dual light-chain expression. If that is the case, "blocking" the Fc binding sites with excess non-fluorescent-labeled IgG antibodies will reduce or eliminate the nonspecific antibody interactions.

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(收稿日期: 2011-06-10)

·综　　述·

Principle of panel design, antibody selection, and fluorochrome choice for immunophenotyping of acute leukemia

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关键词: immunophenotyping; acute leukemia; panel setup

DOI: 10.3969/j.issn.1673-4130.2011.15.019 **文献标识码**: A **文章编号**: 1673-4130(2011)15-1698-03

After working in the field of clinical flow cytometry for many years, I still continue wondering at its ever changing and evolving nature. Like everything else, clinical flow cytometry comes a long way, and I am sure will keep changing, improving, and evolving. Looking back, we see many changes made: new immuno-markers discovered, manufactured, and applied to clinical practice; new instruments developed and put into market; new recommendations proposed; and test methods standardized and improved. Along the way, the flow cytometry has gained undeniable popularity and has become a standard practice in diagnosing diseases, guiding and monitoring treatment in hematopoietic neoplasia such as leukemia and lymphoma.

1 Panel selection

Focusing on the topic of leukemia panel design, antibody selection and fluorochrome choice, I have reviewed a few well known published papers[1-4], and also the leukemia panels from our own flow lab, to get a better understanding of the rationality in why there is variation among clinical labs in panel design. Up to this point, there is not yet a standard panel for acute leukemia diagnosis regardless of many attempts of recommendations and proposals by international and national experts in the field. The reason for this variation I believe is because different labs operate under different circumstances, which lead them to take different approaches to reach the same goal of providing an accurate diagnosis. In general, there are three types of approaches in current practice: comprehensive panel, minimal screen panel, and directly disease oriented panel. Each has its pros and cons and has its rationality.

Comprehensive panel consists of large panel of reagents to include all cell lines and used in the diagnosis of both leukemia and lymphoma. It tends to be used in reference labs which usually do not have sufficient accessibility to patient information and morphology of the samples. This approach provides extensive immunophenotyping information, saves time to almost e-

liminate any add on tests, and produces a faster turnaround time. However it is expensive and costly, especially under the current challenging economical situation. Meanwhile some information derived from this approach may be wasteful in the sense of irrelevant to clinical needs and indications.

Minimal screen panel, on the other hand, consists of relatively small number of reagents, includes only the core reagents and often needs add on tests for further diagnosis. This approach appears to save money, but with additional time and reagents, the final cost is not a lot less expensive. In fact only small percentage of labs in the survey indicate they follow this approach[1-2].

The third approach is the directed or targeted approach. It is based on the clinical indications, including patient medical history, morphology of the sample, and other relevant information from referring clinicians. In this case the selected reagents are used to confirm or classify a disease already suspected or diagnosed. Many hospitals-based clinical flow labs take this approach due to its readily availability of patient information and other clinical correlation tests, easy access and communication with referring physicians, easy access of morphology of samples. With this direct approach, relatively fewer reagents are used therefore lower cost. Our own clinical lab takes this approach because we can satisfy all of above conditions. Our six-color leukemia panel (Table 1) includes the core lineage markers and other supplementary, differentiation markers which we consider as necessary for the diagnosis of acute leukemia.

Table 1　Leukemia panel design

Tube No.	Flurochrome						Purpose
	FITC	PE	PER-CP cy5.5	PE cy7	APC	APC-H7	
Tube 1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	Isotype control
Tube 2	CD7	CD5	CD3	CD4	CD8	CD45	T cell lineage, maturation stage
Tube 3	CD15	CD117	CD19	CD10	CD20	CD45	B cell lineage, maturation stage
Tube 4	CD14	CD33	HLA-DR	CD13	CD34	CD45	Myeloid differentiation
Tube 5	CD71	CD56	CD64	CD38	CD11b	CD45	Others

In my opinion a panel should be constructed based on the clinical needs, especially when cost is of concern. Our panel puts more emphasis on acute myeloid leukemia (AML) since our patient base (over 90% of all the leukemia) is mainly adult leukemia patients. By not including relatively irrelevant markers to AML diagnosis such as TDT, cytoplasmic CD3, surface immunoglobulin, we are cutting the cost of technologist's time and the cost of reagents. Meanwhile, it will not fail to flag acute lymphoid leukemia (ALL) or biophenotipic leukemia. Whenever there is indication of acute B or T lymphocytic leukemia, it will be reflected by current panel, additional markers such as surface immunoglobulin and TDT, cytoplasmic CD3, CD1a can be added accordingly. If there is indication of megakaryocytic leukemia, CD41, CD42b, CD61 will be performed. Furthermore, if there is indication of biphenotipic or mixed lineage population, additional marker such as myeloperoxidase and cytoplasmic CD79a, cytoplamic CD22 will be added to confirm the lineage and maturation stage.

In summary, so far there is no standard panel for every lab to follow, but the general guideline as what and how many of markers should be included do exist and remain the same. It is a general agreement that the number of reagents included in an acute leukemia panel is about 20 to 24[1-3], which sufficiently allows for the recognition of all the major types (if not all types) of abnormal population and its normal counterparts as an internal control. However it is still up to the individual laboratory to make decision as to what and how much antibody to use in their panels. Personally I agree with the guidelines proposed by 2006 Bethesda International consensus and US-Canadian Consensus that the panel should be broad enough to not miss any diagnosis and sometime not waste any antibody.

2　Panel constructions

The construction of a successful leukemia panel is a complex process and depends upon many factors:

2.1　The availability of instrument, monoclonal antibody and corresponding flurochrome conjugates.

2.2　The knowledge and experience of the personnel responsible to construct such panel. Their technical knowledge about the instrument, the flurochromes and related CD markers are critical to properly set up the test. Their professional knowledge of cell development and maturation is the key to guild the panel construction. It is well known that an improper instrument setting will lead to false negative and false positive results. In this regard, there are a few basic principles that must be well understood and followed when one constructs a panel.

2.2.1　Knowing the stain index of CD marker, knowing the characteristic performance of each fluorochorme channel, and matching the brightest flurochrome with the dimmest possible antigen expression　It is always good to become familiar with which CD marker has dim expression. It is now well documented that CD7, CD10, CD11B, CD13, CD23, CD33, CD117. CD64 should be tagged with most bright flurochromes phycoerythrin (PE) or allophycocyanin(APC)[4].

2.2.2　Always keeping signals in linear range[5]　Since linearity is important in fluorescence compensation and quantitative measurements. It is known that the compensation of data in the

last decade involves subtraction of large numbers and a non-linear error can cause a large absolute error in the result. The rule to follow is: run brightest stained sample for each channel and If the population is off scale, lower PMT voltage to bring signal in range.

2.2.3 Minimize spillover from bright population into the detector requiring high sensitivity for those cell populations[5-6] It is not hard to understand that an increased optical background from other fluorochrome (spillover) will decrease the resolution of the signal, therefore causing possible population loss. Knowing the performance character of each florescence channel, knowing each CD marker's stain index, one can balance and match the color with CD marker accordingly. One strategy is to spread or move marker to other channel which is excited by other laser. Example is to move CD marker in the PE channel to APC channel to minimize the large amount of spillover from Fitc when the antigen in PE channel requiring high sensitivity and the signal from Fitc is very strong.

2.2.4 Using tandem dye with consideration of their technical limitation Knowing tandem dye such as APC cy7 and PE cy7 can degrade with exposure to light, temperature and fixation, it is recommended to avoid espousing to those conditions when preparing samples.

2.3 To include a common marker such as CD45 in each tube to follow the population of the interesting. The CD45 versus Side scatter has been proven to be a very powerful tool to separate and gate different hematopoietic populations; furthermore, to use linear specific marker such as CD19 or CD3 as gating markers, combining maturational marker such as CD 20, CD10 for B cell and CD4 and CD8 for T cell, to further characterize the cell and cell subset.

2.4 Controls for validating your panel. There are two types of controls that are recommended: FMO control (fluorescence minus one) and fidelity control. FMO control combines all the reagents in the tube except the one of interest. This control serves as a gate to delineate the positive and negative for that particular reagent. Fidelity control is using the given antibody by itself, and comparing the result to the use of that antibody in the complete cocktail tube. By doing so, you can see the effect of other reagents on that particular reagent. Once the reagent panel has been well validated, it is not necessary to run those control on a daily basis[7].

In summary, there is no standard acute leukemia panel for every lab to follow at this point, but there are general guidelines of what and how many markers to include. As for how to construct a multicolor reagent panel there are also some critical rules to consider and to follow. In general, it is a complex process and requires broad spectrum of knowledge of the instrument, fluorochrome, CD markers and a good understanding of cell maturation and differentiation. With many trials and errors, it is possible to build a sound, multicolor panel.

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(收稿日期: 2011-06-10)

新 书 介 绍

中国人民解放军成都军区总医院检验科吴丽娟教授主编，美国许东升教授、Betty Li 教授审校的《临床流式细胞学检验技术》一书于 2010 年 8 月由人民军医出版社正式出版发行。该书共 15 章 58 节，50 万字，内容涉及流式检验的相关理论、仪器性能特点、常见影响因素、标本的采集/储存与运输、51 个具体流式检验项目的基本原理、主要试剂、检验步骤、仪器参数、注意事项、参考范围、临床意义和质量控制措施等。作为一本按照检验项目的标准化操作程序(SOP)编写的流式检验技术指导性书籍，特别适合检验医师、临床医师日常工作参考，以及生物医学类研究生、检验专业本科生及教师参考。