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Evaluation of a multiple myeloma synthetic cell mimic as an assay control for flow cytometry

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Abstract

The reliability of clinical flow cytometry assays hinges on the availability of robust assay controls that accurately reflect representative samples. Widely used conventional assay controls, such as cell lines, often fail to replicate the phenotypical and biophysical properties of representative primary samples resulting in gating variability and limited confidence in assay performance as certain markers may not be expressed. To address these limitations, we developed a custom synthetic multiple myeloma (MM) cell mimic using Slingshot Biosciences TruCytes[®] technology. Currently, the MM1.S cell line can be used as an assay control in a flow cytometry panel to evaluate key markers, B cell maturation antigen (BCMA) and G protein-coupled receptor, class C, group 5, member D (GPRC5D), on plasma cells (PCs) and B cells in bone marrow aspirate, for MM clinical trials. Although MM.1S shows strong expression of the key markers BCMA and GPRC5D, it lacks CD45 and CD19 which are needed for PC and B cell identification. We aimed to engineer a MM cell mimic to simulate the scatter properties and antigen density for our fresh MM bone marrow aspirate sample testing. Representative MM bone marrow mononuclear cells (BMMCs) were reviewed to find scatter properties and representative median fluorescence intensity (MFI) of CD45, CD19, CD38, CD138, BCMA, GPRC5D, CD56, and PD-L1 on PCs and B cells. We engineered the MM cell mimic with one population representing PCs and another population representing B cells, based on MM BMMC samples. The MM cell mimic overcame the lack of CD45 and CD19 expression and the divergent scatter and MFI profiles observed in traditional MM cell lines like MM1.S. Qualification studies demonstrated intra- and inter-assay precision (%CV ≤20%), robust inter-instrument reproducibility, post-reconstitution stability up to 7 days at 4°C, and closed-vial storage stability of up to 13 months at −20°C. In summary, these results establish the

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MM mimics as an improved and alternate assay control to MM1.S, facilitating longitudinal assay performance monitoring and ongoing quality assurance in clinical flow cytometry.

KEYWORDS

assay control, cell mimic, flow cytometry, multiple myeloma, plasma cell

1 | INTRODUCTION

Reliable flow cytometry assays depend on high quality assay controls to detect deviations in technical protocols and ensure consistent assay performance. Mismatches between controls and clinical samples can compromise assay interpretation, highlighting the importance of control design (Maecker & Trotter, 2006). Traditional assay control materials, such as cell lines and healthy primary donor cells, often fail to reproduce the phenotypic properties of diseased patient samples. Cell lines may lack expression of clinically relevant markers or exhibit abnormal scatter characteristics, resulting in an independent gating strategy from test sample gating. These limitations underscore the need for standardized assay controls that more closely reflect clinical sample behavior and support confident assay interpretation. In multiple myeloma (MM), B cell maturation antigen (BCMA) and G protein-coupled receptor, class C, group 5, member D (GPC5D) on plasma cells (PCs) and B cells have emerged as targets for CAR T-cell therapies, with dual-target strategies actively under clinical investigation (Mailankody et al., 2022; Rodriguez-Otero et al., 2024). Additionally, CD56 and programmed death-ligand 1 (PD-L1) are exploratory biomarkers, as expression of these markers may discern malignant MM PCs from normal PCs (Cottini & Benson, 2023; Tamura et al., 2020). Currently, the MM1.S cell line is commonly used as an assay control for fresh sample testing in MM clinical trials, as it expresses the key CAR T therapy targets BCMA and GPRC5D.

Although the MM1.S cell line strongly expresses BCMA and GPRC5D, it lacks key markers required for PC and B cells identification, such as CD45 and CD19 (Greenstein et al., 2003; Zhou et al., 2024). In addition, the MM1.S cell line exhibits scatter and phenotype profiles that differ from patient samples, often necessitating independent gating strategies. Recent work has demonstrated the feasibility, accuracy, and precision of synthetic, biologically representative materials (Davis et al., 2024; Kotanchiyev et al., 2025; Schneider et al., 2024). To address these limitations, a synthetic MM cell mimic was developed and characterized in collaboration with Slingshot Biosciences, Inc., with the goal of providing a representative, standardized assay control for clinical flow cytometry applications.

2 | MATERIALS AND METHODS

2.1 | Multiple myeloma cell mimic

TruCytes® mimics are fully customizable, lyophilized assay controls composed of hydrogel particles and manufactured by Slingshot

Biosciences, Inc. (Emeryville, CA, USA). A TruCytes® MM cell mimic was developed in collaboration with Slingshot Biosciences to approximate the forward scatter area (FSC-A), side scatter area (SSC-A) of lymphocytes and phenotype of three representative MM bone marrow mononuclear cell (BMMC) samples. As B cells and PCs exhibit varying levels of CD45 density, two populations of cell mimics were created to reflect these distinct immunophenotypes. To reproduce the B cell phenotype, CD45bright and CD19 were added to one population of cell mimic. For the PC phenotype, CD45dim, CD138, CD38, CD56, PD-L1, GPRC5D, and BCMA were added to the second population of cell mimic. The extracellular domain for each marker was incorporated into the mimic, with the exception of GPRC5D, for which the full-length protein was used. The two populations of mimics were mixed and formulated at 75% PC mimics and 25% B cell mimics.

2.2 | Biological samples

Cryopreserved MM BMMCs from three donors were obtained through Discovery Life Sciences (Huntsville, AL, USA). The BMMCs were used as a representative of clinical samples and to check assay performance. MM.1S cell line was acquired from American Type Culture Collection (ATCC) (Manassas, VA, USA).

2.3 | BCMA and GPRC5D flow cytometry panel

An eight-color flow cytometry panel was designed on a 5 laser Cytex Aurora (Cytex Biosciences, Fremont, CA, USA) to monitor

TABLE 1 Qualified B cell maturation antigen (BCMA) and G protein-coupled receptor, class C, group 5, member D (GPC5D) flow cytometry panel.

Marker	Fluor	Vendor	Catalog #
CD138	BV421	BD Biosciences	562935
CD38	FITC	Cytognos	CYT-38F2
CD45	PerCP-Cy5.5	BD Biosciences	340952
BCMA	PE	BioLegend	357504
CD56	PE-Cy7	BD Biosciences	557747
PD-L1	cFluor BYG710	Cytex Biosciences	R7-20613
GPC5D	AF647	BMS	Custom
CD19	APC-H7	BD Biosciences	641395

Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin.

BCMA and GPRC5D expression levels in MM samples (Table 1). CD45brightCD19+ were defined as B cells, while PCs were identified using a Boolean “AND” of CD45dimCD38+ and CD38+CD138+ populations. PD-L1 and CD56 are exploratory markers for PC interrogation. As GPRC5D and BCMA are key markers monitored longitudinally in clinical studies, molecules of equivalent soluble fluorochrome (MESF) values were calculated using Quantum MESF beads for Alexa Fluor 647 (AF647) and PE, respectively (Bangs Laboratories, Inc., Fishers, IN, USA).

2.4 | Qualification study design

The qualification of the MM cell mimic followed a fit-for-purpose analytical validation approach consistent with CLSI H62 (Clinical and Laboratory Standards Institute [CLSI], 2021) and recent method-validation recommendation for flow cytometry assays (Monaghan et al., 2025). Precision was assessed across four approaches: intra-assay precision (replicate measurements within a single run), inter-assay precision (independent runs performed on separate days), inter-instrument precision (sample samples analyzed across multiple Cytex Aurora instruments), and inter-analyst precision (independent preparation and acquisition by different analysts). Stability was assessed using two study designs: a reverse accelerated closed-vial storage using 37°C incubation with Arrhenius-based translation to –20°C storage, performed on a Cytex Aurora at Slingshot Biosciences (Emeryville, CA, USA), and a post-reconstitution study with storage at 4°C, performed on a Cytex Aurora at Bristol Myers Squibb (Seattle, WA, USA). Although the studies were conducted at different sites, instrument performance was standardized using Rainbow calibration beads and the median fluorescence intensity (MFI) values for all evaluated biomarkers were maintained within the designated Rainbow bead peak ranges throughout the study. Longitudinal assay performance was monitored over 20 independent runs using Levey-Jennings analysis. The design of each assessment and predefined acceptance criteria is summarized in Table 2.

3 | RESULTS

3.1 | Phenotype of MM cell mimic

The eight-color flow cytometry panel was applied to the MM mimic to evaluate scatter characteristics and marker expression. As engineered, the median FSC-A and SSC-A of the MM cell mimic (9.50E5 and 5.07E5, respectively) were comparable to those observed in the three representative MM BMMC lymphocyte populations (1.09E6 and 1.88E5, respectively). Consistent with the design intent, the MM cell mimic exhibited two CD45 populations, CD45bright and CD45dim. The CD45bright population was only CD19+ to represent B cells while the CD45dim population was CD19-. The CD45dim population was positive for CD38, CD138, GPRC5D, BCMA, PD-L1, and CD56, thus mimicking PC populations (Figure 1). To further illustrate

TABLE 2 Summary of qualification study design.

Qualification design		
Assessment	Study design	Acceptance criteria
Intra-assay precision	Five replicate measurements within a single run	≤20% CV
Inter-assay precision	Three independent runs on separate days	≤20% CV
Inter-instrument precision	Same samples analyzed on three Cytex Aurora instruments	≤20% CV
Inter-analyst precision	Independent preparation and acquisition by three different analysts	≤20% CV
Closed-vial storage stability	Reverse accelerated aging at 37°C in triplicate with Arrhenius translational to –20°C (0–13 months)	≤20% CV from Day 0
Post-reconstitution stability	Reconstituted mimic stored at 4°C and tested on Days 0, 3, 5, and 7	≤20% change from Day 0
Longitudinal performance	20 independent runs over ~2 months	Within ±3 SD of the mean

Abbreviations: CV, coefficients of variability; SD, standard deviations.

marker specificity, the two mimic populations serve as reciprocal internal negative controls. The PC population was CD19- while the B cell mimic was BCMA-, GPRC5D-, CD38- CD138-, and PD-L1 (Figure 2). MM cell mimics exhibited tight distributions in positive marker expression and were representative of the MM BMMC sample with respect to scatter and marker positivity. The FSC-A of MM1.S was approximately two-fold higher than that of the MM mimic (2.32E6 and 9.50E5). MM1.S lacked expression of CD45 and CD19, whereas both markers were readily detected on the MM cell mimic. Both MM1.S and MM mimic showed strong positive signals of BCMA and GPRC5D. Overall, the MM cell mimic reproduced relevant scatter properties and marker expression patterns observed in MM BMMC samples, overcoming the divergent scatter characteristics and lack of CD45 and CD19 expression in MM1.S cell lines (Figure 3).

3.2 | Precision

MM cell mimics were evaluated for precision, including intra-assay, inter-assay, inter-instrument, and inter-analyst precision (Tables 3–6). GPRC5D AF647 MESF showed coefficients of variability (CV) of 7.46%, 3.82%, 5.26%, and 18.12% in inter-assay, intra-assay, inter-instrument, and inter-analyst precision, respectively. BCMA PE MESF showed CVs of 8.09%, 3.54%, 6.93%, and 14.36% in inter-assay, intra-assay, inter-instrument, and inter-analyst precision, respectively. All reportable parameters met the predefined acceptance criteria of

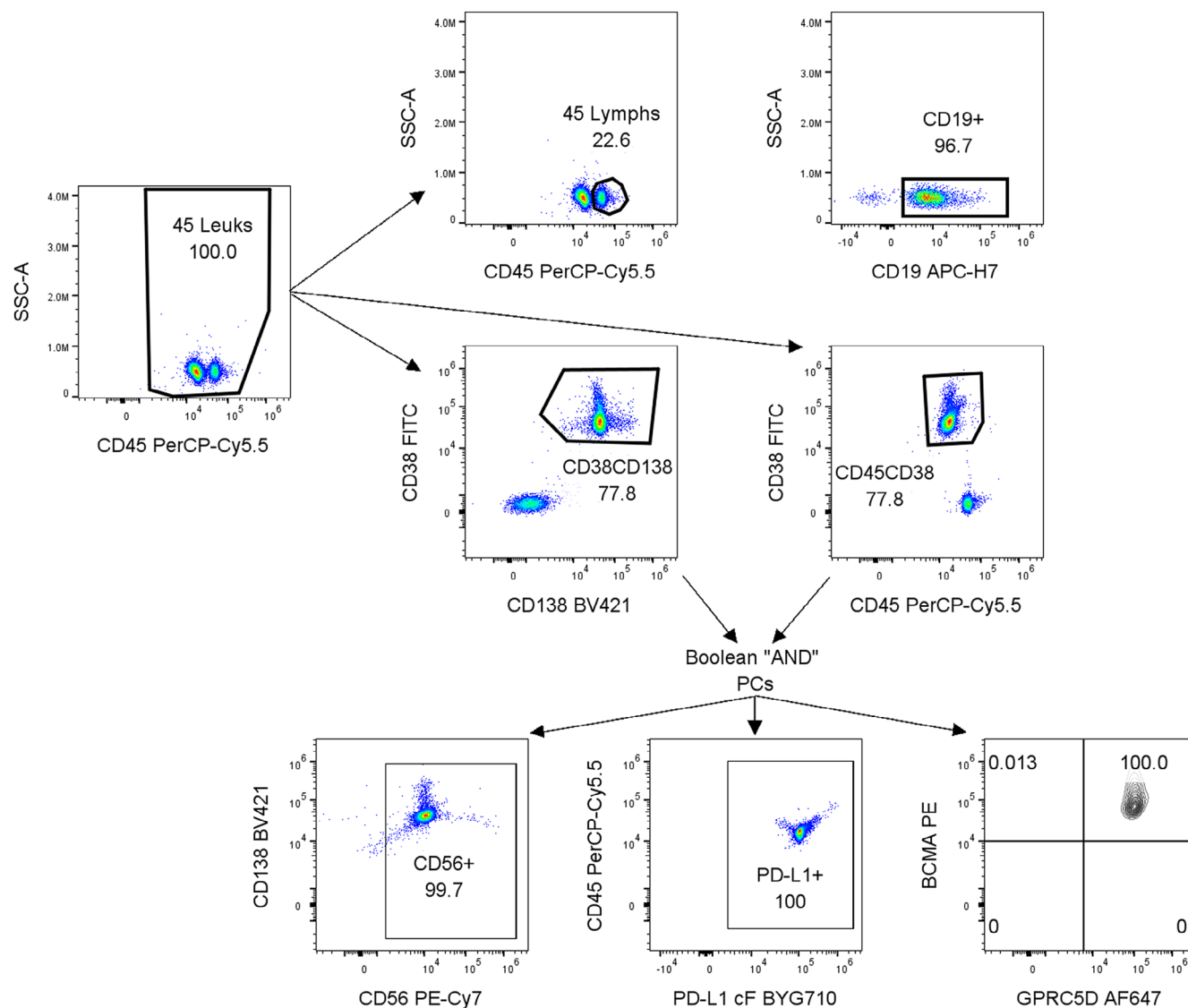


FIGURE 1 Gating strategy of multiple myeloma mimic. Representative pseudocolor dot plots and contour plots are shown. SSC-A, side scatter area. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/cyto.b.20075)]

≤20% CV, demonstrating robust assay precision across analytical parameters.

3.3 | MM cell mimic stability

The MM cell mimic was evaluated under accelerated stability conditions to assess long-term storage stability. Accelerated aging was calculated using the Arrhenius equation, a widely accepted and conservative model for predicting product stability (Lambert & Tang, 2000). A reverse accelerated stability study was performed in which MM cell mimic samples were incubated at 37°C in triplicate for 14, 11, 7, and 4 days until Day 0, at which point all samples were collected simultaneously and analyzed by flow cytometry. According to the Arrhenius equation, incubation at 37°C for 0, 4, 7, 11, and 14 days corresponded to approximately 0, 3, 6, 10, and 13 months of storage at −20°C, respectively. Across all evaluated biomarkers, variability in

MFI remained below 20% CV relative to time 0 (T0) at the 13-month (T13) equivalent time point, with GPRC5D exhibiting the highest CV at 16.92%. Although GPRC5D exceeded 20% CV at the 3- and 10-month equivalent timepoints, all biomarkers met the predefined acceptance criteria at T13 (Table 7). These findings demonstrate that the lyophilized MM cell mimic remains stable for up to 13 months when stored at −20°C (Figure 4).

We also evaluated the post-reconstitution stability of MM cell mimics at 0, 3, 5, and 7 days with storage at 4°C until testing. All markers at Days 3, 5, and 7 showed a less than 20% difference from Day 0, except CD56 MFI at Day 3, which showed a 26.56% increase (Table 8, Figure 5).

3.4 | MM cell mimic assay performance

Longitudinal assay performance was evaluated across 20 independent runs of the MM cell mimic performed over a period of 2 months to

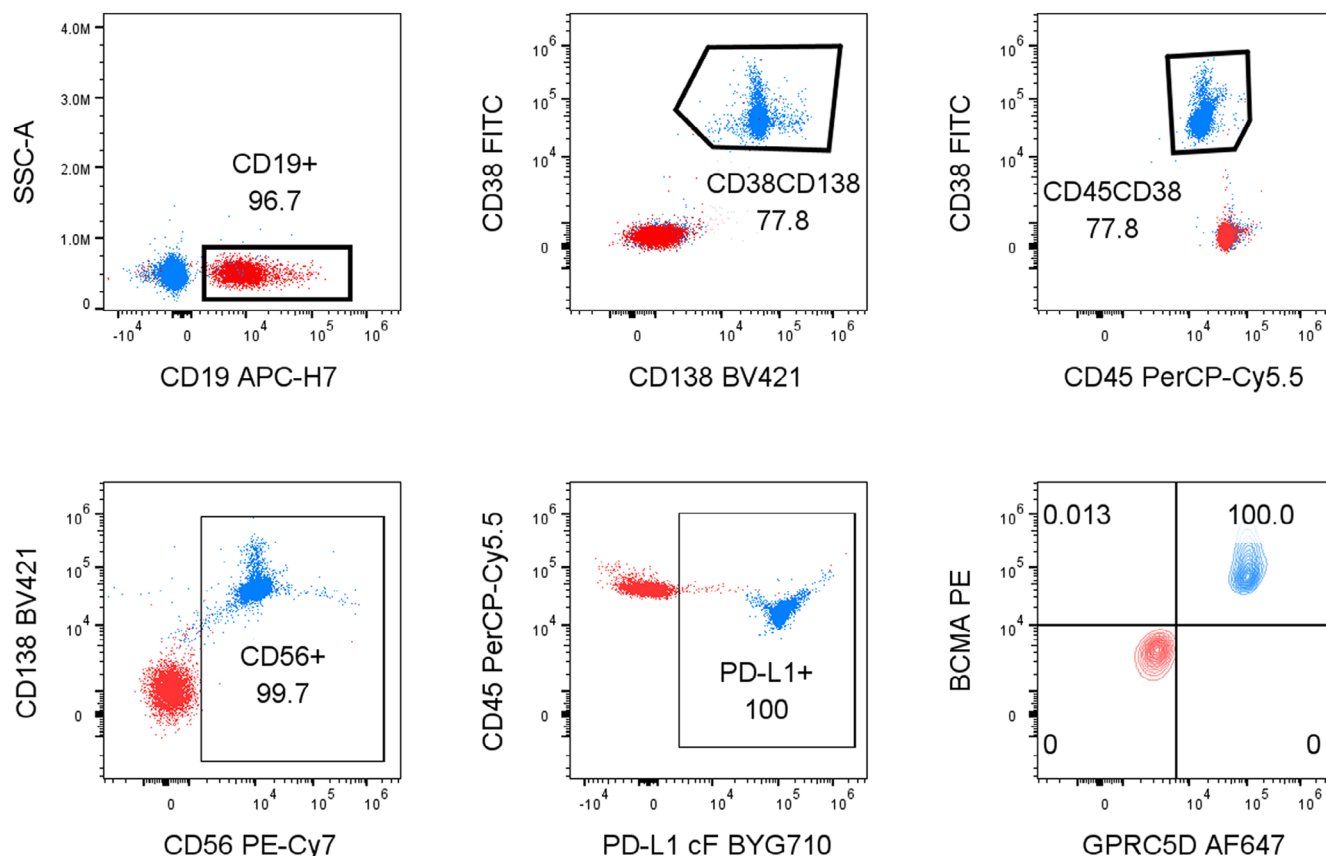


FIGURE 2 Background of mimics. Overlays of plasma cells (PC) mimic (blue) and B cell mimic (red). PC mimic is CD19⁺ while B cell mimic is CD38⁺, CD138⁺, CD56⁺, PD-L1⁺, BCMA⁺, and GPRC5D⁺. BCMA, B cell maturation antigen; SSC-A, side scatter area. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/cyto.b.20075)]

generate a Levey-Jennings plot to identify trends, shifts, or outliers. Across all 20 runs, key parameters of GPRC5D AF647 MESF and BCMA PE MESF values remained ± 3 standard deviations (SD) of the mean (Figures 6 and 7), indicating stable longitudinal performance suitable for routine quality control monitoring.

4 | DISCUSSION

The MM cell mimics addressed key limitations of the MM1.S as an assay control, displaying scatter properties and marker intensity representative of diseased patient samples. Qualification assessments demonstrated robust precision, reproducible performance across instruments and analysts, and stability under both closed-vial stability and post-reconstitution conditions. These stability assessments were performed at separate sites using Cytex Aurora instruments, and the MM cell mimic maintained performance within predefined acceptance criteria across both evaluations. Together, these findings support the suitability of the MM cell mimic for use as a clinical flow cytometry assay control.

A key advantage of the MM cell mimic is its tuneability. While MM1.S expresses BCMA and GPRC5D, it lacks additional markers such as CD45 and CD19 that are required for comprehensive PC and

B cell identification. An important function of an assay control is to confirm the integrity of the antibody cocktail and staining workflow, particularly when clinical samples exhibit phenotypic variability due to disease state, sample processing, or delays to analysis. In such cases, it may be difficult to distinguish between technical issues related to reagent preparation and true biological effects on marker expression. The ability to incorporate markers of interest to a synthetic cell mimic as an assay control enables more confident assessment of assay performance across all reportable parameters. Another advantage of the MM cell mimic is operational ease of use. MM cell mimics are stored at -20°C , eliminating the need for liquid nitrogen storage and reducing associated safety and logistical constraints. The material demonstrated stability for up to 13 months under closed-vial storage at -20°C , supporting long-term storage without compromising assay performance. Upon reconstitution, the material is ready for use within 5 min, whereas cell line-based controls require continuous culture maintenance and batch preparation. Generation of new lots of cell lines as controls can require multiple weeks for thawing, expansion, aliquoting, cryopreservation, and qualification, and storage, whereas synthetic cell mimics can be rapidly ordered, stored, and readily available for use.

Beyond serving as a run control, the tunability of the MM cell mimic also makes it well suited for antibody titration and reagent

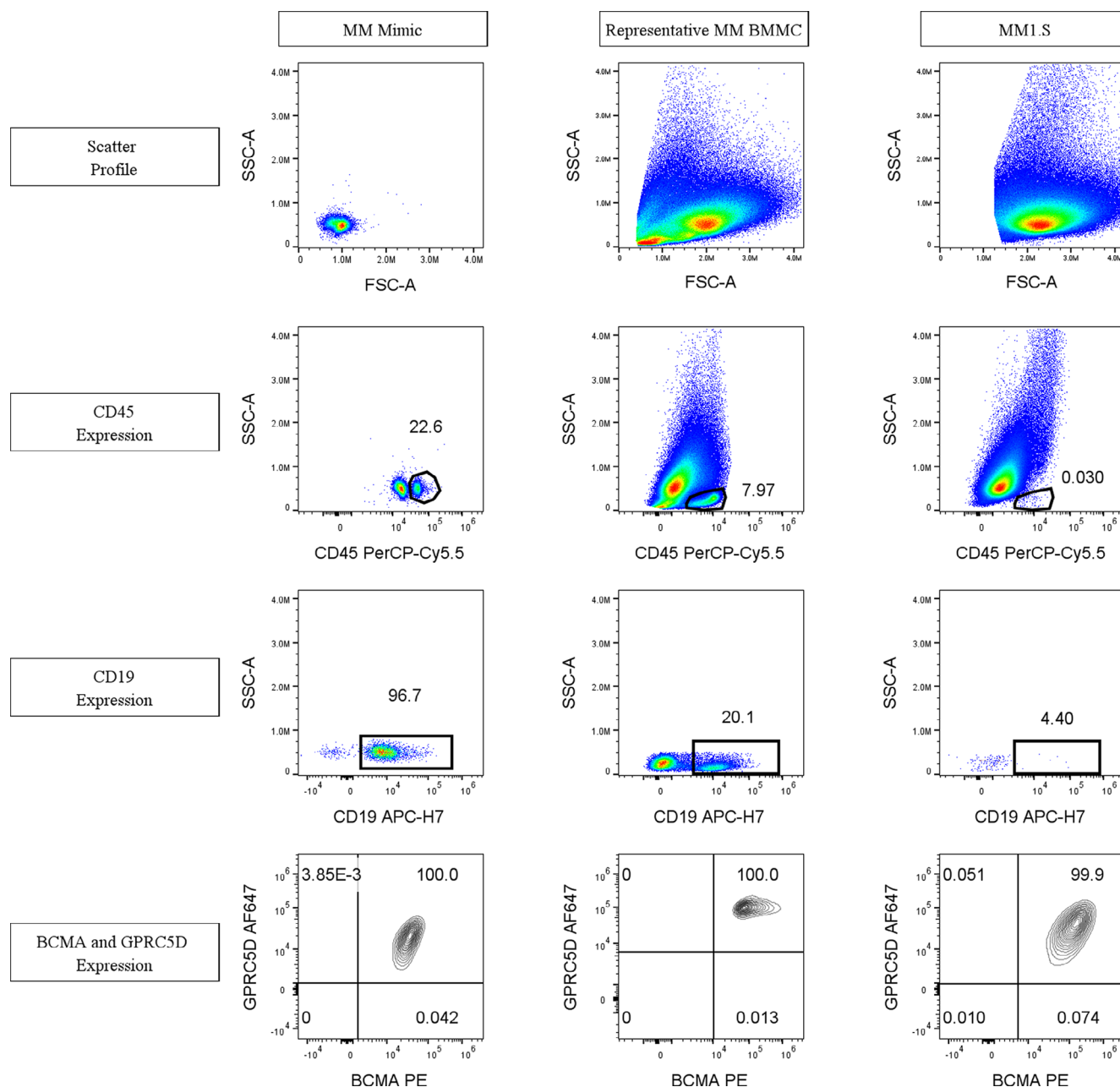


FIGURE 3 Comparison of multiple myeloma (MM) cell mimic with bone marrow mononuclear cells (BMMC) and MM1.S samples. Representative pseudocolor dot plots and contour plots of MM cell mimic, MM BMMC, and MM1.S are shown. From top to bottom, similarities and differences in forward and side scatter profiles, CD45 and CD19 expression, as well as B cell maturation antigen (BCMA) and G protein-coupled receptor, class C, group 5, member D (GPRC5D) expression are shown. FSC-A, forward scatter area; SSC-A, side scatter area. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/cyto.b.20075)]

lot-to-lot bridging. Each marker is engineered and tuned to a defined target antigen density so the mimic provides a stable and reproducible target for determining optimal antibody concentrations for markers that are difficult to obtain on primary cells. Certain limitations should be recognized for this application. Each product represents a single fixed antigen density rather than the physiological range encountered across primary cells and Fc-mediated or nonspecific antibody binding may differ, which should be accounted for when extrapolating titration results to clinical specimens.

The current MM cell mimic was developed as an assay control for an existing exploratory endpoint flow cytometry assay that was already

supporting ongoing MM clinical trials. The established panel was retained without modification and the mimic was therefore engineered to match the markers already in use rather than to introduce additional ones. As a result, the current formulation does not include several markers incorporated into current MM panels such as CD117, CD27, CD28, and CD81 (Flores-Montero et al., 2017). A key advantage of the TruCytes[®] platform is its tunability and these additional markers can be incorporated into further formations as the opportunity arises, further extending the utility of the mimic across a broader range of MM panels.

Although MM cell mimics offer advantages over MM1.S, careful upfront design is required to ensure successful implementation. Precision

TABLE 3 Intra-assay precision of multiple myeloma cell mimic.

Intra-assay precision									
Rep	%CD19 of 45 Leuks	%PCs of 45 Leuks	PC BCMA MFI	BCMA MESF	PC GPRC5D MFI	GPRC5D MESF	%PC PD-L1+ of PC	%PC CD56+ of PC	%PC GPRC5D+ BCMA+ of PC
1	21.5	77.9	98,753	4964	118,099	54,408	100.0	99.7	100.0
2	22.1	77.4	93,132	4676	114,347	52,969	100.0	99.6	100.0
3	22.3	77.2	89,677	4499	108,441	50,687	100.0	99.6	100.0
4	22.1	77.5	94,498	4746	114,010	52,839	100.0	99.7	100.0
5	21.5	77.8	94,774	4760	122,757	56,184	100.0	99.7	100.0
SD	0.37	0.29	3269	168	5310	2039	0.00	0.05	0.00
Avg	21.9	77.6	94,167	4729	115,531	53,417	100.0	99.7	100.0
%CV	1.71	0.37	3.47	3.54	4.60	3.82	0.00	0.05	0.00

Abbreviations: %CV, percent coefficient of variation; %PC, percent plasma cells; BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D; MESF, molecules of equivalent soluble fluorochrome; MFI, median fluorescence intensity; PC, plasma cells.

TABLE 4 Inter-assay precision of multiple myeloma cell mimic.

Inter-assay precision									
Run	%CD19 of 45 Leuks	%PCs of 45 Leuks	PC BCMA MFI	BCMA MESF	PC GPRC5D MFI	GPRC5D MESF	%PC PD-L1+ of PC	%PC CD56+ of PC	%PC GPRC5D+ BCMA+ of PC
1	21.9	77.8	85,213	4361	98,450	46,909	100.0	99.6	100.0
2	21.9	77.4	82,388	4299	106,332	50,075	100.0	99.7	100.0
3	21.5	77.9	98,753	4964	118,099	54,408	100.0	99.7	100.0
SD	0.23	0.26	8748	367	9888	3765	0.00	0.06	0.00
Avg	21.8	77.7	88,785	4542	107,627	50,464	100.0	99.7	100.0
%CV	1.06	0.34	9.85	8.09	9.19	7.46	0.00	0.06	0.00

Abbreviations: %CV, percent coefficient of variation; %PC, percent plasma cells; BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D; MESF, molecules of equivalent soluble fluorochrome; MFI, median fluorescence intensity; PC, plasma cells.

TABLE 5 Inter-instrument precision of multiple myeloma cell mimic.

Inter-Instrument Precision									
Instrument	%CD19 of 45 Leuks	%PCs of 45 Leuks	PC BCMA MFI	BCMA MESF	PC GPRC5D MFI	GPRC5D MESF	%PC PD-L1+ of PC	%PC CD56+ of PC	%PC GPRC5D+ BCMA+ of PC
1	21.9	76.5	75,426	4196	101,362	49,754	100.0	88.7	100.0
2	23.2	76.7	83,404	4384	92,862	50,571	99.4	99.0	99.4
3	22.4	77.2	89,938	4800	109,402	54,822	100.0	99.6	100.0
SD	0.66	0.36	7268	309	8271	2721	0.35	6.13	0.35
Avg	22.5	76.8	82,923	4460	101,209	51,716	99.8	95.8	99.8
%CV	2.91	0.47	8.76	6.93	8.17	5.26	0.35	6.40	0.35

Abbreviations: %CV, percent coefficient of variation; %PC, percent plasma cells; BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D; MESF, molecules of equivalent soluble fluorochrome; MFI, median fluorescence intensity; PC, plasma cells.

assessments for the reportables of interest were within acceptance criteria of less than or equal to 20% CV, with the exception of CD56. CD56 showed a 26.56% change at Day 3 compared to Day 0 when assessing post-reconstitution stability. GPRC5D MFI in closed-vial stability showed 21.7% CV at the 3 month timepoint and 20.9% CV at the 10 month timepoint relative to T0. Additionally, GPRC5D MFI in inter-analyst precision showed a 21.51% CV, but when converted to MESF, the CV was within acceptable criteria. This shows that MFI alone may

not be sufficient for longitudinal assessment with the established acceptance criteria. Key markers to track longitudinally should have MESF designated to the marker of interest. Although there was a slight MFI increase in CD56 on Day 3 which resulted in a more than 20% change from Day 0, it could be due to changes in gain settings on each day on the Cytex Aurora.

The MM mimic was developed for fresh sample testing in MM clinical studies, and more broadly, synthetic cell mimics represent a

TABLE 6 Inter-analyst precision of multiple myeloma cell mimic.

Inter-analyst precision									
Analyst	%CD19 of 45 Leuks	%PCs of 45 Leuks	PC BCMA MFI	BCMA MESF	PC GPRC5D MFI	GPRC5D MESF	%PC PD-L1+ of PC	%PC CD56+ of PC	%PC GPRC5D+ BCMA+ of PC
1	25.9	73.5	104,684	5281	139,919	64,564	100.0	99.7	100.0
2	20.7	77.4	79,655	3991	91,786	45,486	100.0	99.5	100.0
3	22.3	77.0	99,889	5034	133,801	62,210	100.0	99.6	100.0
SD	2.66	2.15	13,284	685	26,203	10,402	0.00	0.10	0.00
Avg	23.0	76.0	94,743	4769	121,835	57,420	100.0	99.6	100.0
%CV	11.60	2.82	14.02	14.36	21.51	18.12	0.00	0.10	0.00

Abbreviations: %CV, percent coefficient of variation; %PC, percent plasma cells; BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D; MESF, molecules of equivalent soluble fluorochrome; MFI, median fluorescence intensity; PC, plasma cells.

TABLE 7 Closed-vial stability %CV of median fluorescence intensity (MFI) compared with timepoint T0.

Closed-vial stability								
%CV MFI relative to T0	CD45	CD19	CD38	CD138	GPRC5D	BCMA	CD56	PD-L1
Month 3	12.8	7.5	13.1	6.3	21.7	2.0	1.8	0.3
Month 6	14.8	9.8	13.4	7.9	12.0	3.0	3.4	0.7
Month 10	15.3	13.7	18.4	13.5	20.9	4.2	9.4	1.0
Month 13	4.7	4.9	10.0	7.0	16.9	5.8	0.7	0.8

Abbreviations: %CV, percent coefficient of variation; BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D; MFI, median fluorescence intensity.

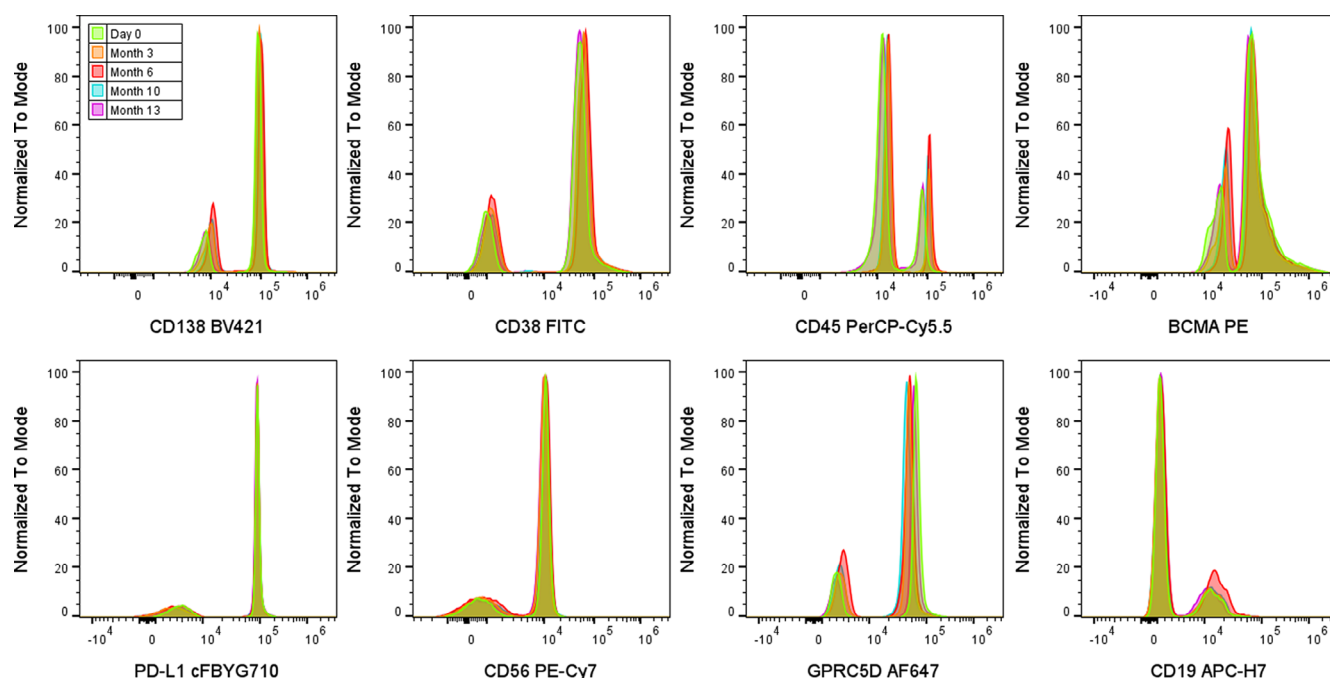


FIGURE 4 Closed-vial storage stability. Vials of multiple myeloma cell mimic were held at 37°C until testing. Timepoints were translated using the Arrhenius equation. Histogram overlay plots on Month 3 (orange), Month 6 (red), Month 10 (blue), and Month 13 (purple) are shown compared to Day 0 (green). BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 8 Post-reconstitution percent change in median fluorescence intensity (MFI) compared to Day 0.

Post-reconstitution stability								
%change in MFI	CD45	CD19	CD38	CD138	GPRC5D	BCMA	CD56	PD-L1
Day 3	−0.89	13.25	13.32	7.07	15.37	15.79	26.56	7.07
Day 5	2.41	9.32	10.97	1.19	−11.15	3.27	−1.87	1.19
Day 7	3.64	11.15	13.32	−0.59	16.41	13.10	6.73	−0.59

Note: Higher %Difference in CD56 MFI observed at Day 3 only.
Abbreviations: BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D.

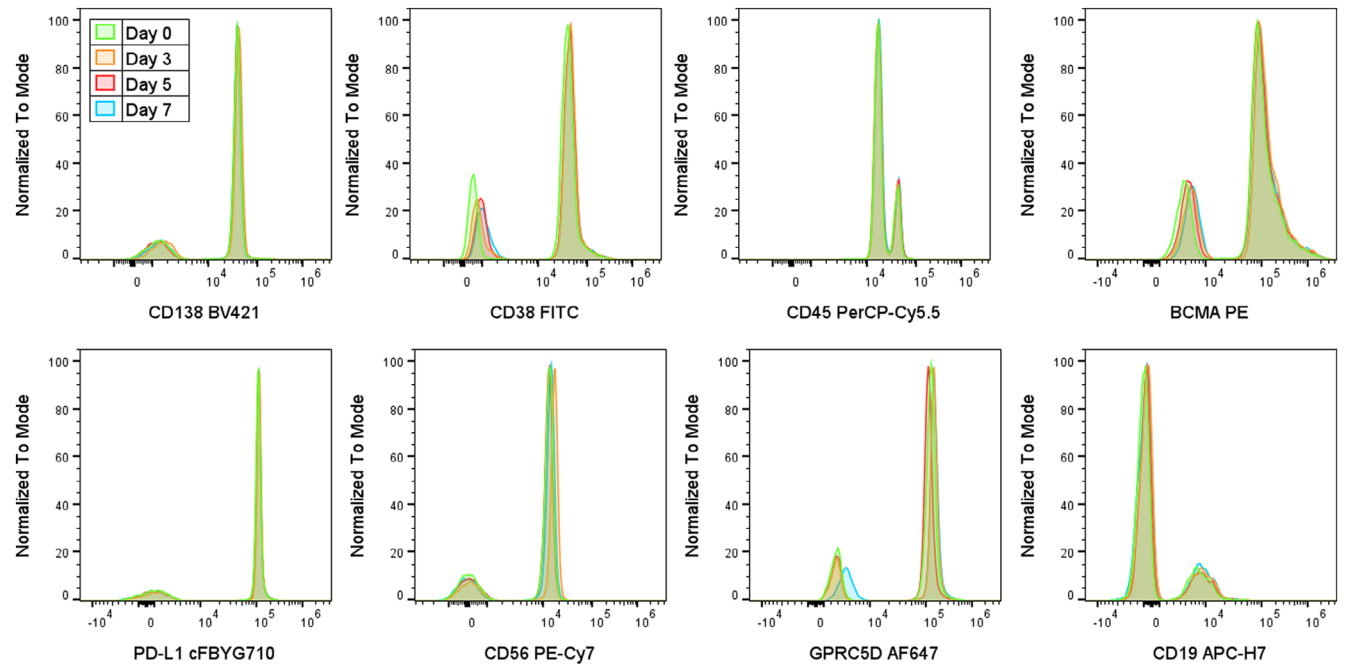


FIGURE 5 Post-reconstitution stability. Vials of multiple myeloma cell mimic were reconstituted and stored at intervals until testing. Histogram overlay plots on Days 3 (orange), 5 (red), and 7 (blue) post-reconstitution are shown compared to Day 0 (green). BCMA, B cell maturation antigen; GPRC5D, G protein-coupled receptor, class C, group 5, member D. [Color figure can be viewed at wileyonlinelibrary.com]

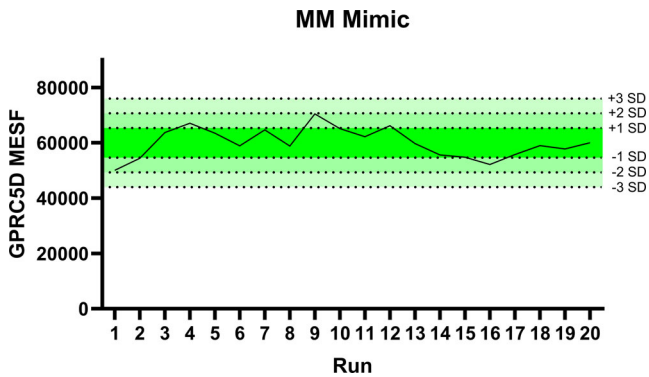


FIGURE 6 Levey-Jennings plot of G protein-coupled receptor, class C, group 5, member D (GPRC5D) molecules of equivalent soluble fluorochrome (MESF) from plasma cells. GPRC5D MESF was within ± 3 standard deviation (SD) of the mean over 20 distinct runs. [Color figure can be viewed at wileyonlinelibrary.com]

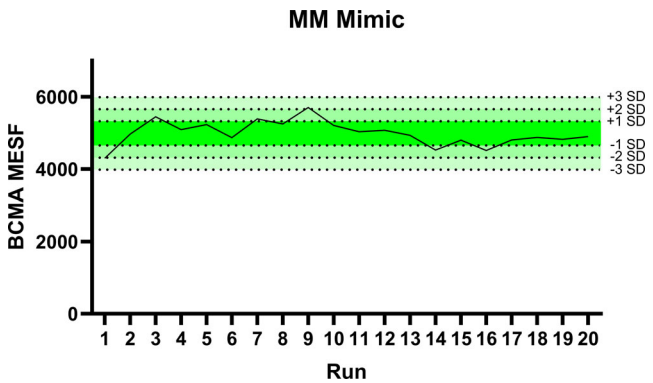


FIGURE 7 Levey-Jennings plot of B cell maturation antigen (BCMA) molecules of equivalent soluble fluorochrome (MESF) from plasma cells. BCMA MESF was within ± 3 standard deviation (SD) of the mean over 20 distinct runs. [Color figure can be viewed at wileyonlinelibrary.com]

promising alternative to traditional assay controls, particularly for markers that are difficult to detect or maintain in cell-line based materials. Overall, the MM cell mimic represents a meaningful advancement in assay control design, addressing technical and operational limitations of conventional cell-line controls while supporting reliable, longitudinal performance monitoring in clinical flow cytometry.

5 | CONCLUSION

In this article, we compared the MM cell mimic to the MM1.S cell line and qualified the MM cell mimic to evaluate its suitability and stability as an assay control. The MM cell mimic overcame limitations of our current assay control with similar scatter profiles of our representative diseased samples and expressed markers that the MM1.S cell line lacked, like CD45 and CD19. Both the MM cell mimic and MM1.S robustly expressed the key markers BCMA and GPRC5D. Ultimately, synthetic cell mimics expand the toolbox and provide a complementary alternative to traditional cell lines.

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