Fast Track Antibody Discovery: a high-throughput method for identifying and isolating rare cells secreting antigen-specific antibodies.

Background

Antibody-derived biologics have become a major class of modern medicine, particularly in the fight against cancer and autoimmune diseases. Highly efficacious immunoglobulin-based drugs have been developed naturally via the antibody-producing B cells of the mammalian immune system, although finding rare cells with the right characteristics has always been challenging.

Traditionally, mouse B cells are immortalized through hybridoma fusion. However, this harbours many challenges as B cells rapidly die off in culture and many do not survive the fusion process. Consequently, the rare B cell of interest may be lost during hybridoma generation process. The production of hybridoma fusions are time consuming, expensive and inefficient as they may not result in finding and immortalising the rare antigen-specific antibody secreting B cells of interest.

Currently, semi-automated technologies such as cell sorting, colony picking, and cell-in-well imagers are used in tandem to screen and isolate rare antigen-specific producing cells. The Cyto-Mine® Single Cell Analysis and Monoclonality Assurance System is the first fully integrated platform designed specifically for biopharma that screens millions of primary B cells or hybridomas for secreted immunoglobulin, identifies and sorts the antigen-specific candidates, and then gently dispenses them into 96- or 384-well microtitre plates with visual proof of cell number, through high-quality imaging (**Figure 1**).

This study shows the method by which Cyto-Mine®, utilising an antigen-specific assay, analyses a heterogeneous B cell or hybridoma population to identify target-specific variants in a high-throughput manner.

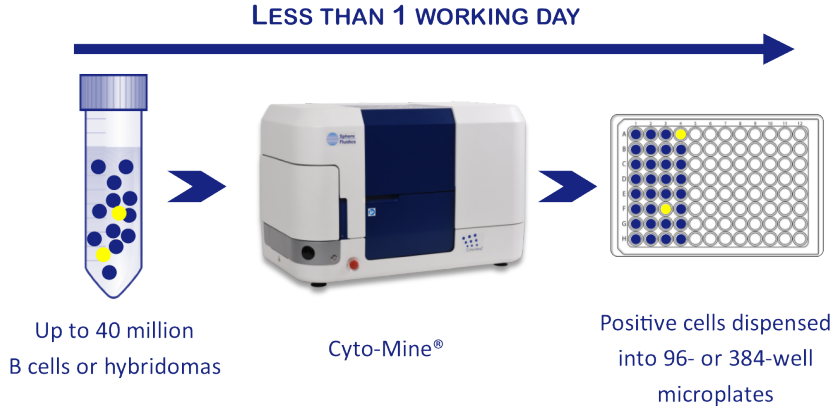


Figure 1 Cyto-Mine® technology finds and isolates cells secreting antigen-specific antibodies from complex cell populations.

Aims & Objectives

This application note will demonstrate how Cyto-Mine® can:

- Accurately identify and isolate 'hit' cells, using an antigen specific assay, from a large hybridoma population.
- Quantify varying concentrations of antigen-specific antibodies.
- Significantly shorten the Antibody Discovery workflow by using a single fully integrated instrument.

Methods

Single Cell Encapsulation

Using Poisson distribution statistics, cells from a hybridoma population were diluted to a concentration which optimizes the number of picodroplets containing only a single cell. For very large populations a dilution can be made to accommodate the encapsulation of pools of cells in one picodroplet (**Figure 2**).

Prior to cell suspension the culture medium was supplemented with OptiPrep™ Density Gradient Medium (Sigma Aldrich Merck) and a pair of detection probes, a fluorescently-conjugated antigen donor probe and an immunoglobulin-specific acceptor probe (see **Box 1**). The diluted mix was then pipetted into a Cyto-Cartridge® and loaded into Cyto-Mine®. Cyto-Mine® initiates the process by encapsulating the cells into 300 pL picodroplets. The number of cells per picodroplet can be varied according to user requirements, *e.g.* 1 cell per picodroplet if single-cell cloning is essential, or more than 1 cell per picodroplet if the population size exceeds 1 million cells, with the latter leading to collection of enriched mini-pools of cells rather than single cells (**Figure 2**).

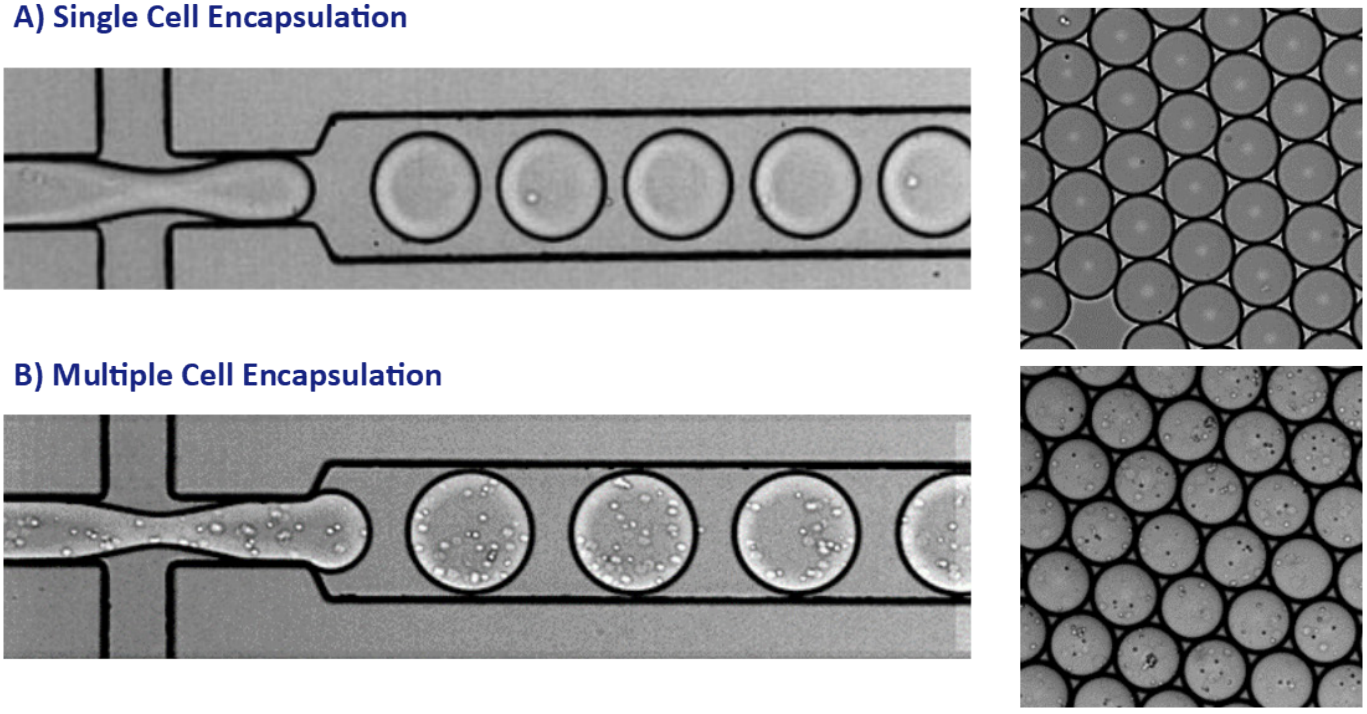


Figure 2 These images show the encapsulation of single cells or multiple cells per picodroplet. A) Cells were diluted to a concentration of $1x10^6$ cells/mL to obtain 1 cell per picodroplet. B) A large population of cells (greater than 1 million) were diluted to a concentration of $1x10^8$ cells/mL in medium resulting in multiple cells per picodroplet.

Antigen-Specific Secretion Assay

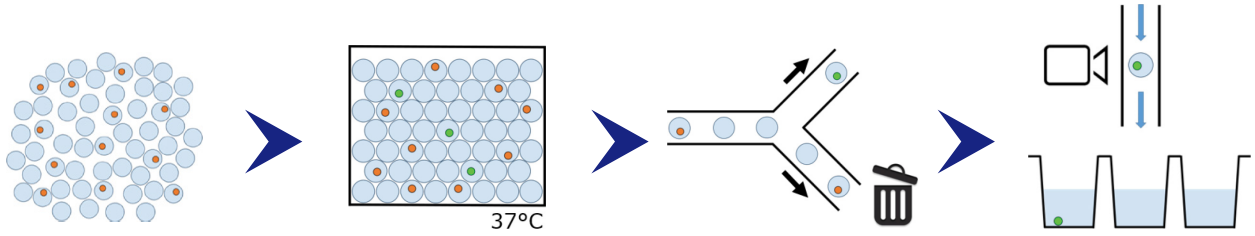
Next Cyto-Mine® incubated the cells *in situ* so that the secreted immunoglobulins accumulated inside the picodroplets and could be detected by the antigen-specific detection reagent present in the medium (**Box 1**). The miniaturized format enables an ultrasensitive, rapid assay (using typically an incubation time 0.5 to 2 hours) depending on the production rate of the cell population.

Sorting

Fluorescent antigen-positive hits are sorted by fluorescence intensity. The integrated stages of the Cyto-Mine® antigen-specific assay process are summarized in **Figure 3**.

Clonality Assurance

Prior to dispensing, as the picodroplet travels through the final dispensing microfluidic channel, the encapsulated cells are imaged multiple times to provide verification of clonality.



Cells encapsulated into picodroplets | Incubation and antigen-specific assay | Sorting Positive cells | Single cell verification during dispensing

Figure 3 The Cyto-Mine® workflow integrates the screening, sorting, isolation and verification of antigen-specific clones into a fully automated process.

Box 1. Antigen-Specific Secretion Assay

A key enabling component of Cyto-Mine® is its ability to analyse the secreted immunoglobulins of millions of cells for antigen specificity while maintaining the cells in a highly viable state with no prior modification. The method by which positives are reported is presented in **Figure 4**. The detection probe pair (fluorescently-conjugated antigen and an immunoglobulin-specific acceptor probe) bind to the secreted antigen-specific immunoglobulin inducing a FRET-mediated shift in fluorescence. Cyto-Mine® then measures the fluorescent signal generated and converts it to a quantitative output. The choice of acceptor probe permits simultaneous screening for antigen specificity and immunoglobulin isotype.

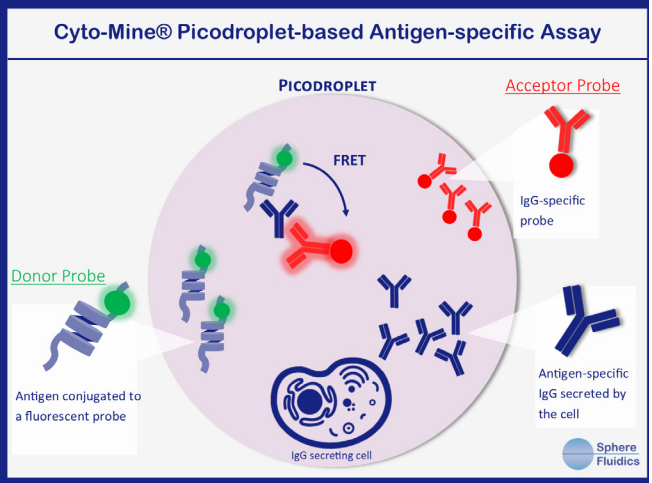


Figure 4 The Cyto-Mine® picodroplet-based antigen-specific assay. This model shows the standard assay to screen for antigen-specific IgG. Antigen-specific IgG secreted from the encapsulated cell during incubation is recognised by both the antigen and the IgG-specific acceptor probe forming a 3-body FRET complex that induces a fluorescent signal.

Results

Antigen-specific Assay Titration Curve

To confirm that the Cyto-Mine® antigen-specific assay is quantitative, titration experiments were carried out using human TNF-alpha as a model antigen. 5 different concentrations of anti-human TNF-alpha and IgG-specific detection probes were encapsulated in 5 separate cell culture medium samples. The highest concentration of TNF-alpha used was 20 µg/mL (equivalent to 133 nM). The samples were then encapsulated and the 5 resulting picodroplet samples were then pooled and analysed by Cyto-Mine® to measure antigen specificity. The results, presented in **Figure 5**, show how the different titers were resolved as discrete populations. As a control, the samples were also analysed using a fluorescent plate reader. The titration curve shown in **Figure 6** demonstrates that the assay is specific and quantitative.

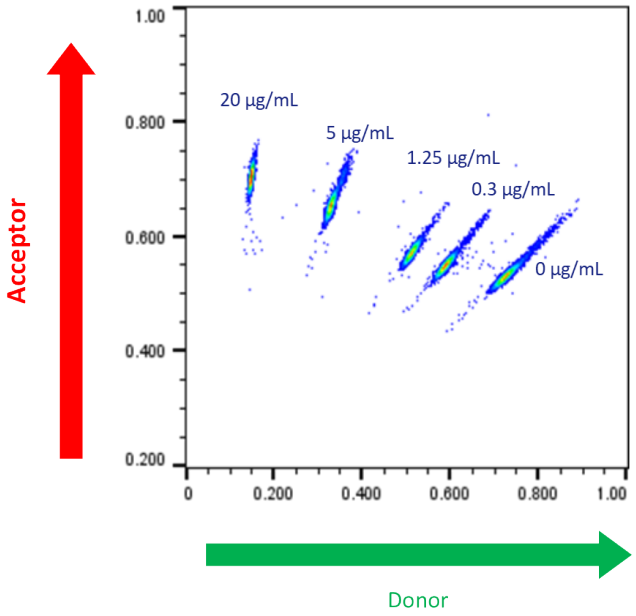


Figure 5 Cyto-Mine® Scatter Plot. Large numbers of individual picodroplets were loaded with the indicated concentrations of anti-human TNF-alpha IgG and then resolved using the Cyto-Mine® antigen-specific assay and analysis.

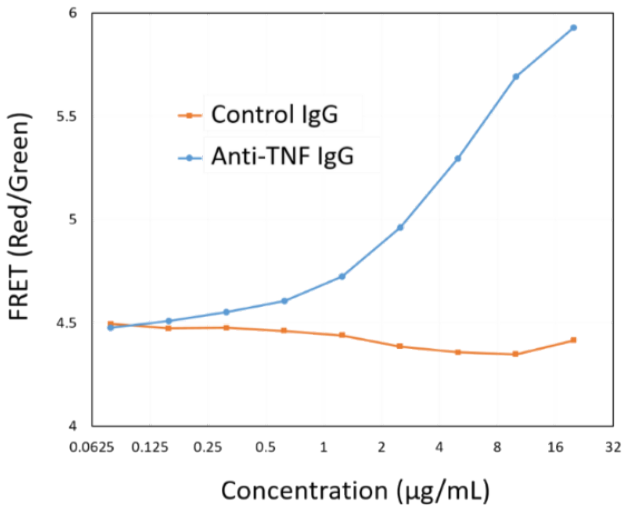
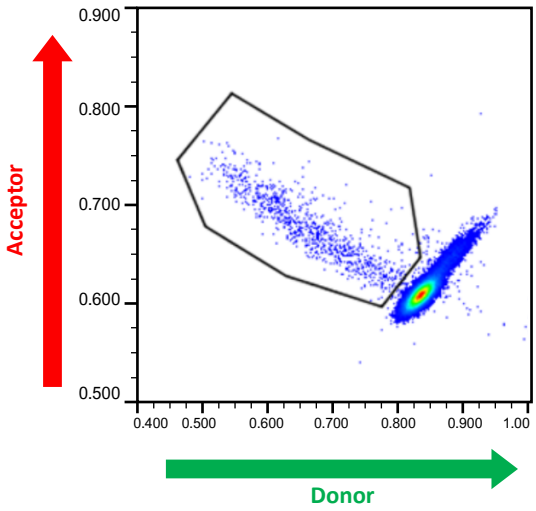


Figure 6 Representative titration curve generated using the Cyto-Mine® antigen-specific assay. In this example, the control IgG confirms specificity of the assay for human TNF-alpha.

Screening Hybridomas for Antigen-specific Clones

A hybridoma fusion sample generated from a mouse immunized with human TNF-alpha was run on Cyto-Mine® to find TNF-alpha specific clones. The cell population was mixed with fluorescently-conjugated human TNF-alpha and acceptor probe against mouse IgG-Fc and analysed using a Cyto-Mine® antigen-specific assay. The results, presented in **Figure 7**, show a population of cells with a high acceptor to donor fluorescence ratio, which have been gated for collection. The bright oval-shaped cluster in the lower-right corner represents the bulk of negative data points, which are comprised of empty picodroplets and picodroplets containing either non-antigen-specific cells or non-secreting cells. In this experiment, the gating polygon has been set to sort all antigen-positive clones but is fully customisable to suit experimental needs.

Figure 7 Cyto-Mine® Scatter Plot of FRET signal generated from hybridomas encapsulated in picodroplets and screened for secretion of human TNF-alpha-specific IgG.



Conclusions

The data presented here shows the value of Cyto-Mine® to find, sort and select rare antigen-specific cells from large populations of B cells and hybridomas. With its picodroplet-based antigen-specific assay, Cyto-Mine® offers the following major benefits:

Quality:
GLP-compliant platform offering deeper immune sampling to find and isolate rare antigen-specific cells based on actual secretion.

Speed and Efficiency:
Shortens the discovery process timeline and enables multiple projects to be run in parallel with no downtime between runs.

Sterility:
Benchtop system compatible for use in Class II biosafety cabinets, with end-to-end sterility and disposable animal origin free (AOF) consumables.

Simplicity:
A seamless, one-step, automated process eliminating the need for multiple instruments.

Cell Compatibility:
Picodroplet encapsulation ensures high viability of selected clones (90–100%) and compatibility with a broad range of human and animal cell types.

Low Operating Costs:
Miniaturisation and automation reduce screening costs by over 10-fold compared to conventional techniques.

Key Point

Cyto-Mine® can rapidly find and isolate rare cells secreting antigen-specific antibodies from millions of primary B cells or hybridoma populations in less than 1 day. Sphere Fluidics' Cyto-Mine® is a powerful fully integrated system that can fast-track antibody discovery by delivering selective cell screening, cell isolation and clonality assurance.

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