Cytometry
PART A
Journal of the
International Society for
Advancement of Cytometry

A Simple Flow Cytometry-Based Barcode for Routine Authentication of Multiple Myeloma and Mantle Cell Lymphoma Cell Lines

To the Editor:

Cell lines are widely used in laboratories for in vitro experiments, especially for investigating abnormal hallmarks in cancer cells and identifying therapeutic targets. Human cell lines are typically derived in academic laboratories from a wide range of cancer samples. To achieve a representation of intra-cancer heterogeneity, several laboratories, including ours, have established cell line collections. However, the establishment and maintenance of such collections significantly increase the risk of cross-contaminations and misidentification of cell lines, leading to the publication of false data/interpretation (1). In addition to the risk of cross-contamination, widely used cell lines can be described in contrasting manners for a particular feature (e.g., the JJN3 myeloma cell line appears either *TP53* ^{wt} or *TP53*^{KO}, depending on the article), suggesting that cell lines may have been misidentified. ICLAC, the international cell line authentication committee, recommends cell lines authentication using single tandem repeat (STR) profiles that are usually performed by suppliers (2). Nevertheless, while cell lines are frozen and thawed at least four times per year, STR profile is assessed upon receipt of cell lines but not for routine assessment of cell lines identity. Because cross-contaminations might happen, a rapid and low-cost method for re-identification after each thawing of cells is required, as it is for mycoplasma detection.

In this letter, we describe a simple and low cost method involving human leukocyte antigen (HLA) typing and phenotyping that could be used for routine re-authentication using flow cytometry. HLA typing is an international worldwide nomenclature dedicated to blood transfusion and organ transplant that identifies the HLA alleles carried by an individual (3,4). Initially performed for research projects in immunology, HLA Class I typing appears to be very useful for identification of cell lines (5,6). The genomic typing is performed at the generic (e.g., HLA-A*02) or specific (e.g., HLA-A*02:01) level with the generic typing usually being sufficient to identify cell lines within a dedicated collection. This genomic identification is particularly useful upon the inclusion of new cell lines within a collection and also for the establishment of derivatives such as drug-resistant cell lines, which could indicate the emergence of cryptic contaminating resistant cells within the parental cell lines. Our laboratory currently uses a large number of both human multiple myeloma cell lines (HMCLs, $n = 17$) and mantle cell lymphoma cell lines (MCLCLs, $n = 8$) that we have collected from ATCC, DSMZ, or from academic laboratories (7–9). Multiple myeloma (MM) and mantle cell lymphoma (MCL) are plasma cell and B cell malignancies, respectively. Independently of the global characterization of cell lines (karyotype, gene expression profile, characterization of *TP53*, and *RAS* mutations), the HLA Class I typing shown in Supporting Information Table S1 confirms that the cell lines examined were derived from independent individuals (7). To routinely check their identity using flow cytometry, we generated an algorithm that is based on HLA-A*02 expression and on the mutually exclusive expression of the kappa or lambda light chain of immunoglobulin. We used HLA-A*02 expression because HLA-A*02 is the most frequent allele in the population and because a specific mAb is commercially available. Expression of HLA-A*02, either positive or negative, segregated cell lines into two groups: kappa or lambda expression then segregated the cell lines into 2–3 subgroups (Tables 1 and 2). A minimum of markers with a global, stable, and selective expression (absent/present or low/bright expression) was then defined within each subgroup. As shown in Table 1

Grant sponsor: Actions Cancer 44.

Additional Supporting Information may be found in the online version of this article.

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Published online 16 February 2015 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22643

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Table 1. Algorithm for HMCLs.

HMCL	HLA-A*02	c-KAPPA	c-LAMBDA	CD81	CD45	CD137	FGFR3	CD117	CD33	CD9	CD27	CD106	TOTAL
KARPAS620	+++	++	−	++	−	−	−	+	−	++	−	−	2
JIM3	+++	−	++	−	−	−	−	−	−	−	−	−	3
U266	+++	−	++	+	+	−	−	−	−	−	−	−	3
KMM1	++	−	++	++	−	+	−	−	−	−	−	−	3
L363	+++	−	++	++	−	−	−	−	−	−	−	−	4
KMS11	−	++	−	−	−	−	+++	−	−	+++	−	−	3
NCIH929	−	++	−	+	−	−	+	−	−	+[a]	−	−	3
AMO1	−	++	−	++	++	−	−	−	−	−	−	−	3
JJN3	−	++	−	+++	−	−	−	+[a]	−	+++[a]	−	−	3
SKMM2	−	++	−	+++	−	−	−	−	++[a]	−	−	−	3
OPM2	−	−	++	+++	−	−	+++	−	−	−	−	−	3
LP1	−	−	++	−	−	−	+	−	−	−	−	−	3
RPMI8226	−	−	++	++	−	−	−	−	++	++	−	−	3
MM1S	−	−	++	−	−	−	−	−	−	+++	−	−	3
ANBL6	−	−	+	−	−	−	−	−	−	+[a]	−	−	5
KMS12BM	−	−	−[b]	+	−	−	−	−	−	+++	++	−	4
KMS12PE	−	−	−[b]	+++	−	−	−	−	−	−	−	+	4

[a]Expressed by a subpopulation.

[b]KMS12BM and KMS12PE are non-secreting cell lines derived from the same patient and express no detectable level of lambda protein (both are weakly *lambda* positive at the mRNA level).

"Total" indicates the minimum number of markers required for cell line identification (the required markers are indicated by gray shaded areas). Expression was determined using flow cytometry. The monoclonal antibodies (mAbs) used were PE-conjugated, except for CD117 and FGFR3 mAbs (APC conjugated) and for CD45 mAb (FITC conjugated). The level of expression was defined by calculating the ratio of fluorescence (specific staining over matched-conjugated isotype staining).

Ratio <2: −.
$2<$ ratio <10: +.
$10<$ ratio <50: ++.
Ratio>50: +++.

and Figure 1A, the HLA-A*02-cytoplasmic kappa/lambda algorithm segregates HMCLs into five groups of 1–7 cell lines. To further identify HMCLs within each group, we looked for surface markers differentially expressed across cell lines. We used gene expression profile to select molecules either acquired or lost by malignant plasma cells and thus heterogeneously expressed by myeloma cells across both patients and cell lines (7,10,11). We found nine markers (CD9, CD27, CD33, CD45, CD81, CD106, CD117, CD137, FGFR3) that were usually absent or present in an entire HMCL population and efficient in segregating cell lines within the groups (Fig. 1A). Of note, some of them (CD27, CD33, CD45, CD117, or FGFR3) are well-known myeloma-related deregulated markers. The use of clonally related markers, such as HLA-A*02 and kappa/lambda, may also help to identify cross-contaminations that may occur after initial genomic authentication. As shown in Table 2 and Figure 1B, the HLA-A*02-surface kappa/lambda algorithm in association with the differential expression of CD28, CD40, or CD5 discriminates the eight MCLCLs (the MAVER-1 and MINO cell lines are discriminated using the differential levels in lambda and CD5 expression). Thus, HMCLs and MCLCLs are identified using at least two (e.g., KARPAS620 or JEKO-1) and at most five markers (ANBL-6).

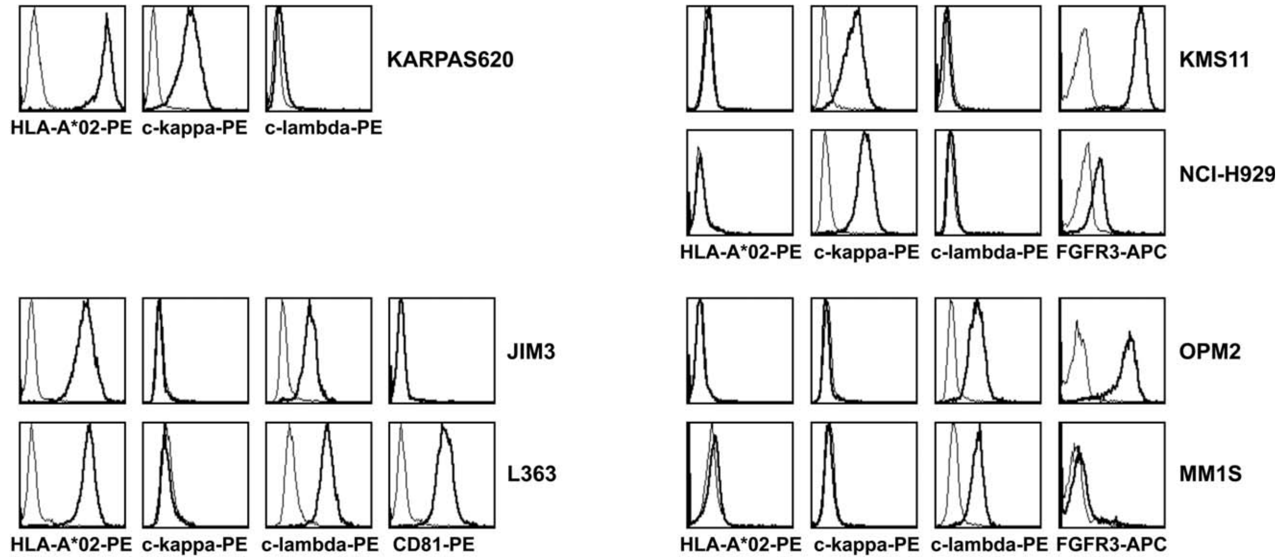
Our authentication procedure of cell lines can be performed in one half-day and does not require DNA. Moreover, flow cytometry is powerful for the detection of very low cross-contamination, which might increase during culture time and repetitive freezing and thawing of cells. This algorithm can also be used for STR-certified cell lines for which HLA Class I

Table 2. Algorithm for MCLCLs

MCLCL	HLA-A*02	KAPPA	LAMBDA	CD28	CD40	CD5	TOTAL
JEKO-1	++	++	−	−	+	+	2
GRANTA-519	+++	−	++	+	+	−	3
JVM2	++	−	+	−	+	−	3
REC-1	−	++	−	−	+	−	3
UPN-1	−	+++	−	−	−	−	3
Z138	−	−	+++	−	+	−	3
MAVER-1	−	−	+++	−	+	+	3
MINO	−	−	++	−	+	++	3

"Total" indicates the minimum number of markers required for cell line identification (the required markers are indicated by gray shaded areas). Expression was determined using flow cytometry. The monoclonal antibodies (mAbs) used were PE-conjugated, except for CD40 mAb (FITC conjugated). The level of expression was defined by calculating the ratio of fluorescence (specific staining over matched-conjugated isotype staining).

Ratio <2: −.
$2<$ ratio <10: +.
$10<$ ratio <50: ++.
Ratio>50: +++.



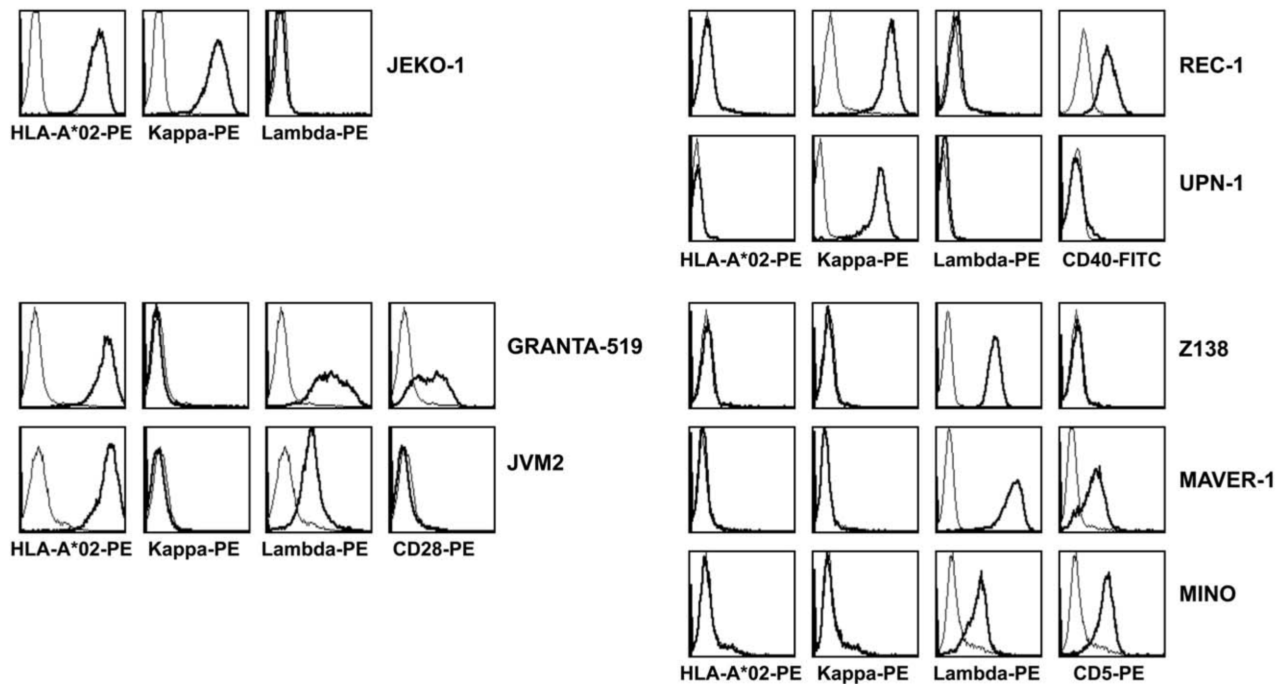


Figure 1. Histograms represent the overlay of specific staining (thick line) over control staining (thin line) in HMCLs (**A**) or MCLCLs (**B**). The monoclonal antibodies (mAbs) used were purchased from Beckman Coulter or Becton Dickinson: they were PE-conjugated, except for CD117 and FGFR3 mAbs (APC conjugated) and for CD40 and CD45 mAbs (FITC conjugated). Cytoplasmic kappa (c-kappa) and c-lambda staining was performed after the permeabilization of cells using the Intraprep Permeabilization Reagent Kit (Beckman Coulter). A single color staining was performed for all markers. Fluorescence acquisition (20,000 events were acquired) and analysis were performed using FACsCalibur (Becton Dickinson) and Cell Quest software (PT Cytocell, SFR Bonamy, Nantes, France).

typing is unknown because flow cytometry directly assesses HLA-A*02 expression. This HLA-A*02-based algorithm is applicable not only to other types of B-cell malignancies but also to other types of cell line collections if both type-specific (such as kappa/lambda for B cells) and selective markers are provided.

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