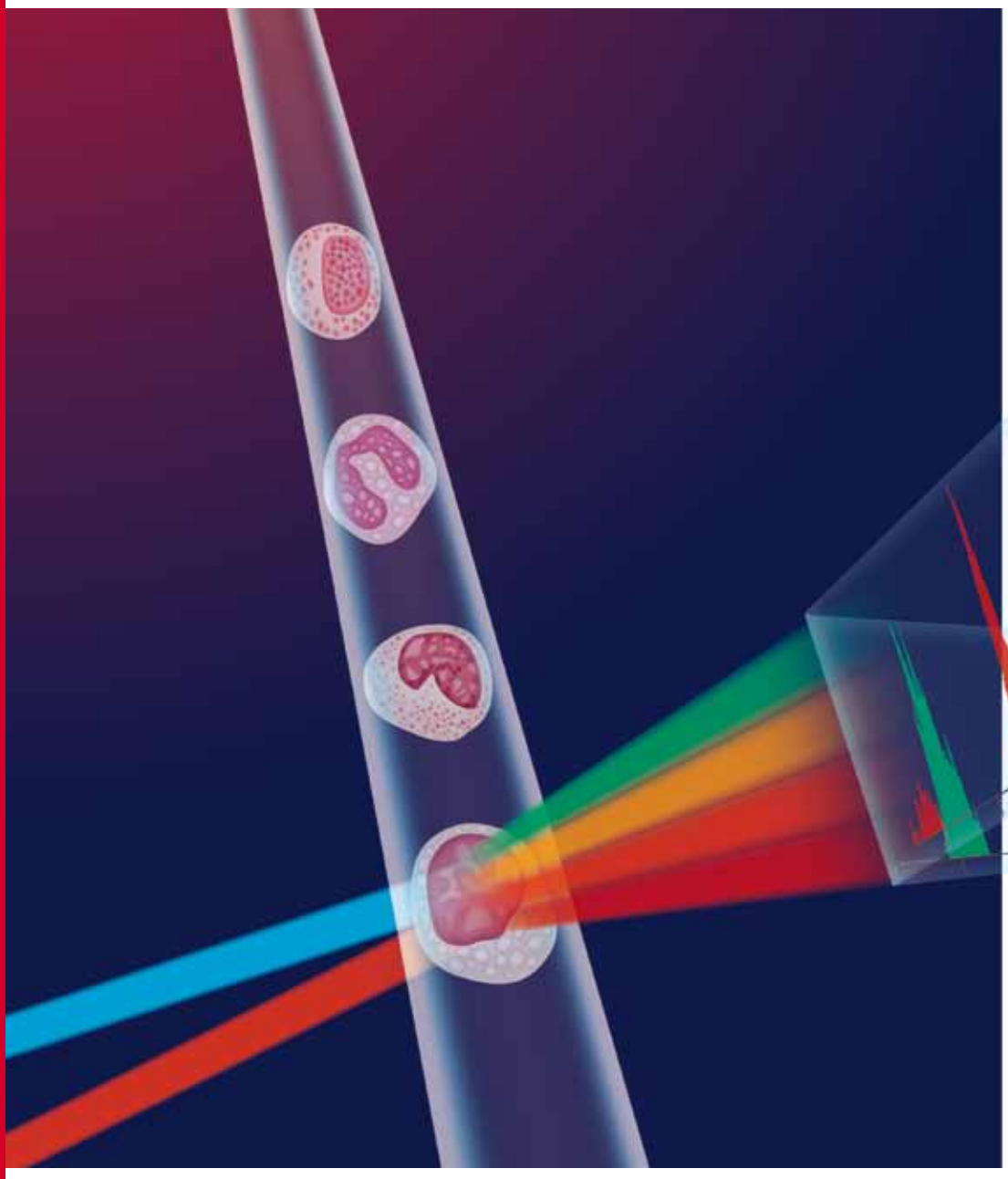
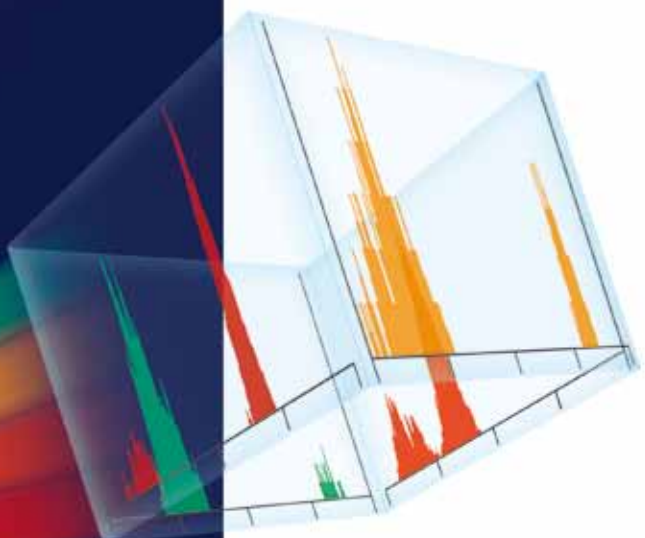
Dako

THE SHAPE OF EXCELLENT
LABORATORY PERFORMANCE

MultiMix™ | Multi Color Panels for Immunophenotyping of Leukemia and Lymphoma for Flow Cytometry



- Enhanced Accuracy
- High Security
- Easy Compensation
- Cost-Effective



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Introduction

This brochure describes the MultiMix™ triple color reagent panels as well as the very useful drop-in reagent CD45/PerCP.

Modern diagnostic characterization of hematologic neoplasms is increasingly dependent on flow cytometric immunophenotyping. Multi-parametric immunophenotyping allows a rapid and objective detection of aberrant antigen co-expression and the analysis of heterogeneity and clonality of malignant cells in leukemias and lymphomas.

The presence or absence of antigens and the intensity of some of the markers are also important for identification of subpopulations of cell lineage. Hence, the right combination of antibodies in the multiparameter analysis is essential.

Many laboratories involved in immunophenotyping have recognized the challenge of building the right antibody combination panel. Thus, many national, as well as international groups have made guidelines for laboratories to use to ease the process.

To facilitate the process of selecting both the right combinations of antibodies and combinations of fluorochromes Dako has tailored “ready-to-use” panels for leukemia and lymphoma immunophenotyping. The panels are carefully designed with the national and international guidelines in mind. Conclusions from the following international and national consensus groups and reference laboratories have been used:

- European Working Group on Clinical Cell Analysis (EWGCCA) (3, 4).
- European Group for the Immunological Characterization of Leukemias (EGIL) (2, 5).
- General Haematology Task Force of the British Committee for Standards in Haematology (BCSH) (6).
- BIOMED-1 Conserted Action ‘Investigation of Minimal Residual Disease in Acute Leukemia: International Standardization and Clinical Evaluation (7).
- U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow cytometry: Selection of Antibody Combinations (8).
- Guidelines of the Dutch Foundation for Immunophenotyping and Hematological Malignancies (SIHON) (9).
- Belgian Consensus Recommendations for Flow Cytometric Immunophenotyping (10).
- Recommendations for Standardization Committee on Clinical Flow of the International Federation of Clinical Chemistry (1).
- Recommendations from laboratories of Jacques M. van Dongen (11), Henk J. Adriaasen (11), Alberto Orfao (12, 13), Raul C. Braylan (12), Michael J. Borowitz (12, 14), Bruce H. Davis (12), Giuseppe Basso (13), Estella Matutes (15), Dario Campana (16), Philip Henon (17).
- 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry (18)

MultiMix™ Triple Color Panels for Immunophenotyping of Leukemia and Lymphoma

The triple color MultiMix leukemia panel is a comprehensive and carefully selected antibody fluorochrome combinations panel that enables identification of hematological malignancies with limited numbers of antibodies.

The panel consists of 2 lines, please see page 5.
The first, the initial evaluation panel, allows evaluation of the presence of normal hematopoietic cells and malignant blast cell populations. The second line is for the detailed classification of cell types. Based on the result of the evaluation panel and additional use of lineage and differentiation markers, it is now possible to classify most leukemias and lymphomas into subgroups. Please notice that interpretation of results must be made within the context of the patient's clinical history and other diagnostic tests by a certified professional.

The antibody and fluorochrome combinations have been designed to gain the best sensitivity of the analysis. The fluorochromes used in the MultiMix™ triple color panels are; Fluorescein isothiocyanate (FITC), R-Phycoerythrin (R-PE) and Allophycocyanin (APC).

Furthermore many laboratories use CD45 in all samples. For this use the Dako CD45/PerCP single reagent (Code PR701) will be useful as drop-in reagent in the samples, which do not contain CD45 already. Drop-in means that the reagent is added to the sample to obtain a cocktail of 4 antibodies with 4 different fluorochromes. Adding CD45 is a useful tool to track cell populations in the different samples (18).

High security on results
To ensure consistent results over time all the fluorochromes are non-tandem fluorochromes. These fluorochromes are very stable, as they do not have the drawback of the tandem fluorochromes which can break down, when exposed to light or other unsuitable conditions.

Easy instrument compensation
When no software tools are available, compensation between three fluorescences is a procedure that for the non-expert user can be problematic.

However for the MultiMix™ triple color panel the only compensation required is between FL1 and FL2 (FITC and RPE). There is no need for compensation on the APC channel. This simplifies compensation and saves a lot of time on the set-up of the instrument.

Fluorochrome	Excitation	Emission	Detection
Fluorescein (FITC)	488 nm argon laser	520	FL-1 channel on most instruments
R-Phycoerythrin (RPE)	488 nm argon laser	575	FL-2 channel on most instruments
Allophycocyanin (APC)	Either by 633 nm He-Ne laser or 635 nm red diode laser	660	Depends on the instrument type

When CD45/PerCP is used as drop-in a compensation step more between PerCP/APC and PerCP/RPE is needed.

Fluorochrome	Excitation	Emission	Detection
Peridinin Chlorophyll-Protein (PerCP)	488 nm argon laser	680	FL-3 channel on most instruments

Enhanced identification and characterization accuracy

The MultiMix™ panels provide a large amount of unique information for each sample studied. The combination of the panels and even the antibody and fluorochrome combinations are deliberately designed to identify and characterize unique cell subsets, even with a high accuracy of rare cell populations.

This is done by matching the antibody and fluorochrome according to the antigen's level of expression and the fluorochromes level of brightness. For antibodies recognizing a marker with low level expression the bright APC or RPE fluorochromes are used to guarantee a sufficiently high signal.

Cost-effective and time-saving reagents

The triple color reagent is premixed and ready to use and there is no need for extra examination and titration between different lots.

References

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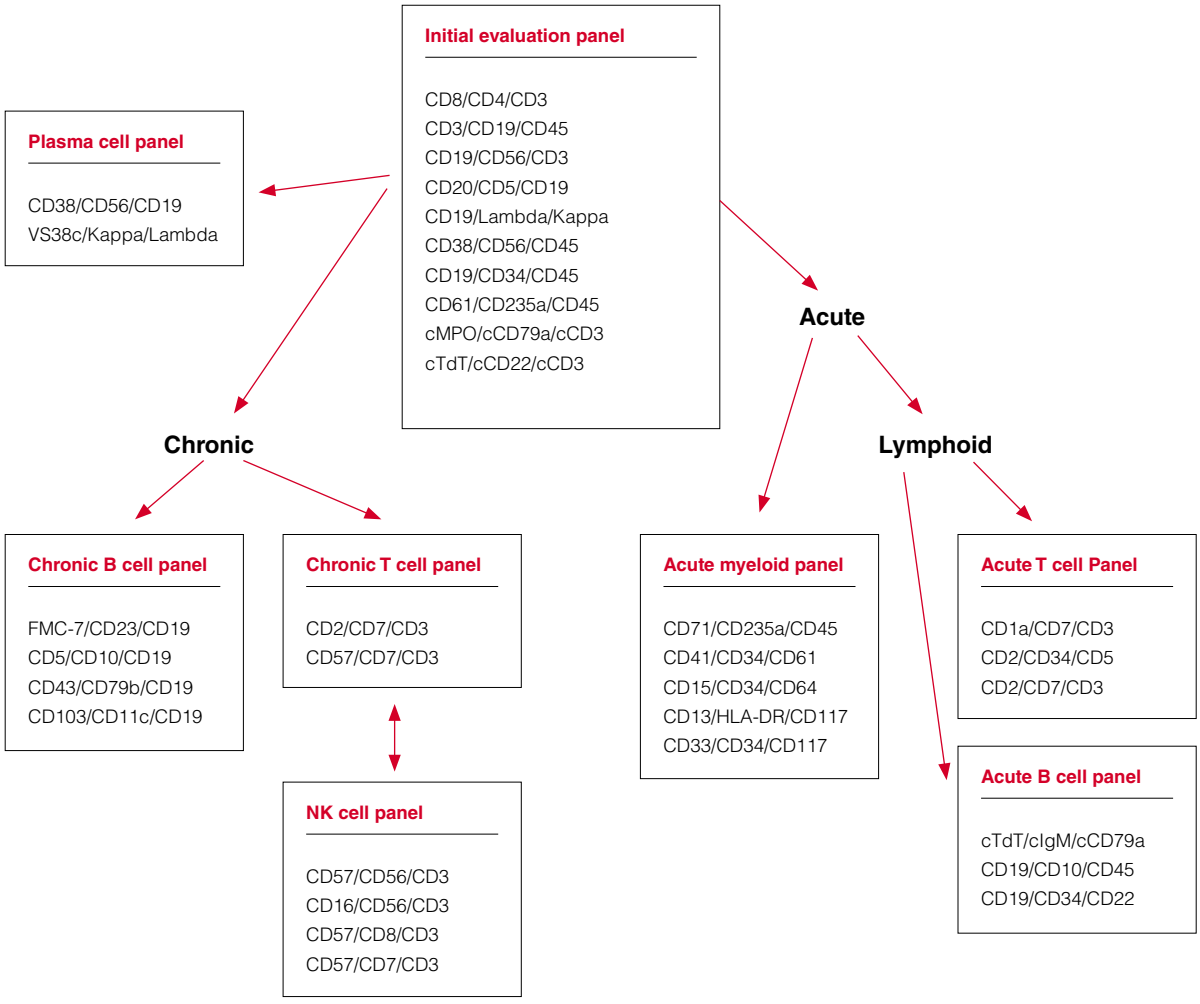
17. *Jung G, Eisenmann J-C, Thiébault S, Hénon P. Cell surface CD43 determination improves diagnostic precision in late B-cell diseases. Br J Haematol 2003;120:496-9.*
18. *Brent L. Wood, Maria Arroz, David Barnett, Joseph DiGiuseppe, Bruce Greig, Steven J. Kussick, Teri Oldaker, Mark Shenkin, Elizabeth Stone, and Paul Wallace. International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry, 2006 Bethesda: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia Cytometry Part B. Clinical Cytometry 72B 2007; S14–S22.*

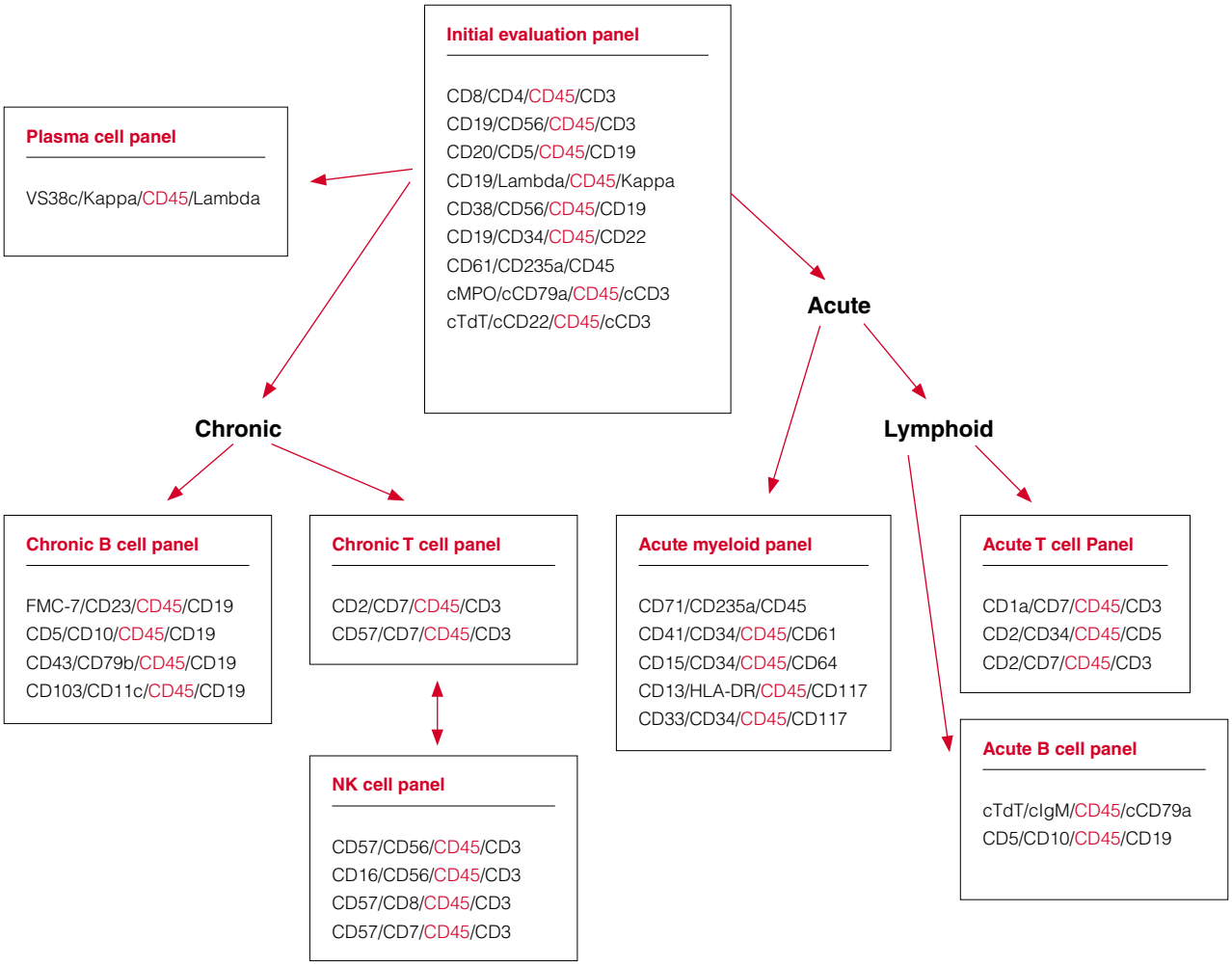
MultiMix™ Triple Color Panels for Immunophenotyping of Leukemia and Lymphoma



CD45/PerCP Code PR701 is recommended as a drop-in reagent, which means that CD45/PerCP reagent is added in all vials, which does not already contain CD45. Please see page 6 for an illustration of four color panels by applying CD45/PerCP as drop-in reagent.

MultiMix™ Color Panels for Immunophenotyping of Leukemia and Lymphoma with CD45/PerCP applied as drop-in reagent

Please notice the reduction in numbers of samples



MultiMix™ Triple Color Panels

Initial evaluation panel
Reagent tubes of this panel are designed to evaluate the unknown patient sample for the following:

- The presence of non-hematopoietic cells
- The presence of normal hematopoietic cells
  – The presence and level of leucocytes (B and T lymphocytes, NK cells, granulocytes, monocytes and platelets)
  – The presence and level of erythrocytes
  – Detection of leukocyte-platelet aggregates
- Discrimination between neoplastic cells and cells arising from reactive conditions or autoimmune disorders
- Identification of malignant blast cell populations
- Identification of B cell clonality (surface kappa or lambda light chain immunoglobulin expression)
- Differentiation between chronic lymphoproliferative disorders and acute leukemias
- First line identification of hematological malignancies based on cell lineage, maturation stage and presence of aberrant phenotypes:
  – Chronic B-cell lymphoproliferative disorders
  – Chronic T-cell lymphoproliferative disorders
  – NK cell disorders
  – B-cell acute lymphoblastic leukemias (B-ALLs)
  – T-Cell acute lymphoblastic leukemias (T-ALLs)
  – Acute myeloid leukemias (AMLs)
  – Multiple myeloma

Plasma cell panel
The panel is designed for immunophenotyping of multiple myeloma and other plasma cell dyscracias. It should be used with antibody combinations from the initial evaluation panel. Multiple myeloma cells are characterized by monoclonality (intracellular expression of kappa or lambda light chains), high expression of CD38, expression of CD56, intracellular expression of VS38c but normally no expression of CD45 and often no expression of CD19.

Chronic B cell panel

This panel is designed to discriminate between various chronic B-cell lymphoproliferative disorders: B-cell chronic lymphocytic leukemia (B-CLL), B-cell prolymphocytic leukemia (B-PLL), hairy cell leukemia (HCL), mantle zone lymphoma (MZL), follicular lymphoma (FL) and splenous marginal zone lymphoma with villous lymphocytes (SLVL). It should be used with antibody combinations from the initial evaluation panel. Typical findings in various chronic B-cell lymphoproliferative disorders are shown in table 1. Findings vary from patient to patient.

Disorders	Characteristic marker expression
B-CLL	sIg dim+, CD5+, CD23+, CD20 dim+, CD43+ but FMC7- and CD79β dim+/-
B-PLL	sIg bright+, FMC7+, CD5-/+, CD23-
HCL	CD103+, CD11c bright+, CD5- and CD25+
MZL	CD5+, CD23-, FMC7+, CD20 bright+, CD19 dim+ and sIg bright+
FL	Cells are usually CD5- but show frequent expression of CD10
SLVL	In contrast to B-CLL, B-PLL and HCL, cells do usually not express CD5 or CD103

Table 1

Chronic T cell panel

The panel is designed to discriminate between various chronic T-cell lymphoproliferative disorders: T-cell chronic lymphocytic leukemia (T-CLL), T-cell prolymphocytic leukemia (T-PLL), adult T-cell leukemia lymphoma (ATLL), cutaneous T-cell leukemia lymphoma (CTLL) and T-cell large granular lymphocyte leukemia (T-LGL). It should be used with antibody combinations from the initial evaluation panel. Typical findings in various chronic T-cell lymphoproliferative disorders are shown in table 2. These findings vary from patient to patient.

Disorders	Characteristic marker expression
T-CLL/T-PLL	CD2+, CD3+, CD4+, CD5+, CD8-, CD7 bright+ and CD57-
ATLL	CD2+, CD3+, CD4+, CD5+, CD8-, CD7 dim+ and CD57-
CTLL	CD2+, CD3+, CD4+, CD5+, CD8-, CD7+ and CD57-
T-LGL	CD2+, CD3+, CD4 dim+/-, CD5+, CD8+, CD7+ and CD57+

Table 2

NK cell panel

This panel is designed to discriminate between NK cell-LGL (NK-LGL) and blastic NK-cell leukemia/lymphoma (NK-LL). It should be used with antibody combinations from the chronic T cell panel and initial evaluation panel. Typical findings in various NK-cell lymphoproliferative disorders are shown in table 3. These findings vary from patient to patient.

Disorders	Characteristic marker expression
NK-LGL	CD2+, CD3-, CD4-, CD8-, CD16+, CD56+ and CD57+
NK-LL	CD4+, CD43+, CD56+

Table 3

Acute myeloid panel

The panel is designed to discriminate between the different subtypes of acute myeloid leukemia (AML): AML M0 (myeloblastic without cytologic maturation), AML M1 (myeloblastic with minimal maturation), AML M2 (myeloblastic with significant maturation), AML M3 (acute promyeloblastic leukemia), AML M4 (acute myelomonocytic leukemia, AML M5 (acute monoblastic leukemia), AML M6 (acute erythroid leukemia) and AML M7 (acute megakaryoblastic leukemia). It should be used with antibody combinations from the initial evaluation panel. Typical findings in various acute myeloproliferative disorders are shown in table 4. These findings vary from patient to patient.

Disorders	Characteristic marker expression
AML M0/M1/M2	CD13+, CD33+, CD34+, CD117+ and TdT+.
AML M1 and M2	MPO+
AML M0	MPO -
AML M2	Strong expression CD15
AML M3	CD13+, CD33+, MPO+ TdT(dim+/-), CD15+, CD34+ and HLA-DR+
AML M4 and M5	CD13+, CD33+, CD64+, CD117+, HLA-DR+, MPO+, TdT+ and CD34 dim+
AML M6	CD13+, CD33+, CD71+, CD235a+, HLA-DR+, MPO+, TdT+, CD34+
AML M7	CD13+, CD33+, CD41+, CD61+, CD235a-, HLA-DR-, MPO-, TdT dim+ and CD34-

Table 4

Acute T cell panel

This panel is designed to discriminate between the four types of acute T-cell lymphoblastic leukemia (T-ALL): pro-T-ALL, early-T-ALL, cortical-T-ALL and late-T-ALL. It should be used with antibody combinations from the initial evaluation panel. Typical findings in various acute T-cell lymphoproliferative disorders are shown in table 5. These findings vary from patient to patient.

Disorders	Characteristic marker expression
All four types of T-ALL	CD2+ and CD7+
Pro-T-ALL	CD34+, TdT+ and cytoplasmic CD3+
Early-T-ALL	TdT+, cytoplasmic CD3+, CD5+ and CD34+ (variable expression)
Cortical T-ALL	CD1a+, cytoplasmic CD3+, CD5+, CD8+, CD34+,and TdT (variable expression)
Late-T-ALL	CD5+, CD4+ or CD8+, surface CD3+ and CD34-

Table 5

Acute B cell panel

This panel is designed to discriminate between the four types of acute B-cell lymphoblastic leukemia (B-ALL): pro-B-ALL, common-B-ALL, pre-B-ALL and B-ALL. It should be used with antibody combinations from the initial evaluation panel. Typical findings in various acute B-cell lymphoproliferative disorders are shown in table 6. These findings vary from patient to patient.

Disorders	Characteristic marker expression
All four types of B-ALL	CD19+, CD22+, HLA-DR+ and cytoplasmic CD79α+
Pro-B-ALL	CD34+, TdT+ and CD10-
Pre-B-ALL	CD34+, TdT+, CD10+, CD20+ and cytoplasmic IgM+
B-ALL	CD34-, TdT-, CD10+, CD20+, cytoplasmic IgM- and surface IgM+

Table 6

MultiMix™ product list:

	Product	Package Size	Regulatory Status EU
Code	**Initial evaluation panel**		
TC660	CD8/ CD4 /CD3	1 mL, 50 tests	CE-IVD*
TC690	CD3/CD19/CD45	1 mL, 50 tests	CE-IVD
TC662	CD19/CD56/CD3	1 mL, 50 tests	CE-IVD
TC663	CD20/CD5/CD19	1 mL, 50 tests	CE-IVD
TC669	CD19/Lambda/Kappa	1 mL, 50 tests	CE-IVD
TC671	CD38/CD56/CD45	1 mL, 50 tests	CE-IVD
TC672	CD19/CD34/CD45	1 mL, 50 tests	CE-IVD
TC673	CD61/CD235a/CD45	1 mL, 50 tests	CE-IVD
TC667	cMPO/cCD79a/cCD3	1 mL, 50 tests	CE-IVD
TC668	cTdT/cCD22/cCD3	1 mL, 50 tests	CE-IVD
Code	**Chronic B cell panel**		
TC683	FMC-7/CD23/CD19	1 mL, 50 tests	CE-IVD
TC664	CD5/CD10/CD19	1 mL, 50 tests	CE-IVD
TC684	CD43/CD79b/CD19	1 mL, 50 tests	CE-IVD
TC665	CD103/CD11c/CD19	1 mL, 50 tests	CE-IVD
Code	**Chronic T cell panel**		
TC677	CD2/CD7/CD3	1 mL, 50 tests	CE-IVD
TC678	CD57/CD7/CD3	1 mL, 50 tests	CE-IVD
Code	**NK cell panel**		
TC679	CD57/CD56/CD3	1 mL, 50 tests	CE-IVD
TC661	CD16/CD56/CD3	1 mL, 50 tests	CE-IVD
TC680	CD57/CD8/CD3	1 mL, 50 tests	CE-IVD
TC678	CD57/CD7/CD3	1 mL, 50 tests	CE-IVD
Code	**Plasma cell panel**		
TC674	CD38/CD56/CD19	1 mL, 50 tests	CE-IVD
TC670	VS38c/Lambda/ Kappa	1 mL, 50 tests	CE-IVD
Code	**Acute myeloid panel**		
TC675	CD71/CD235a/CD45	1 mL, 50 tests	CE-IVD
TC687	CD41/CD34/CD61	1 mL, 50 tests	CE-IVD
TC688	CD15/CD34/CD64	1 mL, 50 tests	CE-IVD
TC685	CD13/HLA-DR/CD117	1 mL, 50 tests	CE-IVD
TC686	CD33/CD34/CD117	1 mL, 50 tests	CE-IVD

* Complies with Directive 98/79/EC of the European Parliament and of the Council on *in vitro* diagnostic medical devices.

	Product	Package Size	Regulatory Status EU
Code	**Acute T cell Panel**		
TC681	CD1a/CD7/CD3	1 mL, 50 tests	CE-IVD
TC666	CD2/CD34/CD5	1 mL, 50 tests	CE-IVD
TC677	CD2/CD7/CD3	1 mL, 50 tests	CE-IVD
Code	**Acute B cell panel**		
TC682	cTdT/cIgM/cCD79a	1 mL, 50 tests	CE-IVD
TC676	CD19/CD10/CD45	1 mL, 50 tests	CE-IVD
TC689	CD19/CD34/CD22	1 mL, 50 tests	CE-IVD
Code	**All panels**		
PR701	CD45/PerCP	1 mL, 100 tests	CE-IVD

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