A Good Way to Count Cells

ECACC carries out hundreds of cell counts every week by direct microscopical examination of cell suspensions in a haemocytometer counting chamber. Viability is assessed by Trypan Blue dye exclusion. This method is laborious and the reproducibility between different operators is typically poor. The error can be greater than 25%, particularly for those cell types that tend to form clumps following trypsin release into suspension. Consequently ECACC scientists have evaluated a number of instruments that are designed to count cells in suspension.

To be suitable for use in ECACC's busy cell culture laboratories an automatic cell counter should:

- **Deliver a fast cycle time with a minimum requirement for specimen preparation.**
- **Be suitable for use with a broad range of different cell types without a need for recalibration between cell types.**
- **Differentiate between viable and dead cells.**
- **Count cells with accuracy and precision.**
- **Be priced at a level that allows the installation of one machine in each of several laboratories.**

Until recently none of the systems ECACC evaluated satisfied many of these requirements. Instruments based on classical image analysis generally had difficulty with the heterogeneity associated with the wide and diverse range of cell types that pass through ECACC laboratories. If such instruments are set up to count a range of cell types of different sizes then the ability to discriminate between dead and viable cells can be compromised. To re-tune the instrument for each cell type is prohibitively time consuming. Finally conventional image analysis instruments are expensive and require frequent maintenance.

THE NUCLEOCOUNTER FROM CHEMOMETEC

The principle of the NucleoCounter technology is to lyse the cells and count the released nuclei after staining with a fluorescent dye. The sample to be counted is first mixed with a lytic reagent and the mixture is injected into a single-use, plastic counting chamber that contains the dye propidium iodide. This means hazards associated with the use and preparation of the dye are avoided.

The chamber is inserted into the NucleoCounter and a CCD camera scans the fluorescing nuclei in the counting chamber. The total cell count is displayed on a screen within 30 seconds. Non-viable cells can be selectively counted by omitting the lytic treatment. The number of viable cells can then be calculated by subtraction.

Similarly, due to the lytic treatment the method is less disturbed by cell clumping. The instrument is simple, compact, requires little maintenance and is reasonably priced.

Initial trials have shown the NucleoCounter to fulfil most of the requirements listed above. It is quick and does not need to be adjusted for different cell types/sizes because it registers only fluorescing nuclei.

The automatic counts broadly agree with the values obtained from haemocytometer counts but are more reproducible (see Chart 1 below). Viable cell counts require 2 machine cycles but the speed of the analysis makes this acceptable. The single use counting chambers introduce a significant consumables cost but this is mitigated by reduced labour costs.

ECACC has now placed 3 machines in its cell banking and QC labs. This will enable us to include objective cell counts in the manufacturing Batch Records and to achieve closer agreement between the Production and Quality Control laboratories.

Another potential application ECACC sees for this technology is for the assessment of blood samples that arrive at ECACC for EBV transformation as part of the many, new human genome projects. ECACC routinely receives 40 or more blood samples each day that need to be processed immediately to separate the lymphocytes ready for EBV transformation. The success of the transformation process is heavily influenced by the quality of the

initial lymphocyte preparation. It is anticipated that the NucleoCounter will enable an objective assessment of every sample as an aid to the early identification of poor quality specimens. This will give ECACC the opportunity to adopt a more suitable protocol and to provide feed-back to the depositor.

In conclusion, the NucleoCounter has the potential to enable rapid, accurate and reproducible cell counts in a busy cell culture laboratory that handles an unusual diversity of cell types.

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Accuracy and Reproduibility of NucleoCounter vs Haemocytometer based on Five Seperate Counts