Flow cytometric immunophenotyping has become an important method in the diagnosis of haematological malignancies, as it facilitates accurate diagnosis in both leukaemias and lymphoid proliferative disorders. Additionally, the techniques may also provide information relevant for prognosis and therapy. In recent years, the many advances made in instrumentation, software analysis tools, and in the availability of a multitude of new fluorochromes have facilitated the jump from single-colour immunophenotyping to multi-colour or multi-parametric immunophenotyping [Figure 1].

The obvious advantage of multi-parametric immunophenotyping is the simultaneous detection of multiple markers on distinct cell populations, resulting in a more rapid and objective detection of aberrant antigen co-expression as well as the ability to analyse the heterogeneity and clonality of the malignant cells. For the adventurous, up to 17-parameter immunophenotyping has been reported [1], but it should be remembered that for each new parameter added, the analysis step gets correspondingly more complicated and time consuming. Fewer colours are needed for routine clinical use.

The use of multiple antibodies per tube requires fewer tubes and thereby less patient material. This is important in some cases where the number of cells available for analysis is limited, e.g., with very ill or lymphopaenic patients, or with paediatric and foetal samples. In addition, the need to prepare fewer tubes means that hands-on time is minimised and the cost of sample preparation is reduced.

Assessing the lineage of the neoplastic cells is the first step in the diagnosis of chronic or acute leukaemia. A few critical markers are sufficient to establish lineage in the majority of cases. However, more extensive immunophenotyping can provide clinical information about the differentiation stage of the neoplastic cells and help assess prognostic factors, thus allowing patients to be stratified into useful therapeutic groups.

A comprehensive understanding of the normal expression pattern of an antigen and its relationship to the expression of other relevant antigens is necessary to perform high-quality immunophenotyping. The presence or absence of antigens and the intensity of some of the markers are also important factors for correct identification of cell lineages as well as for the subsequent subtyping. Hence, selecting the right combination of antibodies for the multi-parameter analysis is essential.

Many laboratories involved in immunophenotyping have recognised the challenge of establishing the right antibody combination panel. Extensive guidelines have been proposed by several national and international groups for this purpose [2-6], but no consensus has been reached and thus the task of selecting panels is an arduous one especially for newcomers to the field.

**Ready-to-use panels for the immunophenotyping of leukemia and lymphoma**

To ease the work of laboratories engaged in multi-parameter immunophenotyping, MultiMix triple-colour panels have been introduced. These are "ready-to-use" panels designed for leukaemia and lymphoma immunophenotyping. The panels are comprehensive and carefully selected combinations of antibodies and fluorochromes that enable identification and characterisation of haematological malignancies with a limited number of antibodies.

**Antibody selection**

The antibodies and clones used in the panels are those recognising the most relevant markers for lineage assessment and sub-classification of leukaemia and lymphoma, as recommended by national and international guidelines.

**Fluorochrome selection**

The fluorochrome combinations used in the panels are: fluorescein isothiocyanate (FITC), R-phycocerythrin (RPE or PE), and allophycocyanin (APC). These fluorochromes have been selected for maximum resolution, stability, and ease of use [Table 1]. The combination specifically takes advantage of the availability of two lasers in modern flow cytometers [Figure 2], one in the blue...
range and one in the red range, so that the spectral overlap is limited to two of the three fluorochromes: FITC and RPE. This aspect of the panels makes compensation as easy as for the conventional FITC and RPE two-colour reagents.

Reliable results
The panels have been carefully designed to identify and characterise unique cell subsets with high accuracy using a limited amount of antibodies. The new system also simplifies the procedure by using only 3 different fluorochromes. The antibody-fluorochrome combinations have been carefully selected according to the level of expression of the antigens and the level of intensity of the fluorochromes. In general, labelling with APC or RPE, which are both strong fluorochromes, is used for antibodies with low affinity or antibodies that recognise epitopes with low expression to ensure a fluorescent signal strong enough to identify positive cells. Likewise, in general, labelling with FITC, which is the weakest of the fluorochromes, is used for antibodies that recognise epitopes with high expression. As the reagents are premixed to optimal concentration and produced according to GMP rules, lot-to-lot variation is minimised.

Initial screening panel and sub panels
The panels consist of 2 lines [Figure 3]. The first line, the initial screening panel, allows screening for the presence of normal haematopoietic cells and malignant cell populations. The second line is for the detailed classification of cell types. Based on the result of the screening panel and subsequent use of the relevant panels of differentiation markers, it is now possible to classify most leukaemias and lymphomas into subgroups.

Interpretation of results
Even though the panels are premixed and easy to use, a comprehensive understanding of flow cytometry and extensive knowledge of the antigen markers are necessary to perform high-quality immunophenotyping. The interpretation of results must be made within the context of the patient’s clinical history and other diagnostic tests performed on samples obtained from the patient.

References

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Figure 3. The MultiMix triple-colour panels for immunophenotyping of leukemia and lymphoma.
* The fluorochromes used in the MultiMix triple colour panels are FITC/RPE/APC.
** Optimised for intracellular staining.
*** Glycophorin A.