The Validity and Utility of a Single, Five-Colour Flow Cytometry Tube in Detecting Plasma Cell Dyscrasias at a Resource-Constrained Laboratory

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ABSTRACT

Introduction: Cost is a major limitation to the utilisation of flow cytometry in resource-poor settings. This study aimed to assess the validity and utility of a single, five-colour immunophenotyping tube, employing dried monoclonal reagents instead of conventional liquid monoclonal reagents, as a tool for the diagnosis of plasma cell malignancies.

Methods: Immunophenotyping results of 26 suspected plasma cell malignancies were compared prospectively using a single customised dried monoclonal reagent tube and the current conventional liquid monoclonal reagent panel.

Results: Cell availability, plasma cell enumeration and monoclonal antigen expression were all comparable except in the expression of the common leucocyte antigen, which was attributed to increased numbers of unlysed red cells on liquid reagent analyses. Mean fluorescence intensity was brighter on the dried monoclonal tube analyses enabling easier identification and characterisation of plasma cells. The final diagnoses obtained correlated in 92% of cases, confirming a sensitivity and specificity of 95% and 86% respectively for the dried monoclonal tube. Total processing time was 38% shorter and costs were 76% lower for the dried monoclonal tube.

Conclusions: This single, dried monoclonal reagent tube showed a high sensitivity and specificity compared to the current reference standard, with potential cost and time- saving benefits. Laboratory verification and implementation of this novel plasma cell dyscrasia tube can be recommended in resource-constrained settings once these dried reagents are commercially validated for clinical use.

KEYWORDS: *immunophenotyping, dried monoclonal antibodies, plasma cell dyscrasia, flow cytometry, diagnostic*

INTRODUCTION

Multiple myeloma is one of the most commonly diagnosed haematological malignancies in South Africa. It is characterised by the expansion of malignant plasma cells in the bone marrow, ultimately resulting in complications such as anaemia, bone lytic lesions, hypercalcaemia and renal failure.[1] Immunophenotyping using multiparametric flow cytometry (MFC) is an invaluable component of the diagnostic work-up of multiple myeloma and many of the other plasma cell dyscrasias (PCDs) such as plasma- cytoma, plasma cell leukaemia and Waldenström macroglobulinaemia.[2]

MFC enables the identification of distinct populations of cells within a heterogenous mixture of cells. This is accomplished using scattered laser light which identifies a cell based on its physical characteristics, such as size (forward scatter) and internal complexity (side scatter), as well as through the expression of specific cellular antigens, which are detected using fluorescently labelled monoclonal antibodies. To enable standardization, monoclonal antibodies are formally classified into clusters of differentiation (or CDs). Haematopoietic cell populations can be detected and differentiated based on their side scatter properties and intensity of expression of the common leucocyte antigen CD45.[3] The technical specifications of flow cytometers employed in immunophenotyping today vary widely and can range from simple single-laser-three-colour instruments on the one end of the spectrum, to highly sophisticated three-laser-ten-colour instruments on the other end.[4]

The value of MFC has been firmly established in the work-up of PCDs and also in the monitoring of PCD patients on therapy. This is due to its unique ability to distinguish normal from clonal plasma cells based on abnormal/aberrant antigen expression or immunoglobulin light chain restriction (LCR).[2]

Normal plasma cells characteristically show intense expression of CD38, CD138, polyclonal cytoplasmic immunoglobulin and low expression of CD19 and CD45. Clonal plasma cells on the other hand typically show monoclonal cytoplasmic immunoglobulin with kappa (K) or lambda (λ) LCR, as well as reduced expression of CD38, absent CD19 and CD45 and aberrant expression of antigens including: CD20, CD56, CD117 and CD200.[5] The EuroFlow Consortium developed a PCD panel incorporating 12 different antibodies in specific combinations to optimise and standardise the immunophenotyping of PCDs.[6]

A major limitation of MFC however, is the cost of the monoclonal antibody reagents and the increasingly sophisticated instrumentation required to analyse the multitude of new diagnostic and prognostic immunophenotypic markers that are continuously being discovered. Healthcare budgets worldwide are facing increasing pressure to optimise benefit, reduce costs and improve efficiency, while maintaining quality, and pathology services have not escaped this pressure.

Liquid monoclonal antibody reagents have traditionally been used for MFC and are widely available. These liquid monoclonal reagents have several disadvantages however including the need for refrigerated transport and storage. Processing of these reagents also requires labour-intensive pipetting. This introduces a potential source of error and variability, and consumes time, which can be more productively applied elsewhere in the laboratory. Additionally, variability in shelf lives of liquid reagents requires complex inventory management and is associated with approximately 5-10% reagent wastage.[7]

In an attempt to address some of these limitations, manufacturers have now started developing pre-mixed dried monoclonal reagents. DuraClone™ dried monoclonal reagents, manufactured by Beckman Coulter Inc. These can be supplied in customised combinations within plastic tubes, as per specification, thereby removing the need for labour-intensive pipetting of liquid reagents. They also have a longer shelf life compared to liquid reagents and are room temperature stable, thereby negating the need for cold chain shipment and storage. These customised combinations of dried reagents have currently only been validated for use in a research capacity and not in the routine diagnostic laboratory.[8] Their efficacy and potential cost and time-saving benefits have however, been investigated and confirmed in articles published by several independent researchers.[9,10]

The National Health Laboratory Service (NHLS) is the sole provider of diagnostic services to the South African public health care system, servicing over 80% of the population.[11] Of the over 260 diagnostic laboratories within the NHLS, more than 50 have access to a basic flow cytometer, which is mainly used for CD4 enumeration and Human Immunodeficiency Virus (HIV) infection monitoring.[12] Many of these flow cytometers have the potential capability to analyse up to five-colour antibody combinations, which could allow for the decentralisation of flow cytometry specimen processing.

Multiple myeloma is commonly diagnosed in SA

The aim of this study was to assess the sensitivity, specificity and financial feasibility of using a single, five-colour PCD immunophenotyping tube, employing DuraClone™ dried monoclonal reagents instead of conventional liquid monoclonal reagents, for the detection and enumeration of clonal plasma cells.

MATERIALS AND METHODS

Study sample and design

The study was conducted at a tertiary academic laboratory in Pretoria over a one-year period ranging from 1 December 2016 to 30 November 2017. Ethical approval was obtained from the Sefako Makgatho Health Sciences University Research Ethics Committee (SMUREC/P/184/2016:PG).

Specimens received for immunophenotyping in cases of suspected or confirmed PCDs or lymphomas with plasmacytic differentiation were identified upon receipt. A census was used as a sampling frame and all cases were included in the study, irrespective of gender or age, provided there was sufficient sample available (>2mL). An observational and analytical design was used with prospective comparison between a single, novel, five-colour, dried monoclonal PCD tube (DMPCDT) and the current in-house four-tube, tenmonoclonal PCD panel using conventional liquid reagents (as a reference standard).

Materials and equipment

The DMPCDT consisted of a customised DuraClone™ dried reagent tube, which was obtained from Beckman Coulter Inc. Each DMPCDT contained CD3-FITC, CD56-PE, CD19-ECD, CD138-PC5 and CD45-PC7, thereby corresponding to the first tube of the conventional PCD panel (CPCDP) in use at DGMTL. This five-colour combination allowed for a basic differential count (using CD45, CD3 and CD19) with easy enumeration of plasma cells (using CD138). And determination of plasma cell clonality based on the aberrant gain of CD56 and/or loss of CD19 expression.[13] The full in-house CPCDP, in addition to the first tube described above, also consisted of three additional tubes containing CD20 and CD38, cytoplasmic kappa and lambda and CD200 respectively. Samples were analysed on a Beckman Coulter FC500™ five-colour flow cytometer and scatter plot analysis was performed using CXP™ software.

Compensation kits were supplied with the DuraClone™ tubes. Setup, compensation and optimisation of the DMPCDT protocol were performed with the assistance of an experienced Beckman Coulter flow cytometry application specialist on site. The current CPCDP was not altered as it is a validated platform.

Liquid reagents were stored in a standard 2-8°C fridge and DuraClone™ tubes were stored in the flow laboratory, at room temperature, as specified by the manufacturer.

Specimen processing

All specimens were processed manually by the designated flow cytometry technologist. Conventional PCD panel

(CPCDP) samples were processed and analysed first, in order not to interfere with the regular workflow and turn-aroundtime of the laboratory.

Specimens with sufficient sample remaining were subsequently processed using the customised DMPCDT and analysed on the same instrument. Dried monoclonal PCD tube (DMPCDT) processing was completed within a maximum period of six hours of the CPCDP processing in each case. All processing and analyses were completed within 24 hours of receipt of the specimen.

The CPCDP was processed as per the current laboratory standard operating procedure (SOP). The DMPCDT was processed according to the manufacturer's assay protocol, as detailed in the accompanying product data sheet (see Table I). Differences in processing between the CPCDP and DMPCDT included the use of: VersaLyse™ lysing solution with the DuraClone™ monoclonals (as per manufacturer's instructions), and Immunoprep™ lysing solution for the liquid reagents (as per current laboratory standard operating procedure). DuraClone™ processing does not require the addition of monoclonal antibodies, as they are already provided in the tube in a dried form.

Data collection

Upon completion of the specimen processing phase, batched analysis of all the DMPCDT scatter plots was performed, followed by batched analysis of all the CPCDP scatter plots, on separate days. Dried monoclonal PCD tube (DMPCDT) samples were identified using unique study identifiers and CPCDP samples using the laboratory episode numbers, in order to avoid bias. All scatter-plot analyses were performed blinded by the researcher, together with a pathologist who is an experienced flow cytometrist.

Enumeration of plasma cells was estimated based on lightscatter properties and CD138 positivity. The percentage expression, as well as mean fluorescence intensity (MFI), obtained for each DMPCDT and the corresponding CPCDP monoclonal antibody, was assessed and recorded on a data collection sheet. A final diagnosis for each DMPCDT specimen, based on these values, was also recorded on the same sheet and compared to the final diagnosis obtained after the full CPCDP.

Cost analysis was performed based on the cost of the monoclonal antibody reagents (Beckman Coulter DuraClone™ and liquid monoclonals) and consumables used (pipette tips, tubes etc.).

Timing of the CPCDP sample processing vs DMPCDT sample processing was performed once sufficient DMPCDT samples had been processed for the technologist to feel comfortable with the steps required. Timing included all processing steps (excluding initial sample dilution) as well as incubation steps up until the sample was ready to run, first for the CPCDP, and then for the DMPCDT. Processing and timing of both samples were performed by the same technologist and under ideal conditions (i.e. no interruptions) using the same electronic timer.

Enumeration of plasma cells was estimated based on light-scatter properties and CD138 positivity

Table I. An outline of the respective processing steps required

CPCDP	DMPCDT
1. Label four plastic tubes *For tubes one-three:* 2. Pipette 10µL of each liquid monoclonal reagent into their respective labelled tubes 3. Add 100µL of patient sample to each tube 4. Incubate for 10 minutes *For the fourth (cytoplasmic) tube:* 5. Pipette 50µL of patient sample into this tube 6. Add 25µL of fixative 7. Vortex and incubate for 20 minutes 8. Vortex and add 300µL permeabilizing reagent 9. Add 10µL Kappa and 10µL Lambda monoclonal reagent 10. Incubate for 15 minutes 11. Add 3ml buffer solution 12. Runon cytometer	1. Add 100µL washed sample to the DuraClone™ tube 2. Vortex and incubate for 15 minutes 3. Add 2ml Versalyse™ 4. Vortex and incubate for 15 minutes 5. Run on cytometer

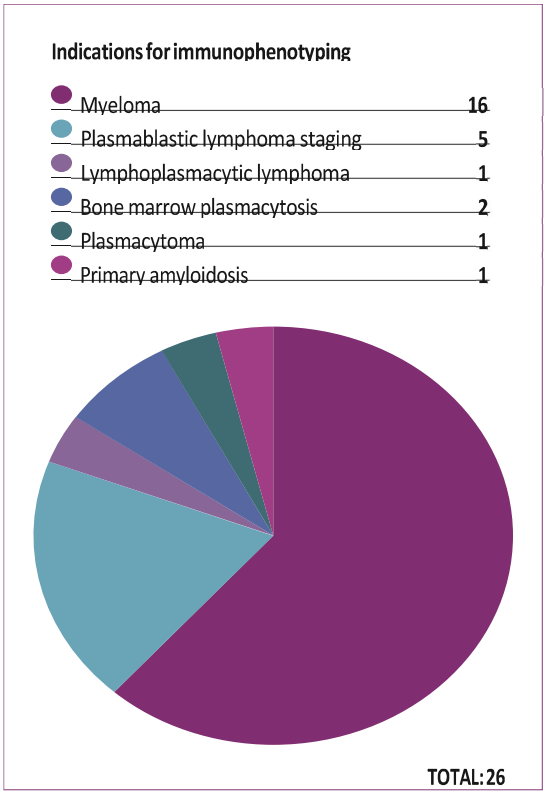

Figure 1. The indications for immunophenotyping of all samples analysed in this study

Data analysis

Frequency tables were constructed and the chi-square test used to compare descriptive variables. Cell availability, plasma cell enumeration, monoclonal antigen expression and mean fluorescence intensity of each of the five monoclonal antibodies were compared using paired samples t-test and correlation test. A significance level of 0.05 was used for all statistical tests. All statistical analyses were performed on IBM SPSS Statistics 23® run under Microsoft Windows® on a personal computer.

RESULTS AND DISCUSSION

Indications for immunophenotyping

There were 26 eligible specimens in the study. The majority (16≈61.5%) were received as part of the work-up for suspected multiple myeloma. Other indications included five (19.2%) specimens for plasmablastic lymphoma staging, two (7.7%) for bone marrow plasmacytosis, and one (3.8%) each for plasmacytoma, primary amyloidosis and suspected lymphoplasmacytic lymphoma (see Figure 1). All the samples (100%) analysed were bone marrow aspirates.

Cell availability

Cells available for analysis was assessed on each specimen using side vs forward scatter properties and all cellular debris was excluded from analysis. The mean cell availability was similar for both methods (57% for the DMPCDT vs 53% for the CPCDP). While cell availability was equal or better preserved in 17 (65%) of the DMPCDT specimens this difference was not found to be statistically significant (p=0.528).

Plasma cell enumeration

The percentage plasma cells enumerated on the DMPCDT was equal or higher than the percentage obtained on the CPCDP analyses in 22 (85%) specimens analysed, as demonstrated in Figure 2. This difference was however, not found to be statistically significant (P=0.704). Comparison of plasma cell enumeration between the DMPCDT and CPCDP showed a good and statistically significant correlation (P<0.0005). Plasma cells, as well as cell availability, were unexpectedly well preserved in the DMPCDT specimens, considering that these tubes were always processed after the CPCDP sample and would therefore have been expected to show a more significant loss of viable plasma cells. This finding has been attributed to the different lysing reagents used in the DMPCDT and CPCDP processing.

Clonality and final diagnoses

The CPCDP analysis demonstrated clonality in 19 (73%) specimens analysed. This study therefore included a good representation of clonal and non-clonal plasmacytoses. Of these 19 clonal specimens, each showed either aberrant CD56 positivity (63%) and/or loss of CD19 (95%) with none relying solely on the demonstration of kappa or lambda light chain restriction. Therestriction. The DMPCDT analyses demonstrated clonality in the same number (73%) of samples. In comparison, aberrant CD56 positivity was congruent on all cases with some variability in loss of CD19 leading to two discrepant final diagnoses. Both of these cases however had very few plasma cells which likely affected interpretation.

Comparable final diagnoses were obtained in 24 (92%) specimens, as demonstrated in Table II. One false positive and one false negative case were obtained with the DMPCDT, giving it a sensitivity of 95% and a specificity of 86%, compared to the reference standard.

Antigen expression and intensity

Statistical significance (P<0.05) was assessed on (1) the difference in antigen expression (Table III) as well as (2) the correlation in antigen expression (Table IV) between the DMPCDT and CPCDP CD3-FITC, CD56-PE, CD19-ECD, CD138PC5 and CD45-PC7 antibodies.

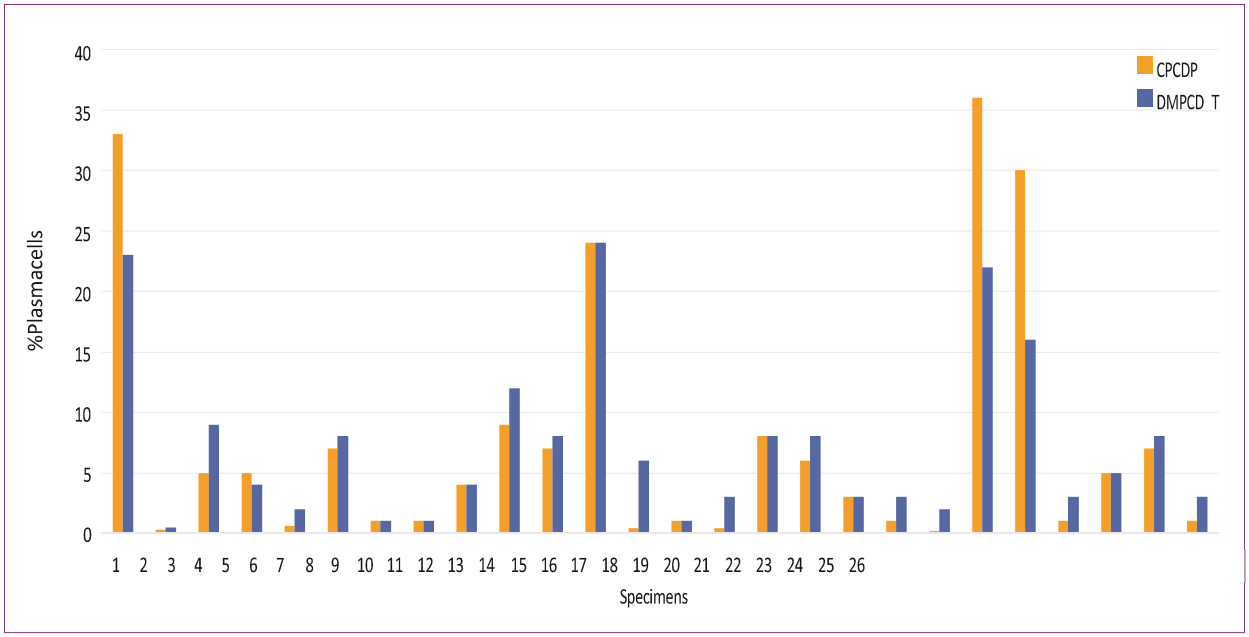

Figure 2. Comparative plasma cell enumerations of the 26 specimens included in the study

Table II. A comparison of the final diagnoses obtained with CPCDP and DMPCDP

No	Indication	Final diagnosis	
		CPCDP	DMPCDT
1	? Myeloma	≥10% Clonal plasma cells with K LCR and aberrant +CD56, +CD200, -CD19	≥10% Clonal plasma cells with aberrant +CD56, -CD19
2	Plasmablastic lymphoma staging	Non-clonal	<10% Clonal with aberrant dim+CD19
3	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
4	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
5	? Myeloma	Non-clonal	Non-clonal
6	Bone marrow plasmacytosis	Non-clonal	Non-clonal
7	Plasmablastic lymphoma staging	Non-clonal	Non-clonal
8	Plasmablastic lymphoma staging	Non-clonal	Non-clonal
9	Bone marrow plasmacytosis	<10% Clonal plasma cells with aberrant +CD56	<10% Clonal plasma cells with aberrant +CD56
10	? Myeloma	<10% Clonal plasma cells with λ LCR and aberrant +CD56, +CD200, -CD19	≥10% Clonal plasma cells with aberrant +CD56, dim+CD19
11	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
12	? Myeloma	≥10% Clonal plasma cells with K LCR and aberrant -CD19	≥10% Clonal plasma cells with aberrant -CD19
13	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant -CD19	<10% Clonal plasma cells with aberrant -CD19
14	Plasmablastic lymphoma staging	Non-clonal	Non-clonal
15	Plasmablastic lymphoma staging	<10% Clonal plasma cells with aberrant dim+CD19	Non-clonal
16	Relapsed myeloma	<10% Clonal plasma cells with λ LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
17---	? Lymphoplasmacytic lymphoma	<10% Clonal plasma cells with aberrant dim+CD19	<10% Clonal plasma cells with aberrant dim+CD19
18	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
19	? Myeloma	<10% Clonal plasma cells with λ LCR with aberrant -CD19	<10% Clonal plasma cells with aberrant dim+CD19
20	? Myeloma	Non-clonal	Non-clonal
21	? Myeloma	≥10% Clonal plasma cells with aberrant +CD56, +CD200, -CD19	≥10% Clonal plasma cells with aberrant +CD56, -CD19
22	Plasmacytoma	≥10% Clonal plasma cells with K LCR and aberrant +CD56, +CD200, -CD19	≥10% Clonal plasma cells with aberrant +CD56, -CD19
23	Primary amyloidosis	<10% Clonal plasma cells with λ LCR and aberrant -CD19	<10% Clonal plasma cells with aberrant dim+CD19
24	? Myeloma	<10% Clonal plasma cells with λ LCR and aberrant +CD56, +CD200, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
25	? Myeloma	<10% Clonal plasma cells with K LCR and aberrant +CD56, -CD19	<10% Clonal plasma cells with aberrant +CD56, -CD19
26	? Myeloma	<10% Clonal plasma cells with aberrant -CD19	<10% Clonal plasma cells with aberrant -CD19

K = kappa; λ = lambda; LCR = light chain restriction

Table III. Difference in antigen expression: paired samples t-test results

Paired samples t-test	P-value	Statistical significance
CD3 DuraClone™ vs Liquid	0.373	Not significant
CD19 DuraClone™ vs Liquid	0.761	Not significant
CD45 DuraClone™ vs Liquid	0.029	Significant
CD56 DuraClone™ vs Liquid	0.055	Not significant
CD138 DuraClone™ vs Liquid	0.831	Not significant

Table IV. Correlation in antigen expression: paired samples correlation results

Paired samples correlations	Correlation	P-value	Statistical significance
CD3 DuraClone™ vs Liquid	0.736	<0.0005	Significant
CD19 DuraClone™ vs Liquid	0.363	0.068	Not significant
CD45 DuraClone™ vs Liquid	-0.116	0.572	Not significant
CD56 DuraClone™ vs Liquid	0.736	<0.0005	Significant
CD138 DuraClone™ vs Liquid	0.942	<0.0005	Significant

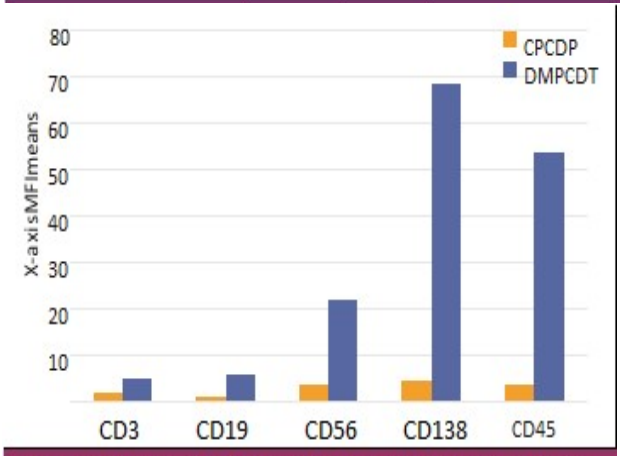

Figure 3. Comparison of the mean MFI percentages obtained for each monoclonal antibody over all 26 specimens.

Statistical significance was assessed on the difference in antigen expression as well as the correlation in antigen expression between the various antibodies

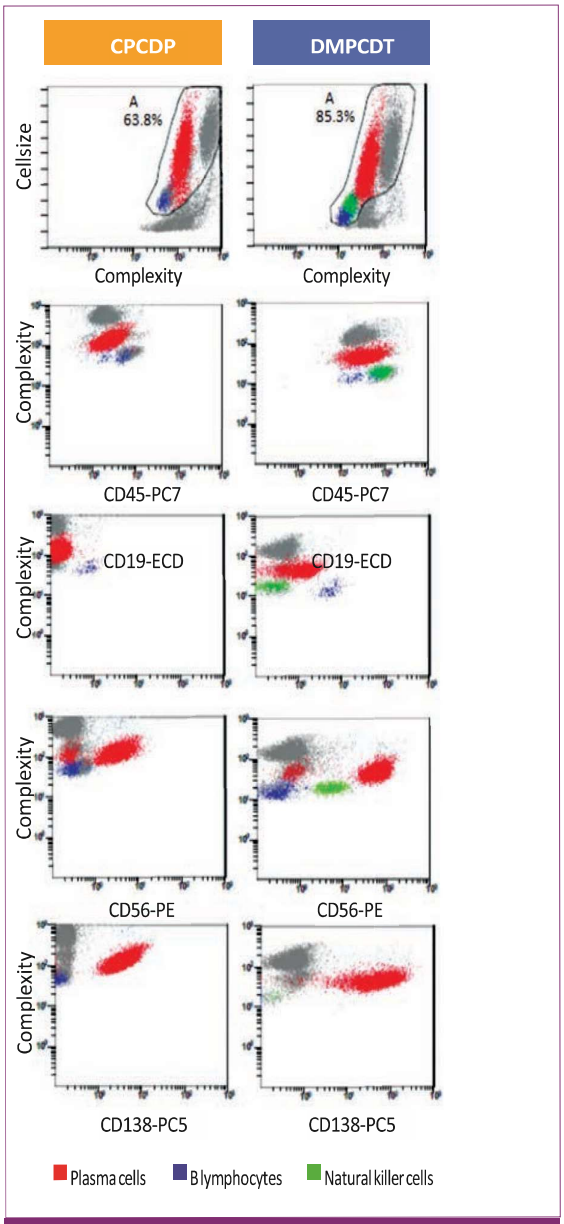

Figure 4 Scatter-plot comparisons on specimen 1 highlighting subtle differences in fluorescence intensity. The brighter CD56-expression enabled differentiation between the two CD56-positive populations (plasma cells and NK cells) on DMPCDT analysis

The former (1) was found to be statistically insignificant on all antigens except CD45 and the latter (2) was statistically significant on all except CD45 as well as CD19. CD45 showed overall poor comparability between the DMPCDT and CPCDP samples. The poor comparability of CD45-PC7 is partly numbers of unlysed red cells seen on some CPCDP samples.

When the collective means of the x-axis MFIs obtained for each monoclonal were compared, DuraClone™ showed a consistently brighter fluorescence intensity as compared to liquid reagents, as demonstrated in Figure 3. This is likely a reflection of higher voltage settings used in the DMPCDP protocol and was especially noticeable when identifying plasma cells (using CD138) as well as when differentiating between plasma cells and natural killer cells (using CD56) as illustrated in Figure 4. This observation was however, not found to be statistically significant (P=0.063).

The brighter DMPCDT CD45-PC7 MFI resulted in a shift of viable cells further into the CD45-positive region, thereby clearly separating them from any unlysed red cells present. This is believed to be another reason for the poor comparability seen on this antigen.

Some minor differences in MFI was also reported in research, published by Hedley *et al*,[9] evaluating the use of a 10-colour dried reagent vs liquid reagent lymphocyte screening tube. Despite these differences, the two methods demonstrated excellent agreement in staining intensity throughout a variety of sample types including blood, bone marrow, tissue and body fluid.

Processing time and cost

The total processing time, starting from the first pipetting of patient sample to the end of the final incubation step, was measured for the full CPCDP and the DMPCDT specimens respectively. The complete processing of a specimen using liquid reagents required 50 minutes whereas, the DMPCDT required 31 minutes (a difference of 19 minutes or 38%). Actual hands-on processing of the DMPCDT required only one minute with the remaining 30 minutes required for two 15-minute incubation steps.

The total cost per specimen using the DMPCDT and CPCDP was calculated and compared as demonstrated below (Table V). Total cost included the cost of all consumables and reagents as well as the liquid monoclonal reagents and customised DuraClone™ tubes (current retail price), obtained at the time of analysis. Total calculated cost per DMPCDT specimen amounted to R135.61, vs R559.03 for the whole CPCDP. The use of the DMPCDT therefore results in a potential cost saving of R423.42 (76%) per specimen processed. Potential savings would likely also be increased by the reduction in labour and the elimination of refrigerated storage.

Our findings correlate with those reported by Correia *et al*[10] who showed a time and cost saving of 15.8% and 12.3% respectively when using a 10-colour dried reagent screening tube for haematological malignancies at their centre. The DMPCDT in our study resulted in a more significant time and cost-saving (38% and 76%) due to the difference in the number of antibodies utilised between the DMPCDT (five) and the CPCDP (14).

Table V. Cost comparison of the CPCDP vs DMPCDT

Consumables used per specimen	Cost (ZAR)	
	CPCDP	DMPCDT
Gloves	1.12	1.12
Plastic tubes	2.86	0
Pipette tips	1	0.07
Gauze	0.2	0
DuraClone™ tube	0	115.56
Versalyse™	0	18.86
PerFix™	34	0
Immunoprep™	24.33	0
Liquid monoclonals	495.52	0
TOTAL	**559.03**	**135.61**

CONCLUSION

The results of this study confirmed the validity and financial feasibility of using the DMPCDT as a tool for the detection and enumeration of clonal plasma cells in patients with a suspected PCD or lymphoma with plasmacytic differentiation. The DMPCDT showed a sensitivity of 95% and a specificity of 86% for detecting clonal plasma cells, which translated into a positive predictive value of 95% and a negative predictive value of 86%. Local implementation of the DMPCDT could result in a potential time saving of 38% as well as a significant reduction in costs from R559.03 to R135.61 (76%) per specimen.

An important limitation of the DMPCDT is the utility of CD19 and CD56 as the only markers of clonality. Loss or dim expression of CD19 is reportedly seen in 96% of clonal plasma cells.[5] CD56 expression is another common aberrancy reported in multiple myeloma. Both the CD19 and CD56 expression in this study correlated with that reported in literature.[5,13] Small numbers of normal/reactive bone marrow plasma cells have however, been reported to show variable expression of CD56. Malignant plasma cells in some aggressive PCDs, such as plasma cell leukaemia, can also be negative for CD56. CD56 and is therefore not always a true or specific indicator of clonality.[5] While the demonstration of cytoplasmic light chain restriction is admittedly a more specific method of confirming clonality, it requires the permeabilisation of cells (necessitating additional reagents and processing steps) and can be difficult to interpret when few abnormal plasma cells are present.[5] Detection of cytoplasmic kappa and lambda light chains would have necessitated inclusion of an additional tube and as such, the DMPCDT relied solely on the demonstration of aberrant loss of CD19 or gain of CD56 as markers of clonality to avoid this additional expense. In practice however, immunohisto- chemical analysis of light chain restriction would also be performed on the bone marrow trephine biopsy, which could clarify those cases of uncertain clonality.

A limitation of the DMPCDT is the utility of CD19 and CD56 as the only markers of clonality

Resource-constrained laboratories often rely on the referral of flow cytometry samples to distant tertiary and academic laboratories for analysis. The delayed time to processing leads to an inevitable and often significant reduction in viable cells, which reduces the sensitivity of this expensive investigation and often results in specimen rejection. The novel DMPCDT used in this study offers a valid and cost-effective alternative in which referring laboratories themselves can screen patients for plasma cell dyscrasias, such as multiple myeloma, using a single immunophenotyping tube and a basic five-colour flow cytometer and limited technical expertise. The flow cytometry data obtained could then be referred electronically to an academic or tertiary laboratory with the necessary skill required for analysis and interpretation.

DuraClone™ customised tubes have, as of yet, not been validated for diagnostic use by the manufacturer, which restricts its current implementation. Once validated however, a formal verification of the DMPCDT can be recommended. Broader application of these DuraClone™ dried monoclonal antibodies in the immunophenotyping of PCDs as well as other malignancies deserves consideration. As seen in this study, they have shown comparable, and in some instances, superior performance to the conventional liquid monoclonal reagents used in this study, with an additional potential cost- and time-saving benefit.

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