Cell therapy

Automated detection of residual CTS Dynabeads magnetic beads on the Attune CytPix Flow Cytometer enables rapid and reproducible results

Abstract

In bead-based *ex vivo* isolation and activation processes, accurate quantification of residual magnetic beads is a key quality attribute in cell therapy workflow development and manufacturing. This application note presents a semiautomated approach using the Invitrogen™ Attune™ CytPix™ Flow Cytometer, combining flow cytometry and brightfield imaging for residual bead detection and quantification. Performance was evaluated for accuracy, linearity, and reproducibility across a dynamic range with the Gibco™ DynaDetect™ Model embedded in Invitrogen™ Attune™ Cytometric Software. Results demonstrate robust detection at both high and low bead concentrations, with consistent quantification in the presence or absence of cells. This workflow was developed following established analytical principles to provide customers with reference-quality data for designing their own validated assays in regulated environments.

Introduction

Gibco™ CTS™ Dynabeads™ magnetic beads have been successfully used across multiple studies and clinical applications. In T cell isolation, activation, and expansion workflows, these beads play a critical role. However, post-manufacturing, bead removal and residual bead quantification are necessary to provide a final drug product that satisfies safety and regulatory requirements for process-related impurities [1]. To address the need for rapid, objective bead detection, a machine learning (ML)-based algorithm—the DynaDetect Model—was developed and implemented within the Attune CytPix Flow Cytometer. The Attune CytPix Flow Cytometer combines conventional flow cytometry with high-resolution brightfield images for advanced image-based analysis. The image processing software for CTS Dynabeads magnetic bead detection helps enable consistent, high-throughput data acquisition (Figure 1). This study presents a data-driven approach for residual bead quantification developed to align with industry best practices for high accuracy, precision, and repeatability across results.

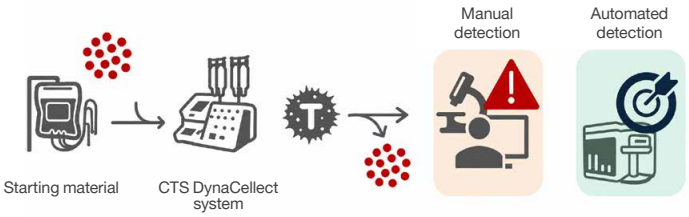

Figure 1. Automated residual CTS Dynabeads magnetic bead detection supports operator-independent, consistent data analysis.

Materials and methods

To evaluate the performance of automated, DynaDetect platform–based residual bead detection and quantification, samples were prepared under three conditions: CTS Dynabeads magnetic beads alone, CTS Dynabeads magnetic beads mixed with T cells, and a blank control to establish a baseline signal. Data quality and acquisition stability were confirmed using six quality control (QC) gates that removed debris, optimized signal-to-noise ratio, and verified uniform event distribution, signal stability, and accurate image segmentation without artifacts, thereby supporting streamlined data acquisition. Samples were vortexed between each acquisition to enable homogeneous bead suspension and Invitrogen™ Attune™ Performance Tracking Beads were run before each sample to ensure proper instrument performance.

Results

Performance remained robust across both bead-only and cell-containing samples at high bead concentrations, demonstrating that the image-based detection algorithm reliably differentiated beads from cells while maintaining accuracy and precision (Figures 2 and 3). Sensitivity was preserved at low concentrations, where detected bead counts closely matched expected values, demonstrating the system's ability to accurately capture near–limit-of-detection events with consistent data output (Figure 4). To confirm the consistency of the automated residual bead quantification assay with manual analysis, bead quantification was also performed using a hemocytometer-based counting method on de-beaded samples (Figure 5). The manual bead counting assay follows the method of harvesting a representative sample

from de-beaded cells and using a hemocytometer, or KOVA™ slide, to count cells under an inverted light microscope. The manual bead quantification protocol can be referenced **here** for additional details. Comparison of automated and manual counting across de-beaded samples showed moderately strong correlation and reproducibility between the two approaches.

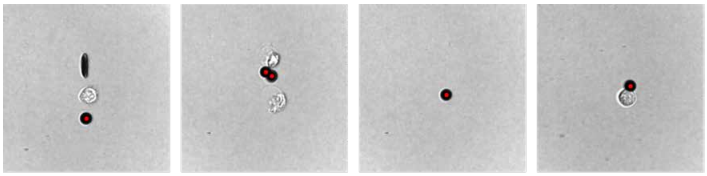

Figure 2. The residual bead quantification assay, automated by the DynaDetect platform, identifies single and clustered CTS Dynabeads magnetic beads detected in bead-only and cell-containing samples. From left to right, images show accurate detection of beads near cellular debris, in proximity to multiple cells, as an isolated event, and adjacent to a single cell.

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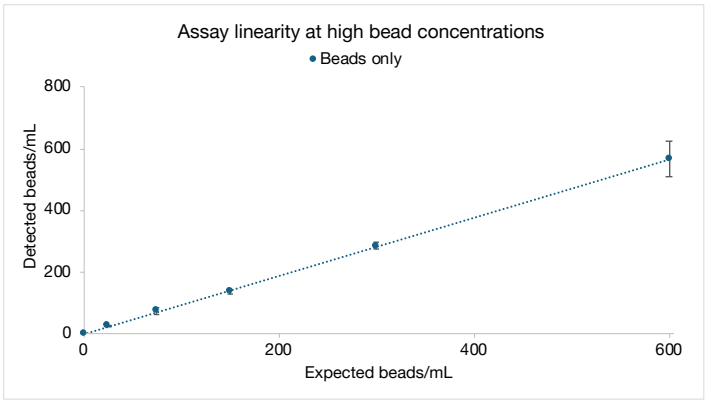

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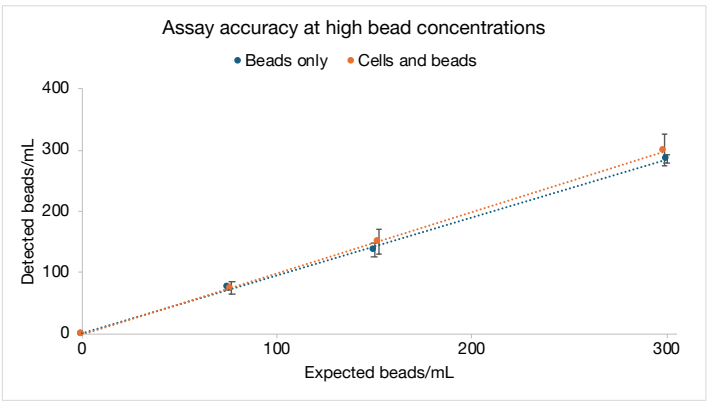

Figure 3. Residual CTS Dynabeads magnetic beads quantification assay demonstrates strong linearity, accuracy, and precision at high CTS Dynabeads magnetic bead concentrations. (A) Run-to-run linearity of bead detection at high concentrations (0–600 beads/mL) in bead-only samples. **(B)** Run-to-run assay accuracy and precision of both bead-only samples (blue) and cell and bead suspensions (orange). Samples exceeding 7.5 x 10^5 cells/mL were diluted with buffer to maintain optimal flow, and the acquired sample size was 250 µL. Error bars represent standard deviation (n = 5–6 replicates).

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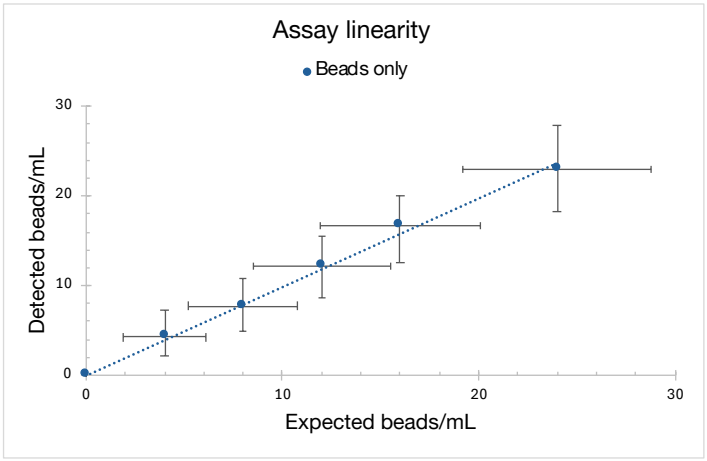

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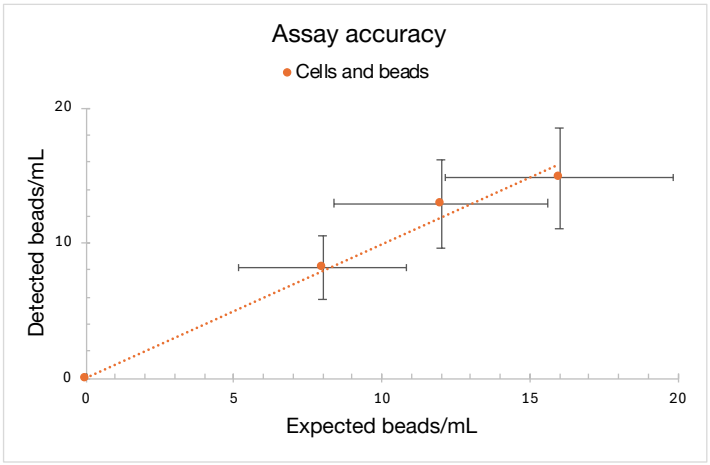

Figure 4. Residual CTS Dynabeads magnetic beads quantification assay demonstrates strong linearity and accuracy at low CTS Dynabeads magnetic bead concentrations. (A) Run-to-run linearity of bead detection in bead-only samples. **(B)** Run-to-run assay accuracy in cell and bead suspensions. Cell concentration is 7.5 x 10^5 cells/mL, with a sample size of 800 µL. The 0 data point indicates a sample without beads. Error bars on the y-axis represent standard deviation (n = 15–18 replicates). Error bars on the x-axis represent the theoretical variation predicted by the Poisson distribution, reflecting the low number of beads present in the samples during sample preparation.

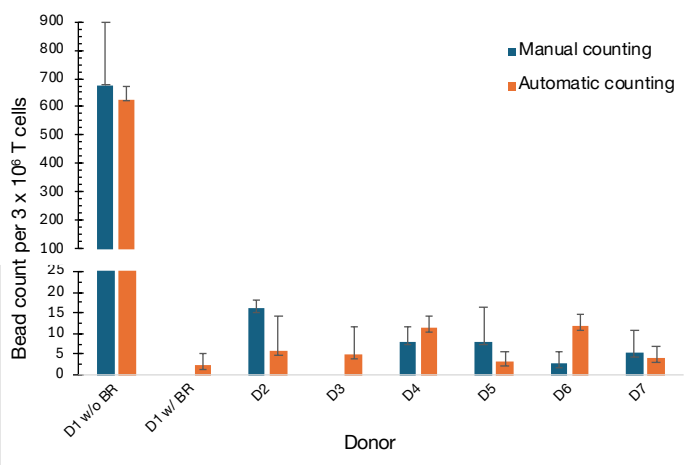

Figure 5. Automated and manual bead counts are comparable across independent donor samples. The manual and automated residual CTS Dynabeads magnetic bead counting methods were compared across seven independent donor cells that underwent T cell isolation by using the Gibco™ CTS™ DynaCellect™ Magnetic Separation System and Gibco™ CTS™ Detachable Dynabeads™ CD4 and CD8. Samples from donor 1 (D1) were collected before and after the bead removal (BR) process to demonstrate the ability of the assay to detect samples ranging from low to high bead counts with high consistency. All remaining samples (D2–D7) were collected after the de-beading process with the DynaCellect system. Error bars represent standard deviation (n = 3–6 technical replicates).

Conclusion

In combination with the Attune CytPix Flow Cytometer, the automated residual bead quantification and the DynaDetect Model are specialized for the detection and quantification of CTS Dynabeads magnetic beads and CTS Detachable Dynabeads products.

- ML-based software detects a broad dynamic range with reliable quantifications at the evaluated high and low bead concentrations.
- The specificity of the residual bead assay helps enable accurate data to confirm bead removal with confidence.
- A widely trusted residual bead assay can support streamlined analysis workflows.

Automated residual bead imaging and analysis can enhance the control of process impurities in a confident and consistent manner, which is essential within cell therapy manufacturing. Combined with short sample preparation time, this approach supports rapid, high-throughput data analysis, enabling streamlined release timelines and shorter workflows. This study is intended to demonstrate a potential approach that customers may adapt to their own processes. The Attune CytPix instrument used in this study is for Research Use Only (RUO) and has not been validated for use in cGMP. This instrument can support regulatory compliance through an optional 21 CFR Part 11 software module. End users should perform qualification and validation as needed to confirm compatibility with their specific manufacturing workflows or quality control processes. The user manual will provide recommendations on how to perform the qualification of the assay, as it was developed by following the ICH Q2(R2) guidelines for quantification of impurities (Type 3).

For additional information and experimental details, a data summary presentation is available. To learn more, please see the user guide or reach out to your Thermo Fisher Scientific sales representative.

Reference

1. European Medicines Agency (EMA) (2024) Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (Revision 1). European Medicines Agency, Amsterdam.

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