



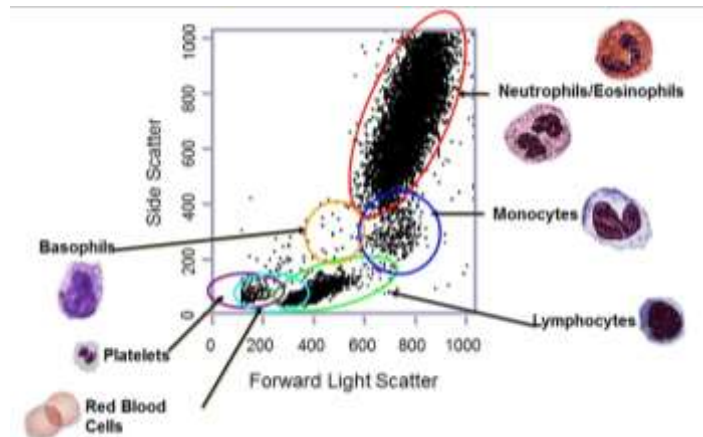
# New Alternatives to Laboratory Developed Tests in Flow Cytometry

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BD Life Sciences

October 2, 2017

# What is flow cytometry?

- Measuring the physical characteristics of a cell in a moving fluid stream using light.
- We can measure a cells size, granularity and fluorescence.
- Flow cytometry is based on pattern recognition.



# Common clinical applications

## CE-IVD registered

- CD4 immune status
- CD34 stem cell enumeration
- HLA-B27
- Residual white blood count
- TBNK
- BD OneFlow™ (EuroFlow™)

## Lab Developed Tests

- HLA crossmatch
- Fetal hemoglobin
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Leukemia & lymphoma
- Various immune function assays

# The power of standardization

Clinical results must be:

- Reproducible, accurate, validated and documented.
- Comparable over time, over different instruments in the same lab as well as over different care venues.
- Comparable in multi-centre clinical treatment trials.

**Standardization in Clinical Flow Cytometry:**

**Not a Luxury, but a Requirement**

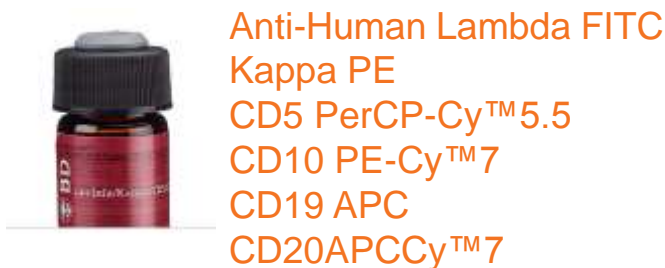
# First attempts at standardization

In 2004, BD launched the first 6-colour pre-combined reagents for hematological malignancies.

- Pros: based on current guidelines, cocktail efficiencies, common markers used.
- Cons: not a full solution, developed by a company.

## LESSONS LEARNED

- A complete solution should be provided which is *evidence-based* on peer-reviewed articles.



# Why use LDTs as a clinical diagnostic tool in flow?

- Clinical trials are expensive and the process is lengthy.
- The scientific knowledge about leukemia and lymphoma diagnosis is advancing at a rapid pace.
- Rapid and accurate diagnosis of rare blood cancers.
- Technology has made significant improvements in past 10 years.
- Antibody panels may be adapted to suit changing needs.

# Oversight of LDTs is changing

FDA reconsiders enforcement of LDTs

- 2010: FDA holds workshops to receive stakeholder input
- 2014: Public workshops held and draft guidelines released
- 2017: Discussion paper released
- TODAY: A work in progress...

There are concerns with high-risk LDTs and a balance needs to be struck between the qualities of LDTs while providing analytically and clinically valid tests.

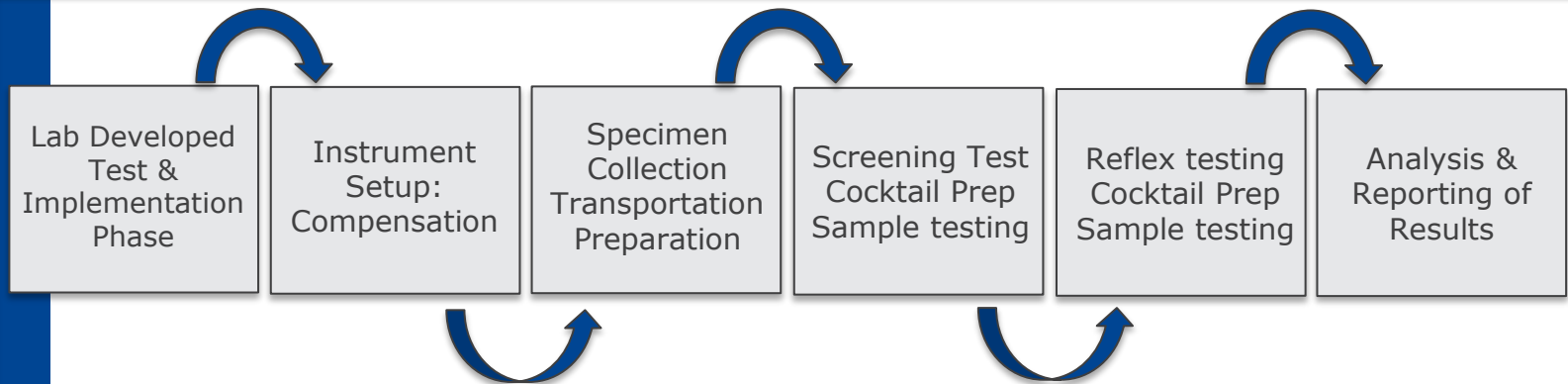
# Important considerations for LDTs

1. Marker selection.
  - Use scientific evidence to evaluate unique staining pattern for each distinct cell lineage.
  - Choose ideal clones for each marker.
2. Optimization of combined reagents.
3. Robust assay validation.
  - Accuracy
  - Sensitivity
  - Reproducibility (low prevalence of diseases)
4. Cocktail validation.
5. Cocktail stability.
6. Quality and process controls.
7. Accreditation (CAP, IQMH, ISO15189 etc...).



# Observational study examining error-prone steps

## High Level Process Map

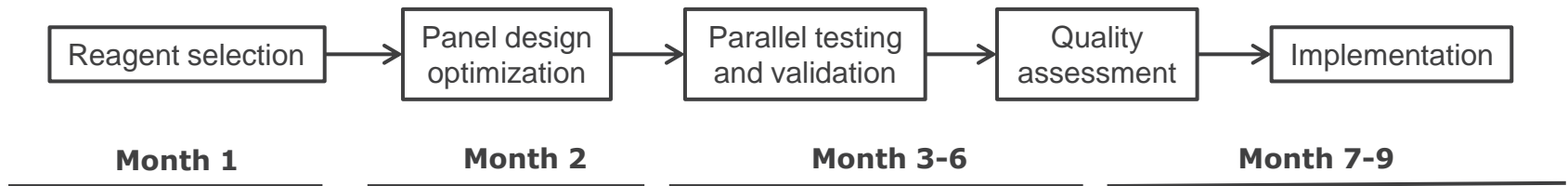


High level process map of Hematological Disorder testing:  
**Complex process** requiring specialized resources to achieve patient testing

# Implementation phase

## Current methodology for diagnostic panel development - lengthy implementation

- liquid vials, lab developed tests



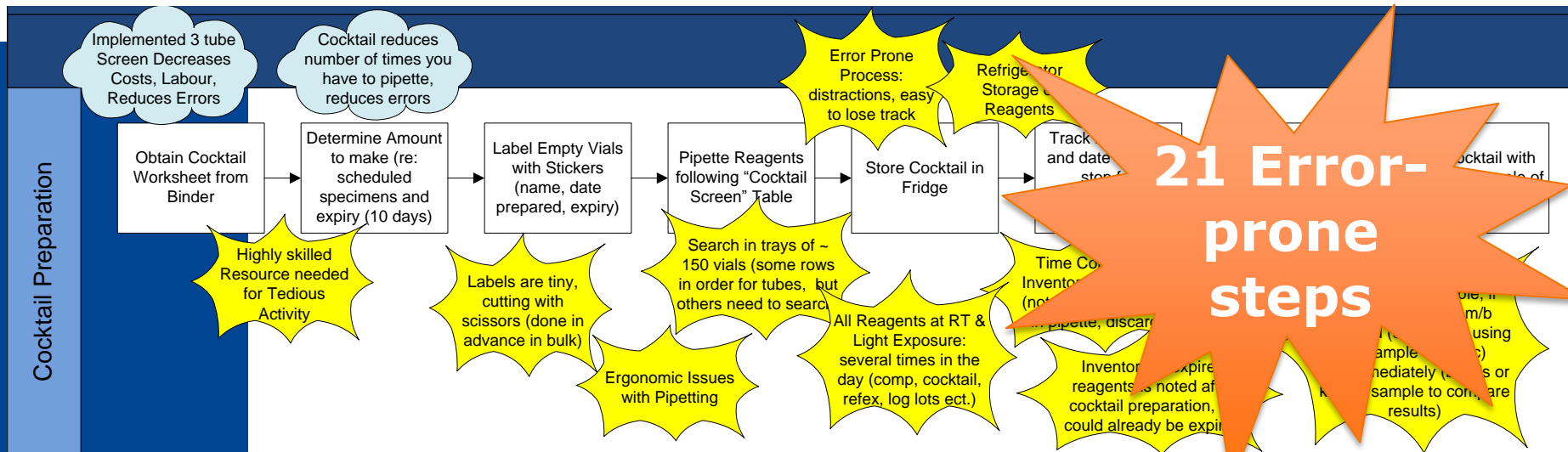
- Reliant on varied experience and knowledge of individual experts
- Non-standardized approach
- Requires highly skilled resources
- Lengthy process to implement- 6 months or longer
- Prolonged panel assessment phase due to low incidence of disease states for validation

# Inventory management



- Inventory management time consuming.
- Many reagents & expiry dates need to be tracked.
- Costs not always captured for remake of cocktails, pipetting errors and dead volumes.
- Reagent waste: minimum 1 vial / month up to \$1,000 if vial full
- Reagents require refrigerated storage.
- Need to stock markers for diagnosing low prevalence disease.

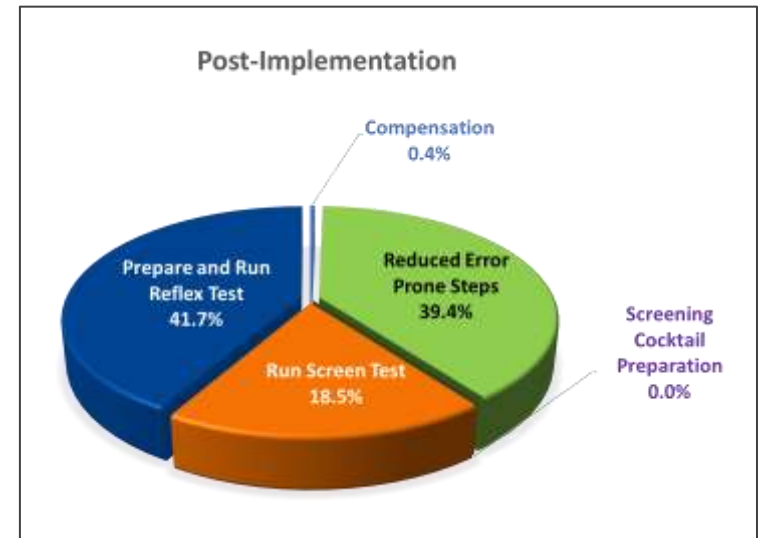
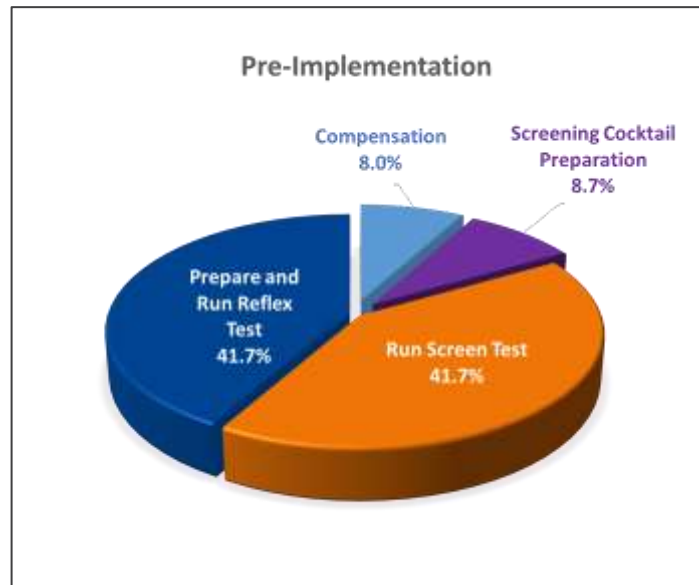
# Errors during reagent preparation



- 21 error prone steps – small volume pipette or add drops
- Annual error prone steps = 1,008
- Annual Hands-On Time = 15:28 (h:mm)

# Results: Error reduction

Study assessing the implementation of dry-tube technology.

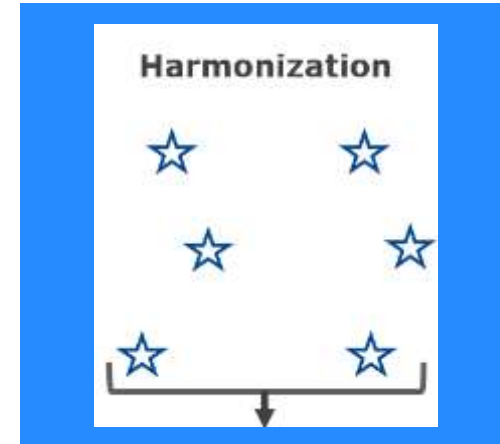
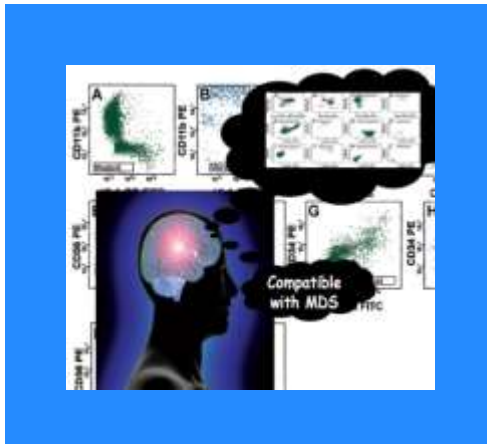


# Diagnostic Approach Today: Use of Lab Developed Tests

**Complex**

**Lack of  
Standardization**

**Subjective**



**Limited patient  
accessibility**

**Delay in delivery  
patient results**

**Need for patient  
testing repetition**

# Standardization vs. harmonization

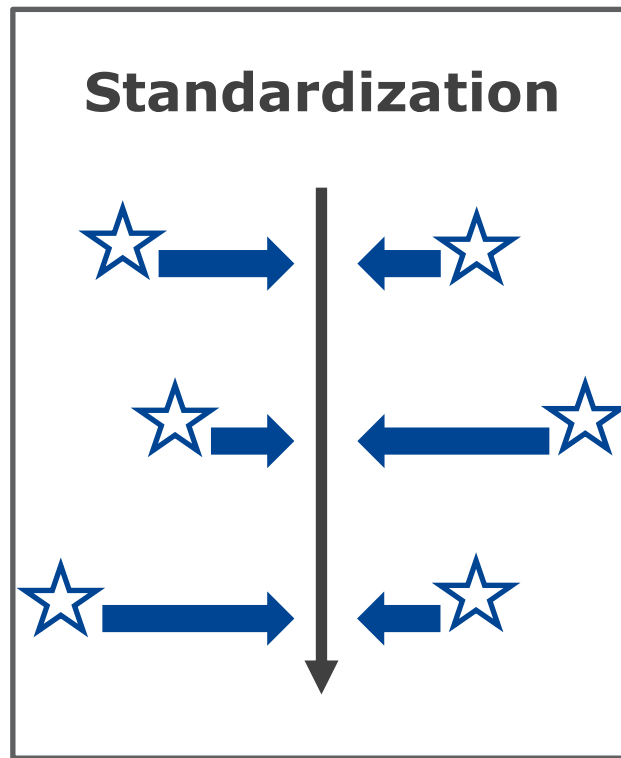
## Standardization

- Minimize variability
- Maximize agreements
- Delivers confidence
- Intra- and inter-laboratory comparison
- Use of database for comparison

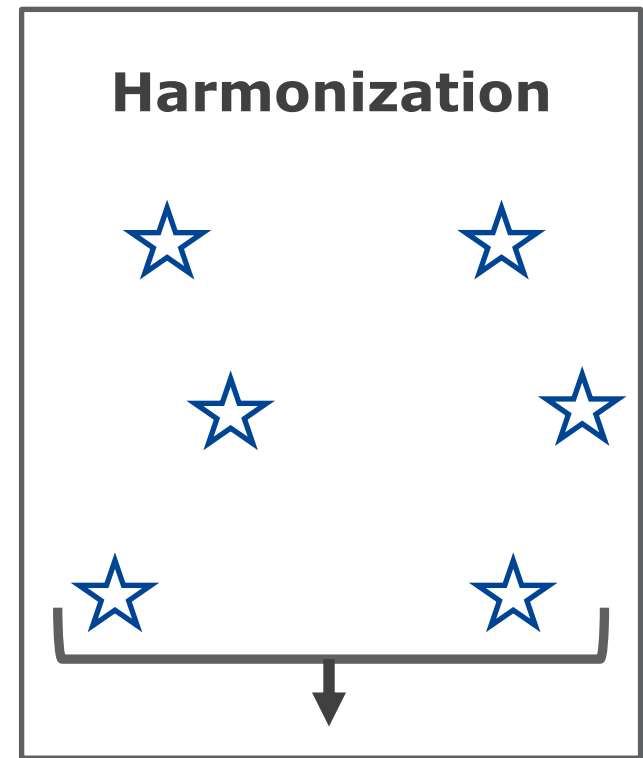
## Harmonization

- Accepts variability
- Provides common goals and outcomes
- Leaves uncertainty
- Laboratory developed test (LDT) methodologies
- No real comparison, especially for MFI
- No use of database for comparison

# Standardization Vs. Harmonization



**To conform with a standard**



**To bring into agreement**

Graham  
Beastall



# The EuroFlow™ Consortium

- Development and evaluation of novel versus 'classical' **antibodies**.
- Definition of multicolor flow cytometry **protocols**.
- Definition of comprehensive **antibody panels** for the diagnosis and classification of hematological disorders.
- Novel **software tools** for recognition of complex flow cytometry data.
- Promote **standardization** of flow cytometric immunophenotyping.

van Dongen J.J.M., and Orfao A. on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708).  
EuroFlow : resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine.  
Leukemia 2012; 26(9):1899-1907

EuroFlow trademark and Logo are property of the EuroFlow Consortium.

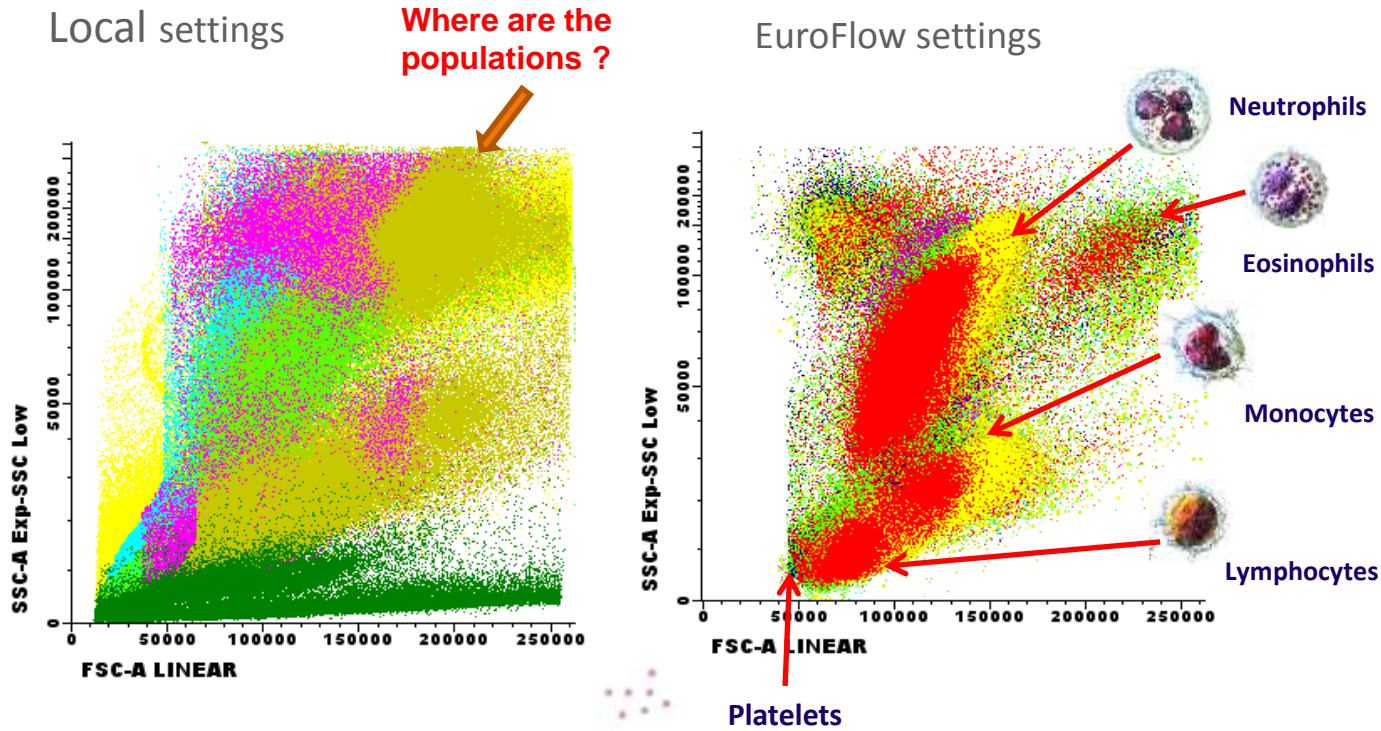
# The EuroFlow™ Consortium

- Founded in April 2006
- >40 researchers from 8 public university hospitals
- Consensus markers were agreed upon based on expert opinions.
- Multiple rounds of testing and redesign as well as testing of new informative markers.
- Findings published in *Leukemia* 2012

van Dongen J.J.M., and Orfao A. on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708).  
EuroFlow : resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine.  
*Leukemia* 2012; 26(9):1899-1907

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# Why the need to standardize ?



7 different normal PB blood samples from 7 centers

# EuroFlow™ Reagent Selection

Antibody selection

Antibody-dye combination

Reagent backbone definition

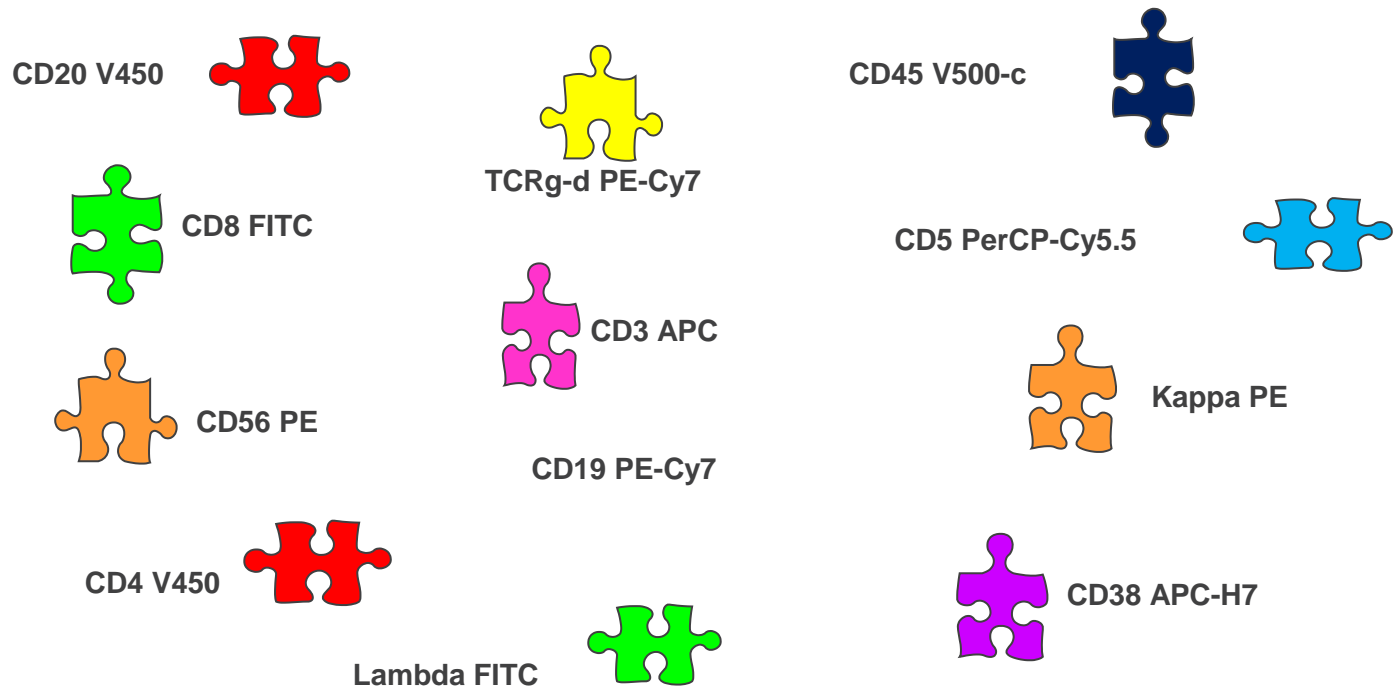
Reagent diagnostic population identifiers

Reagent cocktail concept



# EuroFlow™ Antibody Solution

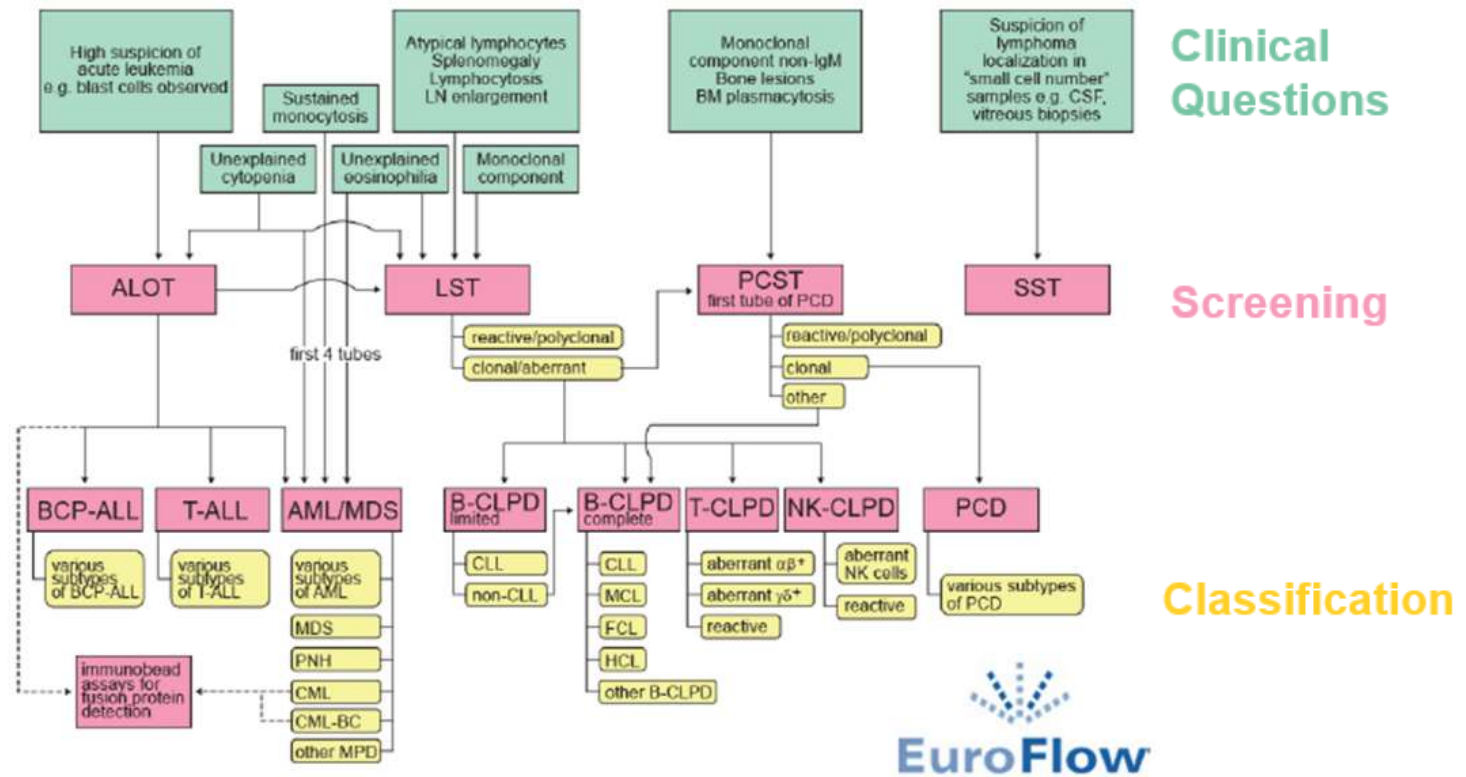
## Single Vial Assay Construction based on Lymphocyte Screening Tube (LST) combination



EuroFlow trademark and Logo are property of the EuroFlow Consortium.

# The EuroFlow™ Algorithm

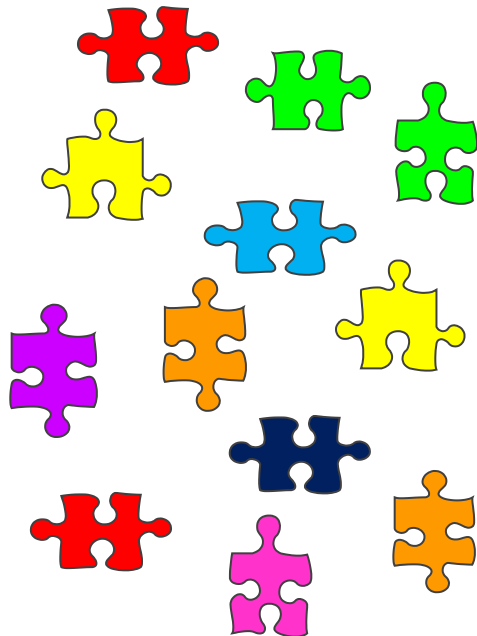
The EuroFlow™ Consortium identified thirty three (33) disease-specific combinations



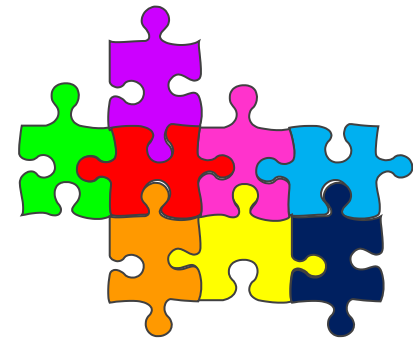
EuroFlow trademark and Logo are property of the EuroFlow Consortium.

# BD Reagent Cocktail Concept

**Single Vial Assay Construction**



**Cocktail Construction**



**Single Tube 'dried' concept**



# Discover an Optimized Standard

BD OneFlow™

Lab  
Efficiency  
& Accuracy

Built on the research  
and validation work of  
the EuroFlow™  
Consortium,

The BD OneFlow™  
brings the  
**standardization** of  
**hematological  
disorder diagnosis**  
**one step forward**



EuroFlow logo and trademark are property of the EuroFlow™ Consortium.

  
**EuroFlow**  
approved

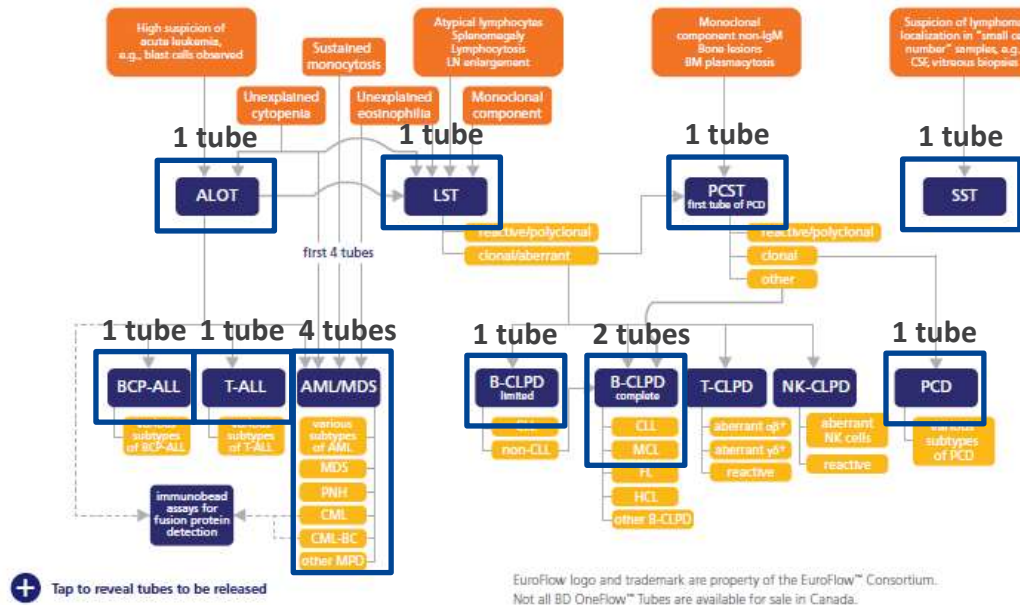




# EuroFlow Consortium Approach

The EuroFlow™ Consortium identified 33 disease-specific combinations<sup>7</sup>

Fourteen BD OneFlow™ tubes will screen 100% and classify 95% of hematological malignancies.



“Around 900 institutions around the world today use this system of early diagnosis and standardized diagnosis of leukemia and lymphoma”\*

[http://www.uniovi.es/comunicacion/noticias/-/asset\\_publisher/33ICSSzZmx4V/content/el-doctor-alberto-orfao-y-la-empresa-cytognos-recogen-el-iii-premio-en-biomedicina-aplicada-valdes-salas](http://www.uniovi.es/comunicacion/noticias/-/asset_publisher/33ICSSzZmx4V/content/el-doctor-alberto-orfao-y-la-empresa-cytognos-recogen-el-iii-premio-en-biomedicina-aplicada-valdes-salas)

# BD OneFlow™ LST (Lymphoid Screening Tube)

BD OneFlow™

ONEFLOW™

## Intended use:

For flow cytometric immunophenotyping of normal and aberrant mature lymphocytic populations of B, T and NK cell lineages as an aid in diagnosis of hematological disorders.

Built on the standard defined by the EuroFlow™ Consortium, LST provide high diagnostic utility *detecting aberrant B-, T- and NK-cells immunophenotypes in > 99% of abnormal B-cell populations and in ≥ 94% of T-/NK-CPLD with an overall frequency of 97.4%<sup>4</sup>*



# BD OneFlow™ LST (Lymphoid Screening Tube)

BD OneFlow™

ONEFLOW™

HV450	HV500	FITC	PE	PerCP Cy5.5	PECy7	APC	APC-H7
CD20	CD45	CD8	CD56	CD5	CD19	CD3	CD38
CD4		SmIgλ	SmIgκ		TCRγδ		

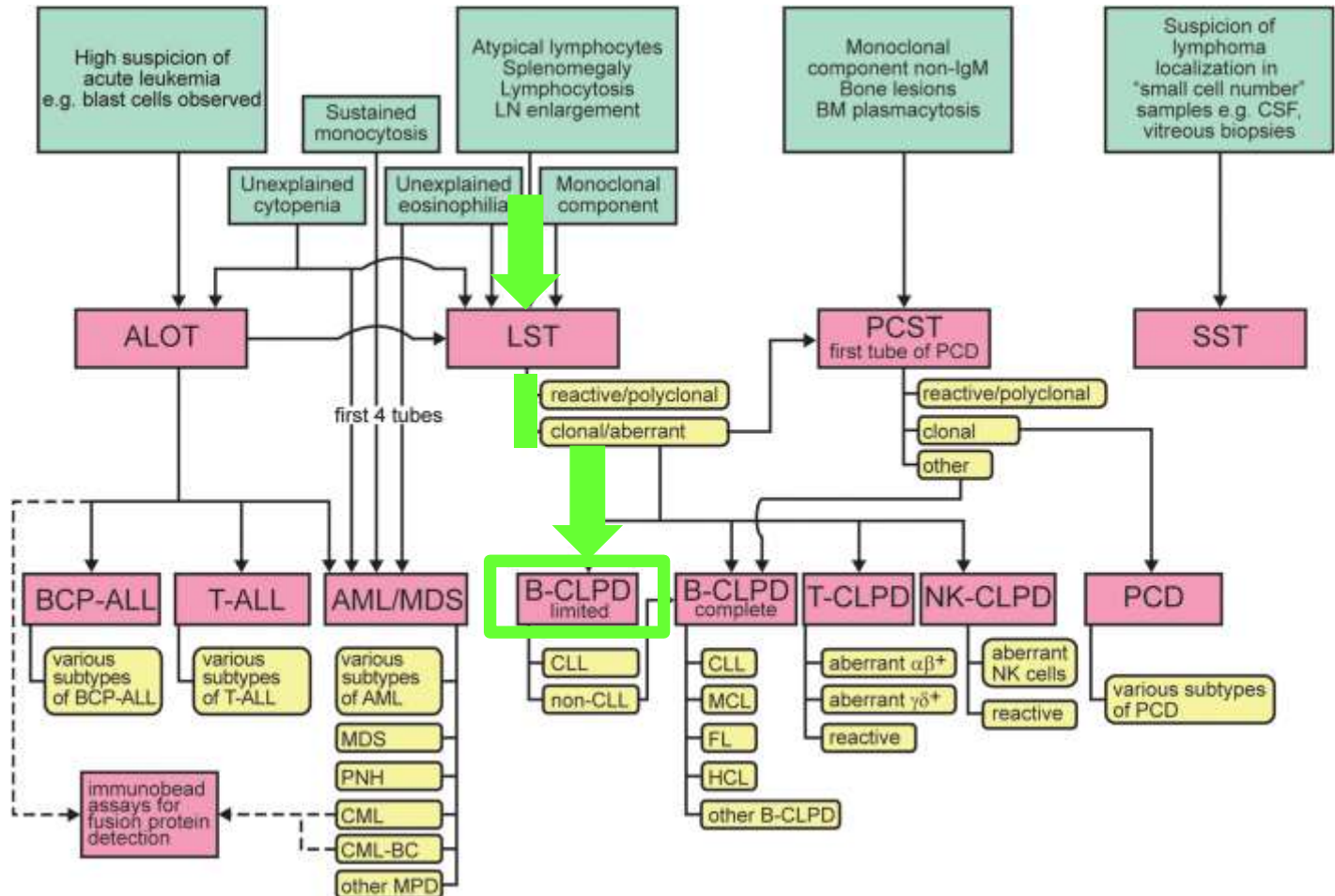
Antibodies were chosen for their ability to separate lymphocytes in their major subpopulations.

- CD45 → mature lymphocytes, B-cell precursors
- CD19, CD20 → B cells, T- and NK- by exclusion
- Kappa & lambda → B cell Clonality
- CD4+, CD8+, CD4+/CD8+, TCRgd + → mature T
- CD3-, CD56+, CD45++ → NK cells
- CD38 --> Plasma cells

# The Algorithm of CLL

BD OneFlow™

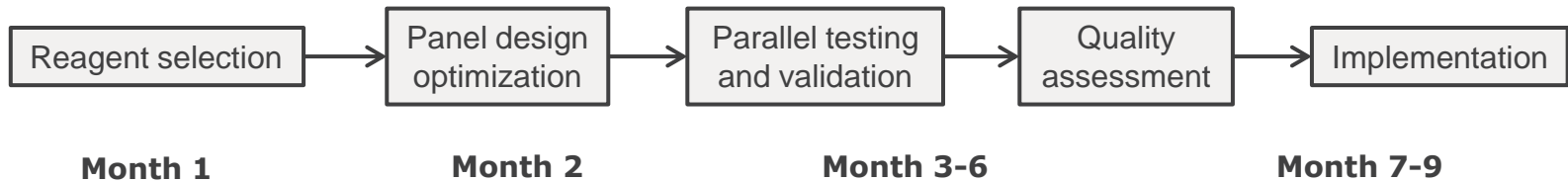
ONEFLOW™



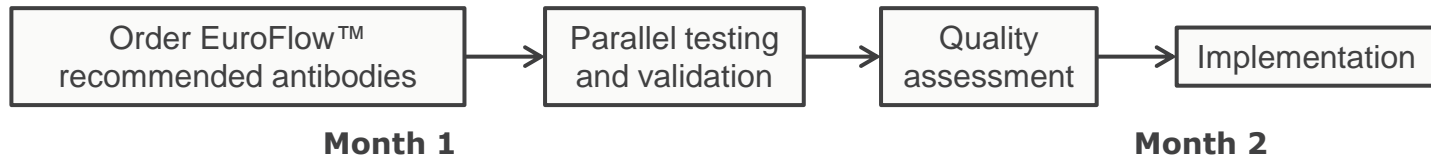
# Summary of diagnostic approaches

## Current methodology for diagnostic panel development - lengthy implementation

- liquid vials, lab developed tests

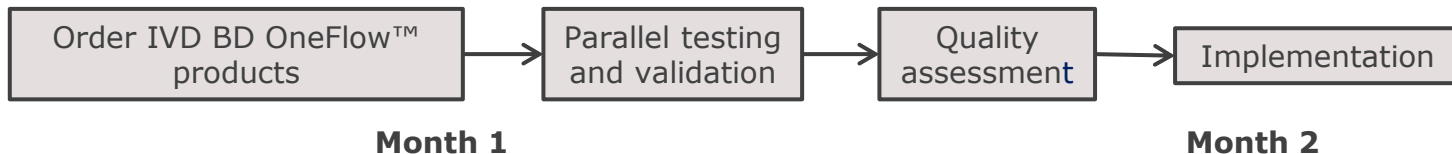


## EuroFlow™ – short implementation, liquid cocktails



## BD OneFlow™ – short implementation

- dry technology, IVD solution, no antibody / cocktail management



# Evaluate the impact

ESCCA Sept 24-27, 2017

Abstract 023

## **Evaluation of the impact of standardized 8-color flow cytometry protocols (EuroFlow) on the diagnostic accuracy of poorly differentiated acute leukemias**

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# Questions?

