Application Note

Determination of Cell Aggregation in Bacteria Cultures for Contact Lens Disinfection Efficacy Testing

By Andres Lobeiras
Product Manager
Beckman Coulter, Inc

INTRODUCTION

The percentage of aggregates in bacteria cultures may be determined by employing the Coulter Principle, also known as Electrical Sensing Zone (ESZ) technique. The ISO International Standard 13319 describes this technique. A suitable electrolyte solution is required to perform the analysis, in many cases the Phosphate Buffered Saline (PBS) solution used to suspend the cells may be used as the electrolyte. The sample is prepared and then analyzed using a Beckman Coulter Multisizer 3 to determine the size distribution of the cells. The results are reported as number percent of aggregates.

The use of the Beckman Coulter™ Multisizer™ 3 provides a fast, easy, accurate and automatic method to determine cell aggregation. This instrument also provides reliable results not dependent of the operator's judgment as opposed to results reported when using a hemocytometer.

Contact lens care disinfecting products must be evaluated for their safety and effectiveness. Procedures used to evaluate the efficacy of lens care disinfection solutions routinely employ a specific panel of potentially pathogenic challenge organism suspensions. Microscopic examination of challenge organism suspensions has demonstrated the presence of cell clumps. These aggregates prevent uniform exposure to the test disinfectant and result in underestimation of the true number of challenge organisms. In order to reduce the number of clumps, and therefore produce a more uniform cell suspension, some methods suggests filtration of bacterial challenge organism suspensions prior to use as test inoculate. The method also allows the use of suspension mediums containing 0.05% Tween 80 as a dispersant. The Multisizer 3 Coulter Counter may be used to conduct analysis on cell suspensions pre- and post-filtration to evaluate filtration effectiveness by determining the size distribution and relative number of cell clumps in the bacterial cell suspension. The effectiveness of the Tween 80 may also be evaluated.

MATERIALS AND METHODS

- **Instrument Set up and Calibration**

A 30 μm and a 100 μm aperture tubes were used for the analysis. The linear dynamic range for any aperture is 2% to 60% of its diameter. A 30 μm aperture tube will be capable of analyzing the particle concentration and size distribution from 0.6 μm to 18 μm and a 100 μm from 2 μm to 60 μm. The two apertures combined yields an analysis range of 0.6 μm to 60 μm.

The instrument was calibrated according to the Multisizer 3 Operator's Manual. For determining number percent size distribution the control mode for the instrument was Time Mode selecting 60 seconds as the run time.

- **Procedure**

1. Electrolyte Preparation

Isoton® II is a balanced electrolytic solution manufactured by Beckman Coulter. It is approximately 1% total salt concentration, (sodium and potassium chloride plus a phosphate buffer system). Isoton II was used to suspend the cells for analysis on the Multisizer 3.

If a different saline solution is used, there will be two possibilities:

a) The custom made saline solution contains approximately 1% total salt concentration.

In this case the electrolyte in the external container may be Isoton® II or the custom made saline solution.

b) The custom made saline solution total salt concentration is different from 1%.

In this case the electrolyte in the external container must be the custom made saline solution.

In both cases the cells may be analyzed using the custom made saline solution as electrolyte for the beaker containing the sample.

2. Sample Analysis.

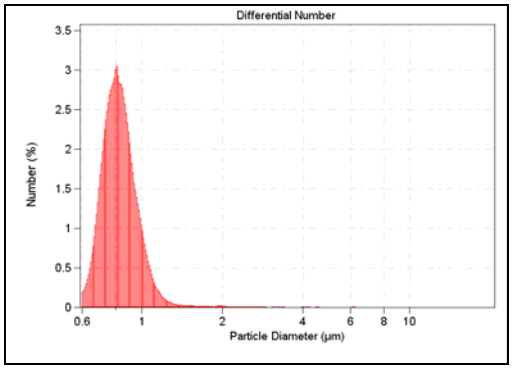
A small round bottom beaker containing the cells was analyzed using a 100 µm aperture. The beaker containing the sample was placed into the analyzer, using the Multisizer stirrer to ensure a uniform distribution in the beaker. The aperture tube was flushed before each analysis. After each run the aperture and electrode were rinsed before proceeding to the next sample.

When all the samples were run, each sample was filtered using a 10 µm filter. The filtrates were analyzed using a 30 µm aperture tube.

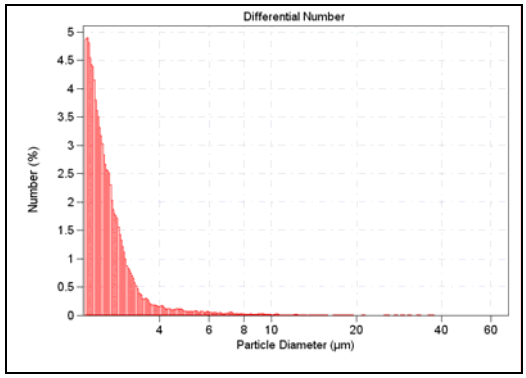
RESULTS

The "Multi-tube Overlap" function in the Multisizer 3 software allows combining results from different aperture tubes.

The results from the analyses using the 30 µm and 100 µm apertures for each sample are presented as a single size distribution covering the combined range for both apertures.



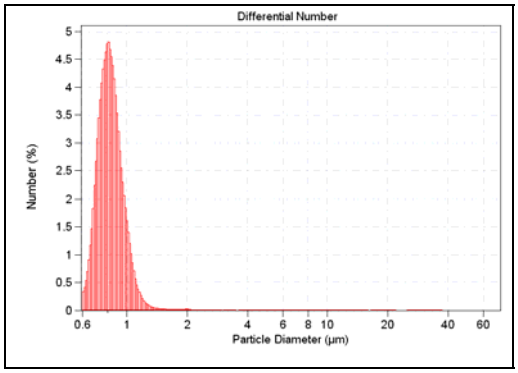
Results using a 30 µm aperture



Results using a 100 µm aperture

Results may be represented as the number percentage of cells above selected size categories in the range of the size distribution by using the Interpolation feature in the Multisizer 3 Software.

Defining which one is the larger size for the distribution of the normal cell population, it is possible to calculate the percentage of agglomerates. We considered that above 3 µm the cells are present in agglomerates. From this assumption the cell aggregation was 1.37% of the total population.



Combined results from the 30 µm and 100 µm apertures

Particle Diameter (µm)	Number % > Particle Diameter
0.6	100.00
1	23.76
2	3.23
3	1.37
5	1.18
10	0.84
15	0.63

BECKMAN COULTER

CONCLUSIONS

By comparing the percentage of aglomarates present in a sample it is posible to control the filtration efficiency and therefore the potential effectiveness of the antimicrobial agent.

It is also possible to refine filtration process and the effectiveness of detergents in preventing cell clumps.