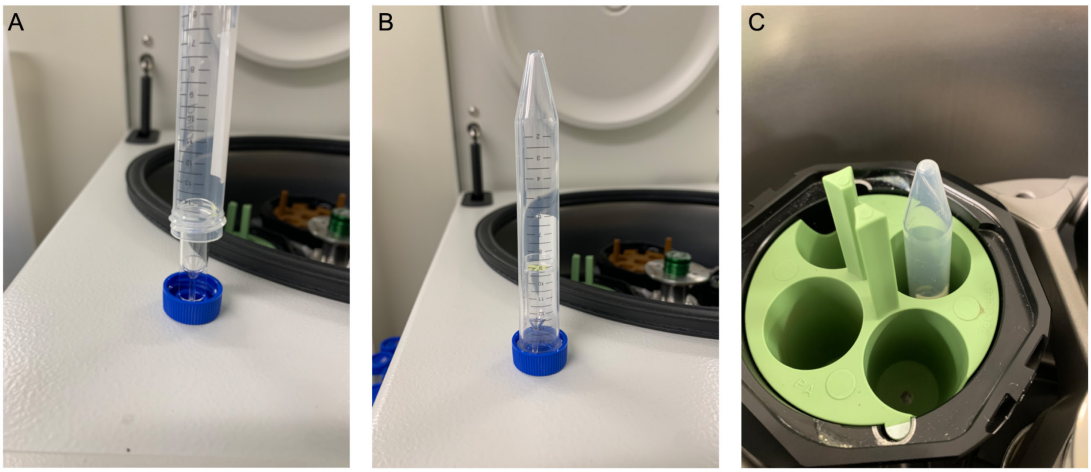

Figure 1. Spinning PCV tubes at a horizontal angle. (A) After loading with cells, place PCV tube into 15 ml conical tube as shown. (B) Screw cap onto 15 ml conical tube. (C) Place into centrifuge. This technique allows cells to be spun at a horizontal angle while also allowing PCV tubes to be easily removed.

   b. 4,696 × *g* for 1 min.
   c. 4,696 × *g* for 5 min.
      Maximum speed of TX-400 Rotor in Sorvall Legend X1R centrifuge.
   d. 17,000 × *g* for 1 min.
      i. Remove PCV tubes from the 15 ml conical tubes and place directly into accuSpin Micro 17 bench top centrifuge.
      ii. Perform steps C2d and C2e in an accuSpin Micro 17 bench top centrifuge to achieve a

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high centrifugal force of 17,000 × *g*.

e. 17,000 × *g* for 5 min.

Data analysis

Each cell line was tested with three replicates in Particle Analyzer measurements and six replicates in PCV tubes. The recorded volume after each step of centrifugation was compared to the theoretical 2-2.5 µl volume and displayed as the percentage of theoretical PCV as determined by Coulter Counter. Average values were then graphed against the centrifugation step (Figure 2).

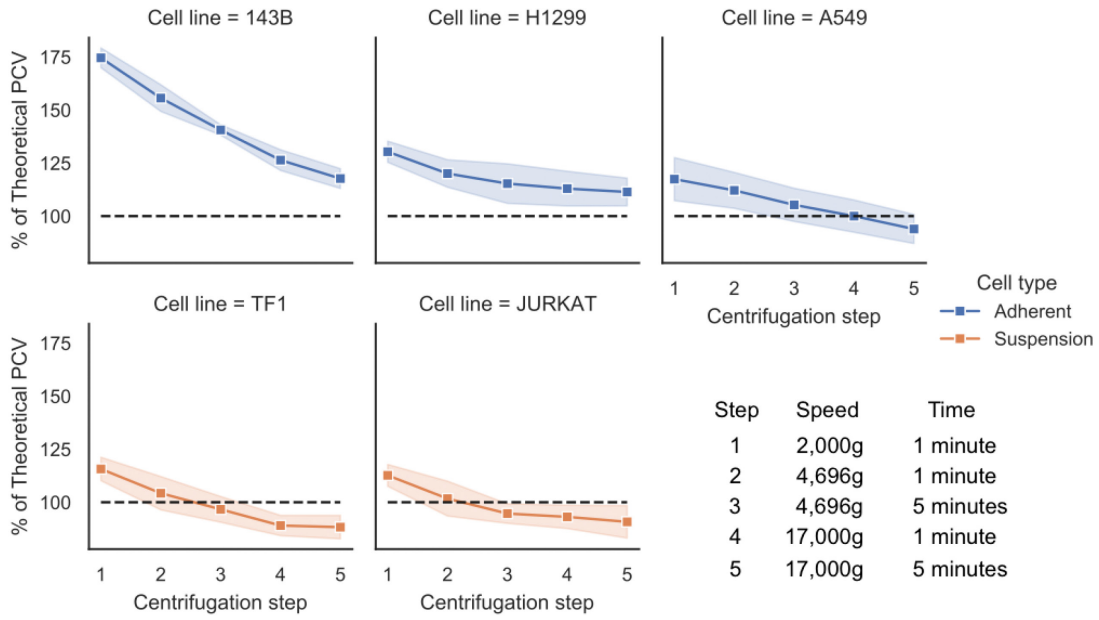

Figure 2. The difference between measured PCV by PCV tubes and the expected cell volume determined by Coulter Counter measurements. Dashed line is where PCV equals the volume of cells as determined by Coulter Counter volume measurements. Error bands are shown as the colored area and represent the standard deviation.

Notes

Our results indicate that PCV consistently overestimates cell volume compared to measurements using a Coulter counter. The overestimate for suspension cells is smaller than that for adherent cells, and the PCV measurements in suspension cells can approach Coulter counter measurements with higher centrifugation force and time. This overestimate in PCV is likely due to incomplete packing, which our data indicate is particularly problematic for adherent cell lines. Therefore, we suggest that future studies relying on accurate cell volume measurements, especially for adherent cell lines, use the Coulter principle to determine cell volume.

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Acknowledgments

I.A.E. acknowledges support from a Levinson Emerging Scholars Fellowship, a Mary Gates Research Scholarship, and the Ed F. And Clara M. Degering Scholarship. L.B.S. acknowledges support from the NIH (R00CA218679) and a CARE Award from the Andy Hill Cancer Research Endowment.

Competing interests

The authors do not have any competing interests to disclose.

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