MARTIN JORDAN

A NOVEL DISPOSABLE MICROTUBE FOR RAPID ASSESSMENT OF BIOMASS IN CELL CULTURES

Swiss Federal Institute of Technology Lausanne, Faculty of Basic Sciences, Institute of Biological and Chemical Process Sciences

1. INTRODUCTION

Accurate and reproducible values of cell number form the basis of all experimental work with mammalian cells. I am presenting a prototype of a microtube system for the assessment of cell mass based on packed cell volume (PCV). Glass PCV tubes are currently being used, but they require large sample volumes (5-10 ml) and are dependent on swinging bucket rotors for centrifugation. The microtubes presented here were manufactured by Techno Plastic Products AG in Trasadingen, Switzerland and have been designed according to the following criteria:

1.) all cells settle into the calibrated capillary during centrifugation (up to 10'000g).
2.) the tubes are compatible with common fixed angle and swinging bucket rotors.
3.) up to 1 ml of sample fits into the tube.

2. RESULTS

The prototype of the PCV microtube is depicted in Fig. 1. Manufacturing of the final version is in progress and will have a distinct marking for direct reading of total cell volume in µl. Prototype tubes were rigorously tested using a centrifuge with a swinging bucket rotor for 1.5/2 ml tubes (Eppendorf 5417C, A-8-11 rotor). After a brief centrifugation, all cells were found within the defined capillary. The height of the cell pellet, visible to the naked eye, defines the volume that is occupied by the cells (Fig. 1). This volume is transformed into PCV (%) using the formula:

PCV (%) = (volume of cell pellet/volume of sample) x 100

After centrifugation the column of packed cells within the capillary still contains a given fraction of medium (filling the space between the cells). This fraction depends on the time and speed of centrifugation. Both parameters were studied separately and eventually defined to get reproducible results. Initially, 300 µl of CHO-DG44 suspension cells (at a cell density of 4.2 x 10^6 cells/ml and a viability of 98%) were pipetted into microtubes and centrifuged at different speeds (850 g = 3000 rpm, 9500 g = 10'000 rpm) (Fig. 2, left panel). The experiment was done in duplicate (series A and B), and each point represents the average of two PCV microtubes.

Even though the cells were pelleted within 30 seconds a 1 minute centrifugation period was chosen as the standard time. For this centrifugation period there was a

strong effect on the PCV up to a speed of 2000 g (Fig. 2, left panel). Beyond 2000 g the volume of the pellet was further decreased, but to a lesser extent. Based on these results a speed of 2380 g (5000 rpm) was chosen as the standard for all further experiments.

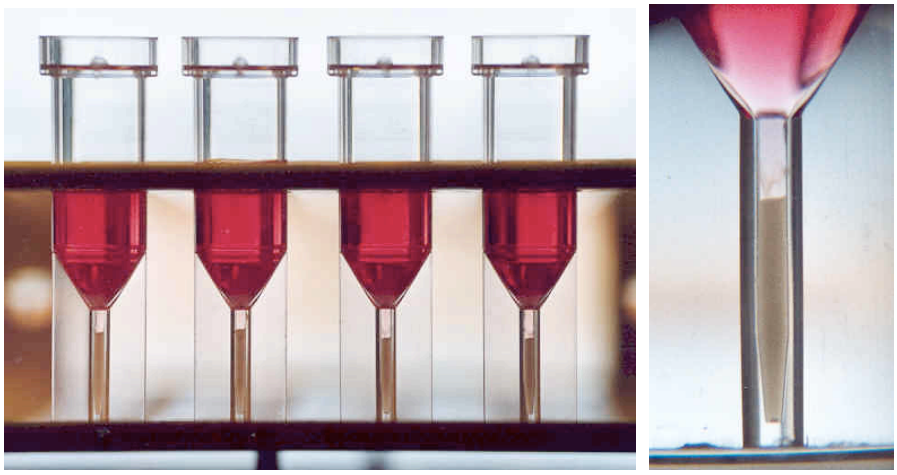

Figure 1. *Prototype PCV microtube. The depicted tube is manufactured from transparent polystyrene. The capillary has a volume of 5 µl. A graduated scale for easily reading the PCV will be printed onto the tubes. Left: 2 samples in duplicate. Right: A sample with 3.6 µl of packed cells corresponding to 1.6 million CHO cells.*

Next, the effect of centrifugation time was studied for speeds of 5000 rpm and 10000 rpm (Fig. 2, right panel). Centrifugation for 30 seconds at 5000 rpm was not sufficient to achieve a compact pellet, but pellet size did not vary for centrifugation times between 1 and 10 minutes. At 10000 rpm the pellet became more compact with longer periods of centrifugation.

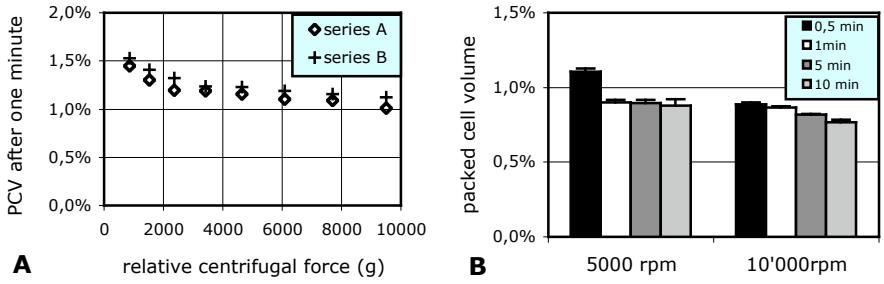

Figure 2. *The packed cell volume of cells as a function of speed and centrifugation time. A) Effect of centrifugation speed. Per tube 300 ul of CHO cells at 4.2 x 10^6 cells per ml were added. B) Effect of centrifugation time. Per tube 400 µl of CHO cells at 3.2 x 10^6 cells per ml were added (n=4).*

The PCV can be calibrated as cell number per ml, making it a powerful method for quantitating cells. In order to demonstrate the reliability of the PCV method two different cell lines (NS0 and CHO) were used in a comparative study. Serial dilutions of the cells were carefully prepared and analysed by the trypan blue method, an automated counting method (Casy, Schärfe Systems), and PCV microtubes. All three methods correlated linearly with the dilutions (Fig. 3). Since the PCV was calibrated against the manual cell counts, the slope of these curves was identical. The Casy counts were close to the manual counts for the NS0 cells, a culture in which no aggregate formation occurred. For the CHO cells grown in serum-free medium we routinely saw small aggregates of about10 cells at densities above 1 million cells per ml. This caused a systematic underestimation of the cell number when cells were counted with the Casy.

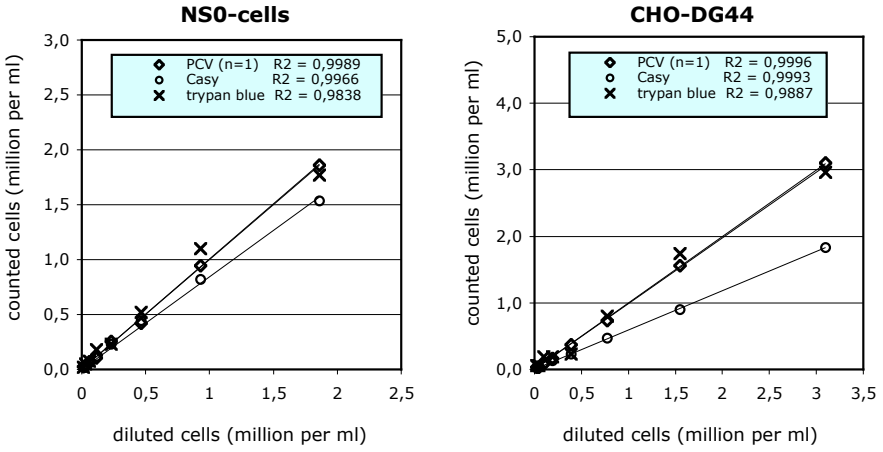

Figure 3. *Quantitation of cell number by PCV in comparison with two established counting methods. NSO cells and CHO cells were serially diluted and counted in parallel by PCV, Casy and trypan blue methods.*

3. CONCLUSION

The disposable PCV microtubes represent a rapid and accurate approach for measuring the packed cell volume using only a small volume of sample. A centrifugation step of 1 minute at more than 2000g is sufficient to pellet the cells. The method covers a wide dynamic range starting at a detection minimum that is below 100'000 cells per ml. Since the capillary holds about 2 million cells and the loading volume is 1 ml or less, there is no upper detection limit. No dilution or further manipulation of the samples is needed in order to rapidly obtain a result that represents an absolute value. PCV can be calibrated against any desired value such as cell number, protein content or metabolic activity. With these features the assay is one of the simplest and most rapid for obtaining precise information about a cell culture.