

**EUTOS Molecular Monitoring Meeting  
International Standardization of RQ-PCR  
Berlin, 7 June 2009**

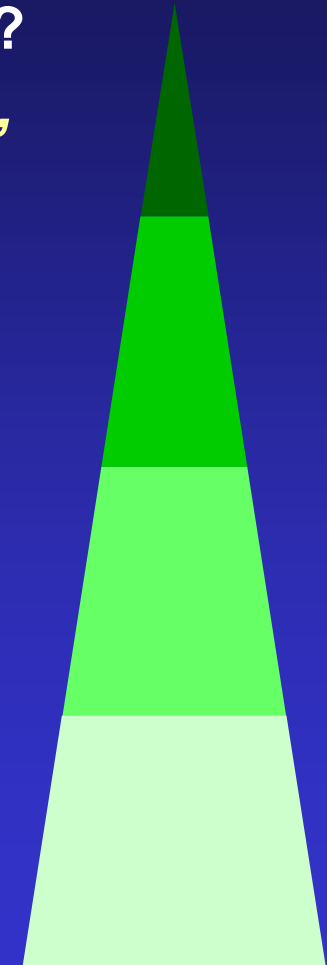
**Standardization of molecular monitoring -  
rationale for the EUTOS for CML**

**Martin C. Müller**

Medizinische Fakultät Mannheim  
Ruprecht-Karls-Universität Heidelberg  
Mannheim, Germany

# Standardization - how ?

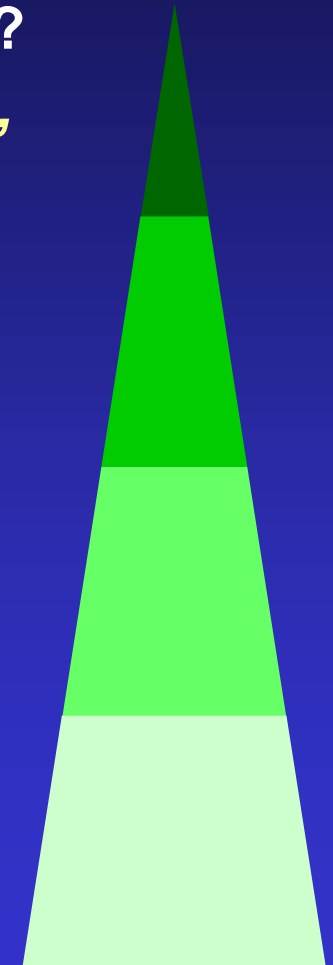
- Unification of all preanalytic and analytic steps ?  
*(RNA extraction, cDNA synthesis, PCR protocol, housekeeping gene, evaluation method)*  
↔ give up established methods
- Use of a uniform plasmid as external control ?  
*(housekeeping gene)*
- Adaptation to the 'International Scale' by sample exchange with standardized lab ?  
*(no change of established methods necessary)*



degree of  
freedom

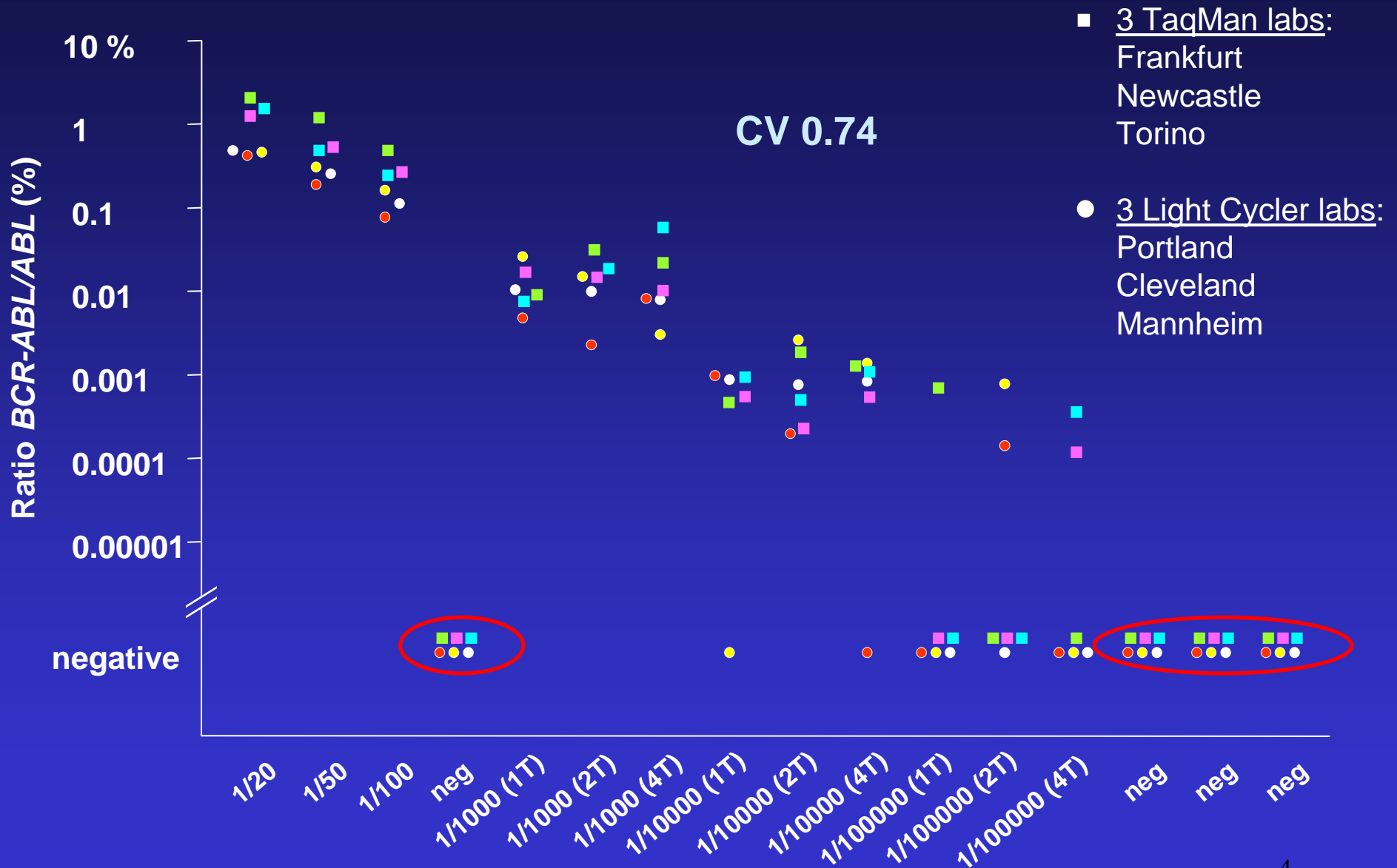
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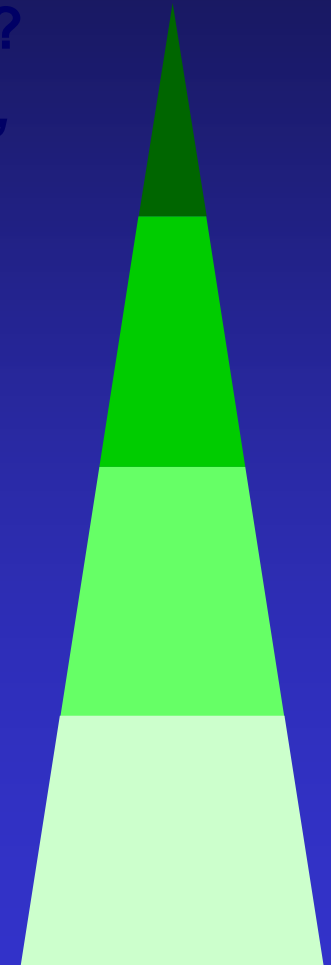
degree of  
freedom

# 6 international labs using unified methods



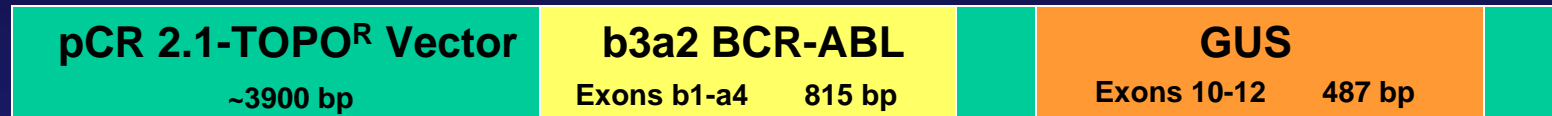
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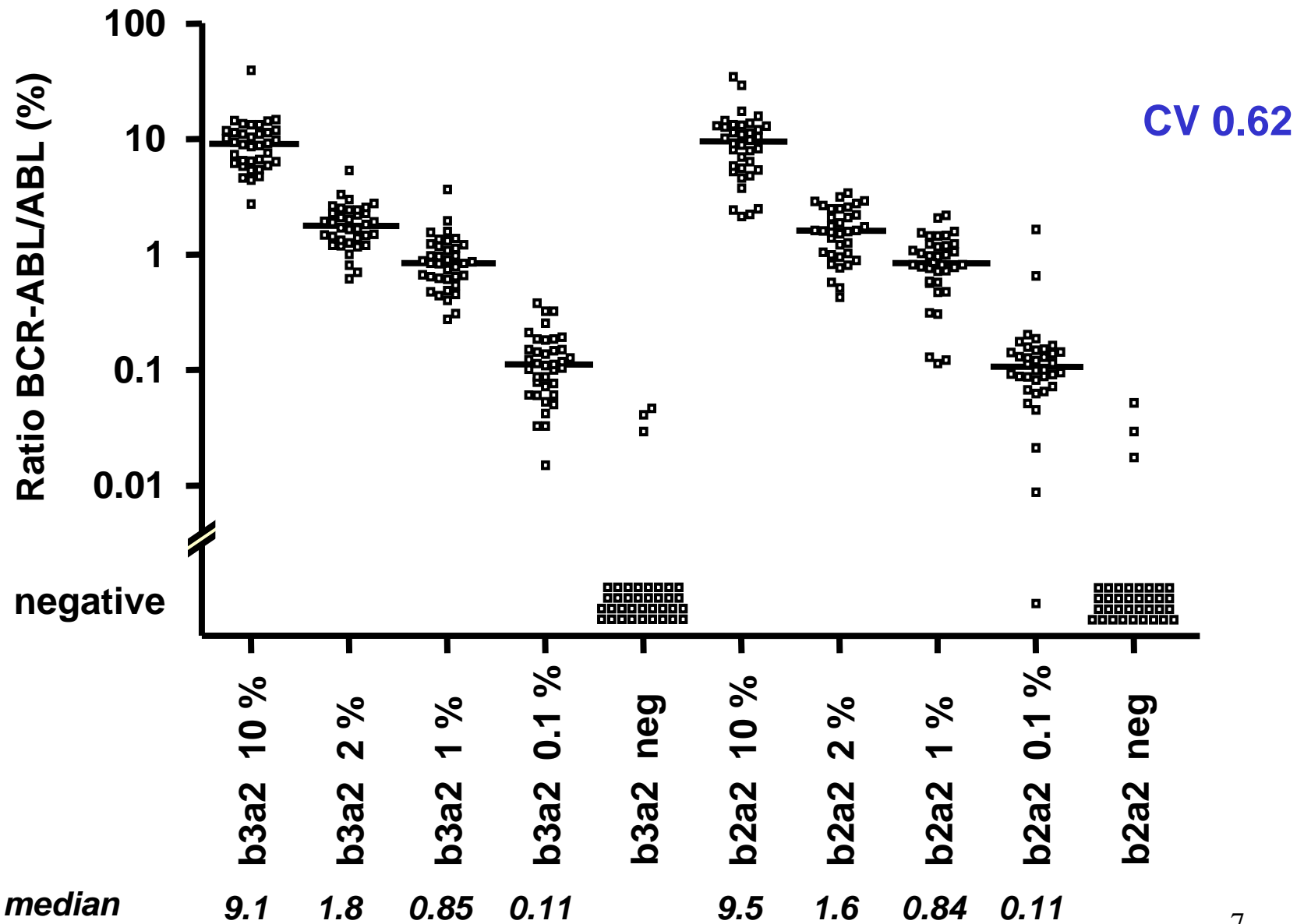
# Construction of a new plasmid (pME-2)



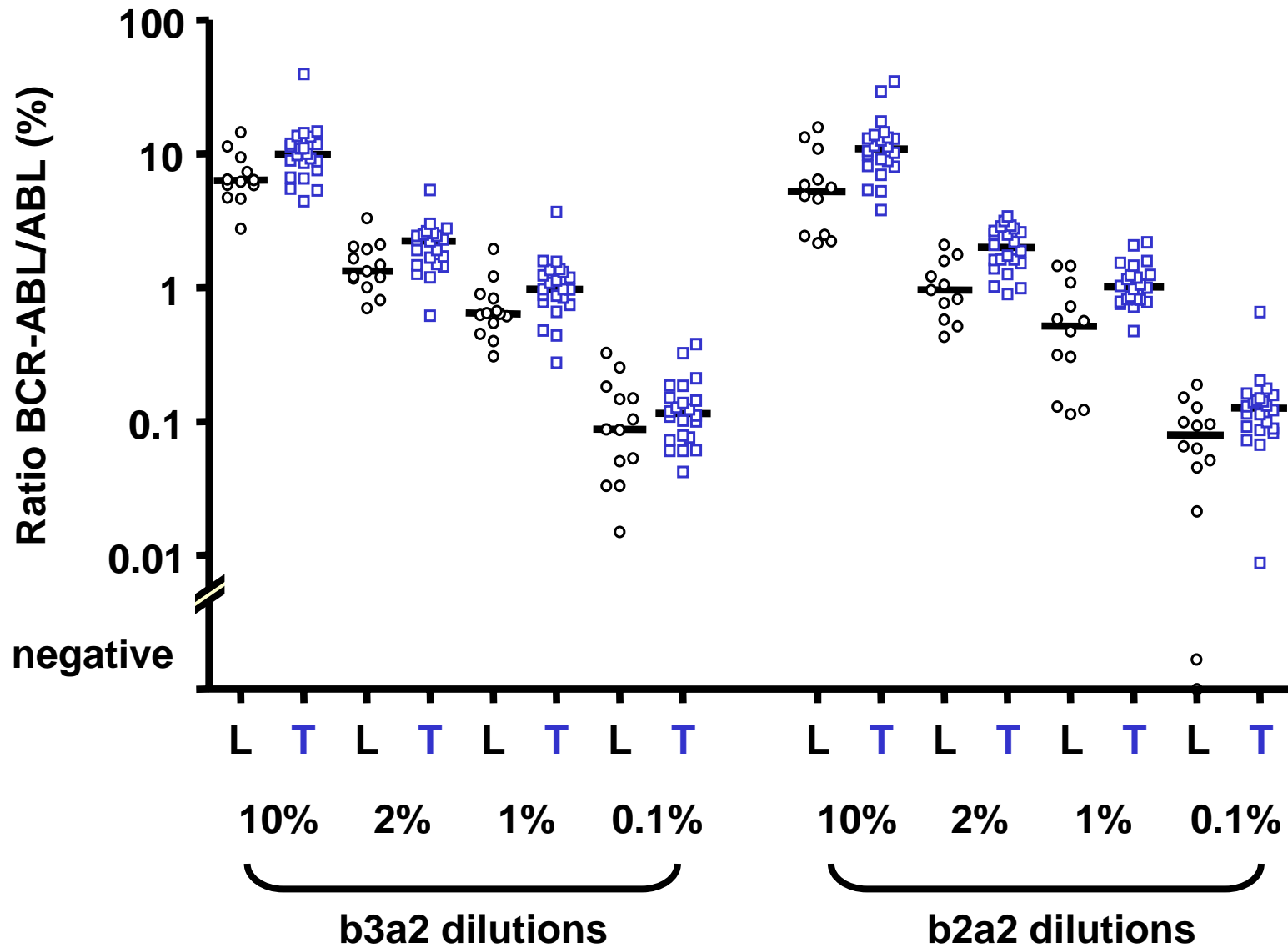
AGAAACGTTCTGGTCTGCCGTGAACAGTCCAGGAGGCACCTTGTTCTGCTGCTGTGGAAGTCGCC  
 CTGACTCGGGGAGGAAGGGACACGCAGGTGGTATCAGTCTTGCTCAAGTAAACAGGCTGTTTTC  
 Exon 12 CAAACATTGTGACTTGGCTACTGAGTGGGATACTGGTTTCATTGGCAATCTTCCAGTATCTC  
 TCTCGCAAAGGAACGCTGCACTTTTTGGTTGTCTCTG **CCGAGTGAAGATCCCCTTTTATTCC**  
 CCAGCACTCTCGTCGGTGACTGTTCAAGTCATGAAATCGGCAAATTC **AATGAGCTCTCCAAC**  
 Exon 11 **CACGTATTTTC**TGCGTTTTTGATCCAGACCAGATGGTACTGCTCTAGCAGACTTTTCTGGTAC  
 TCTTCAGTGAACATCAGAGGTGGATCT **GTGAAACCCTGCAATCGTTTCT**GCTCCATACTCGC  
 Exon 10 TCTGAATAATGGCTTCTGATACTTCTTATAACAGTTCT

	ENR1162	ENF1102	
TaqMan	<b>CCGAGTGAAGATCCCCTTTTATTCC</b>	<b>AATGAGCTCTCCAACCACGTATTTTC</b>	101 bp
	ENR1162	GUS10-LC	
LightCycler	<b>CCGAGTGAAGATCCCCTTTTATTCC</b>	<b>GTGAAACCCTGCAATCGTTTCT</b>	205 bp

# 37 international labs using uniform plasmid (pME-2)

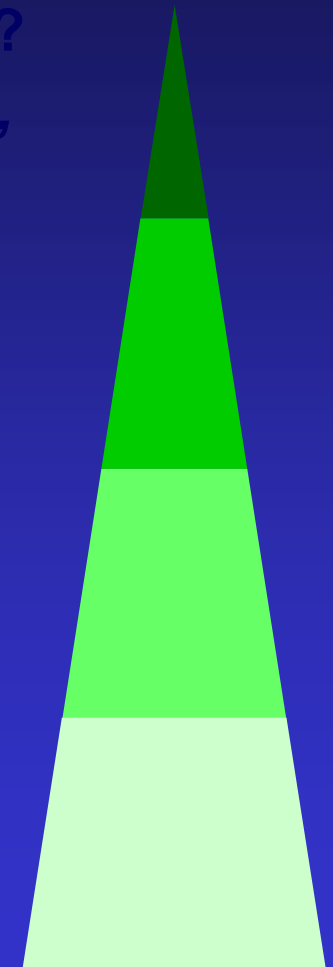


# Higher BCR-ABL expression using TaqMan compared to LightCycler



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degree of  
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## Review article

### Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet

Michele Baccarani, Giuseppe Saglio, John Goldman, Andreas Hochhaus, Bengt Simonsson, Frederick Appelbaum, Jane Apperley, Francisco Cervantes, Jorge Cortes, Michael Deininger, Alois Gratwohl, François Guilhot, Mary Horowitz, Timothy Hughes, Hagop Kantarjian, Richard Larson, Dietger Niederwieser, Richard Silver, and Rudiger Hehlmann

BLOOD, 15 SEPTEMBER 2006 • VOLUME 108, NUMBER 6

## Review in translational hematology

### Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting *BCR-ABL* transcripts and kinase domain mutations and for expressing results

Timothy Hughes, Michael Deininger, Andreas Hochhaus, Susan Branford, Jerald Radich, Jaspal Kaeda, Michele Baccarani, Jorge Cortes, Nicholas C. P. Cross, Brian J. Druker, Jean Gabert, David Grimwade, Rüdiger Hehlmann, Suzanne Kamel-Reid, Jeffrey H. Lipton, Janina Longtine, Giovanni Martinelli, Giuseppe Saglio, Simona Soverini, Wendy Stock, and John M. Goldman

BLOOD, 1 JULY 2006 • VOLUME 108, NUMBER 1

# Proposal for the expression of results

Hughes et al., Blood 2006

- We propose that the value corresponding to a MMR in each laboratory be mathematically converted to 0.1% (reduction of 3 log from baseline of 100%).

- Therefore the International Scale (IS) will be fixed to an absolute value as defined in the IRIS trial.

**History:** the mean BCR-ABL expression of 30 early diagnosed CML patients in the year 2000 (start of IRIS study) was mathematically converted to 100% in each of the three participating laboratories.

- **Benefit:** no adaptation of methods necessary

# Stability of the RQ-PCR system in Adelaide

- Quality control samples have been shown to be stable since 2001.

Table 2. Yearly mean and coefficient of variation (CV) values for the quality control (QC) samples of the Adelaide reference laboratory

Control	QC target values	2001	2002	2003	2004	2005	2006 (to March 28)	New QC target values	2006 (from March 29)	2007
<b>Low b2a2</b>										
Mean	0.07	0.07	0.07	0.09	0.08	0.06	0.07	0.06	0.06	0.06
CV, %		32	55	34	35	40	34		30	29
<b>High b2a2</b>										
Mean	56	48	53	69	50	42	52	22	21	20
CV, %		33	33	22	25	28	29		26	29
<b>Low b3a2</b>										
Mean	0.07	0.07	0.06	0.08	0.06	0.08	0.09	0.08	0.08	0.08
CV, %		54	52	37	41	35	34		34	35
<b>High b3a2</b>										
Mean	85	82	77	93	69	94	93	27	26	24
CV, %		26	38	20	18	23	31		26	22
Number of RQ-PCR runs		34	110	147	157	212	77		257	192

All values were recorded whether the run was accepted or rejected. A new batch of QC samples was in use from March 2006.

# Worldwide distribution of the “International Scale”

Branford et al., Blood 2008

- **Transmission of conversion factors to 38 laboratories worldwide, validation in 19 laboratories to date.**

**blood**

Prepublished online Aug 6, 2008;  
doi:10.1182/blood-2008-04-150680

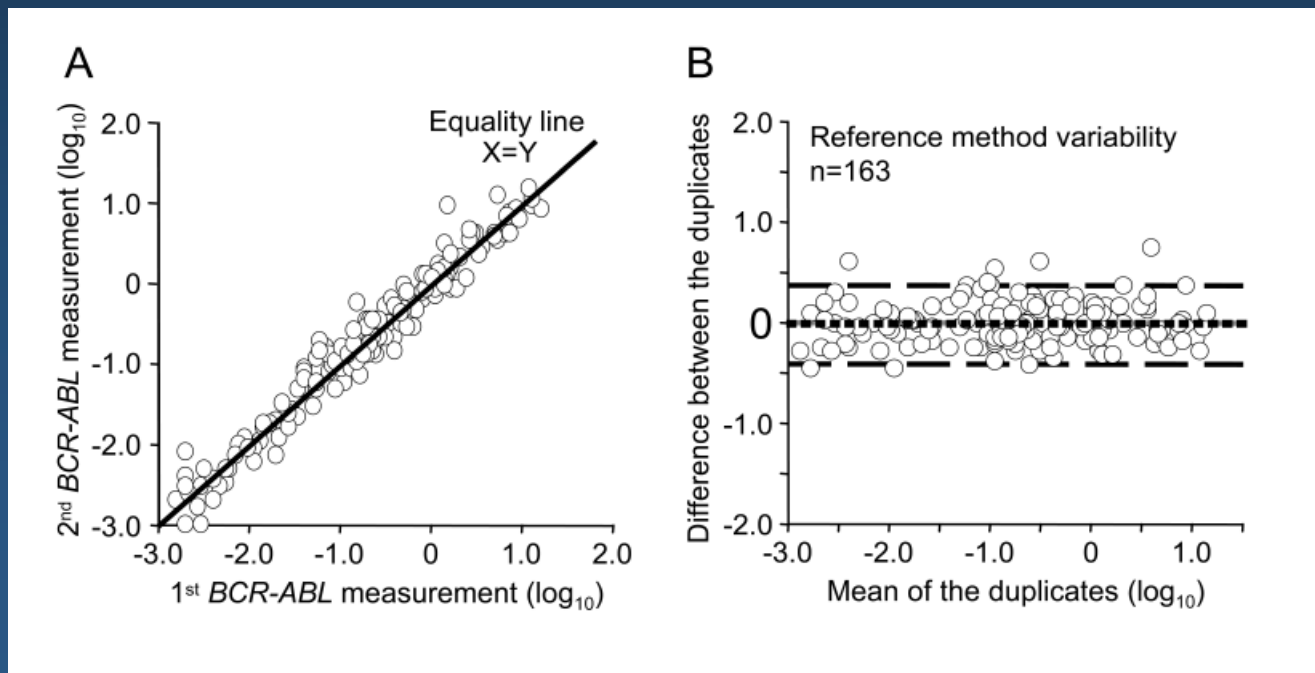
**Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials**

Susan Branford, Linda Fletcher, Nicholas CP Cross, Martin C Muller, Andreas Hochhaus, Dong-Wook Kim, Jerald P. Radich, Giuseppe Saglio, Fabrizio Pane, Suzanne Kamel-Reid, Y. Lynn Wang, Richard D Press, Kevin Lynch, Zbigniew Rudzki, John M Goldman and Timothy Hughes

# Worldwide distribution of the “International Scale”

Branford et al., Blood 2008

- Two methods with optimal agreement of results
- The bias is close to zero



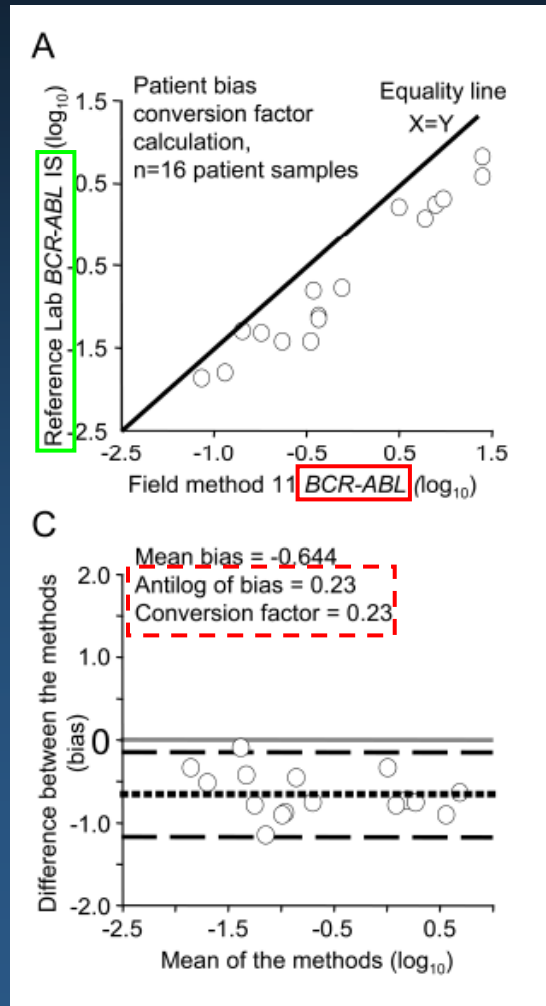
# Worldwide distribution of the “International Scale”

Branford et al., Blood 2008

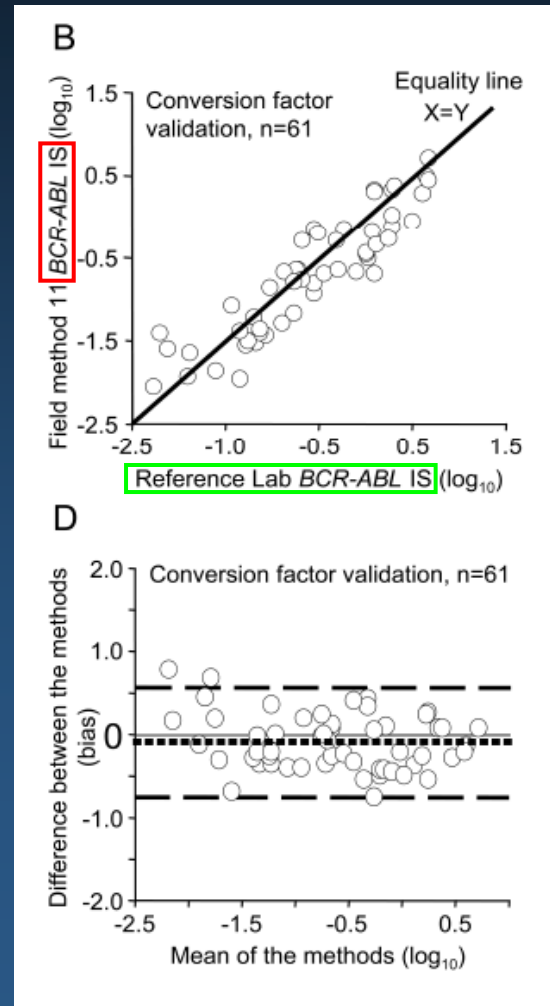
- **Round 1:** patient samples (n=20) have been sent to the central lab
- After processing, results were plotted against each other and conversion factors were calculated applying the Bland-Altman bias method
- **Round 2:** evaluation of a new series of patient samples (n=30) after 7 months
- Conversion factors from round 1 were applied and the bias was calculated using Bland-Altman for validation

# Branford et al., Blood 2008

## Round 1: CF calculation



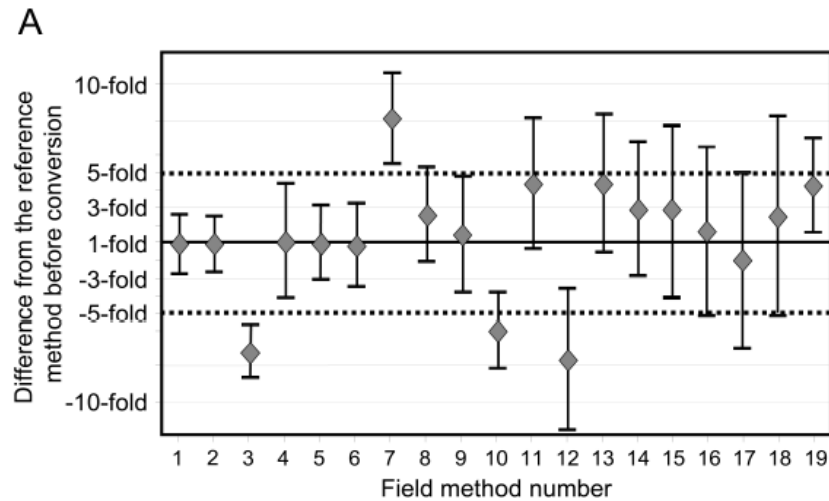
## Round 2: CF validation



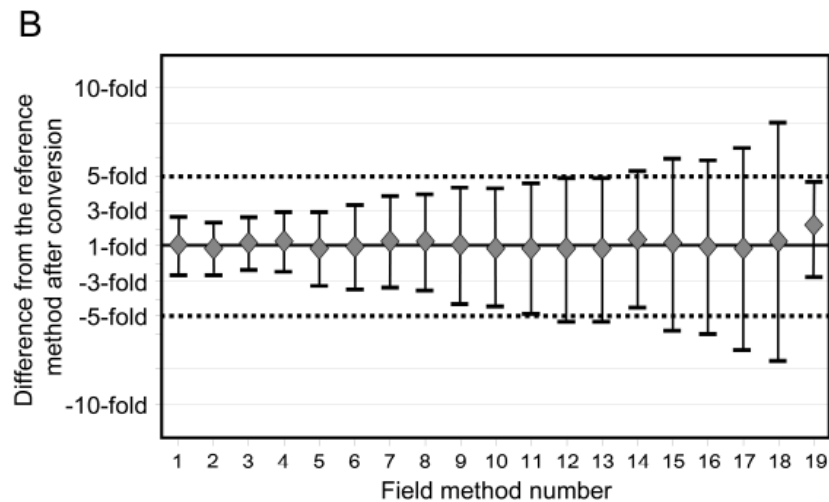
# X-fold Average Difference between Reference Lab and Participating Labs

- Desirable criteria:
- average difference within +/- 1.2-fold
  - 95% limits of agreement within +/- 5-fold

before  
conversion



after  
conversion



## Performance Characteristics translate into MMR concordance

**Desirable criteria:** → average difference within +/- 1.2-fold  
 → 95% limits of agreement within +/- 5-fold

**Table 4. Concordance of MMR IS between the reference method and the field methods according to the performance characteristics of the 19 field methods that have undertaken the validation procedure for their current analytical systems.**

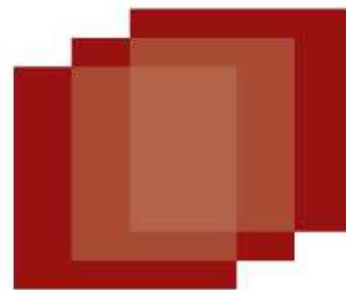
Field method group	Performance characteristics		Number of field methods	Number of validation samples	MMR by either the reference method and/or the field method	MMR by the reference method (% of all validation samples)	MMR by the field methods (% of all validation samples)	MMR concordance
	Average difference	95% limits of agreement						
Group 1	±1.2-fold	±5-fold	11 (58%)	318	159	152 (48%)	151 (47%)	144 (91%)
Group 2	±1.2-fold	> ±5-fold	6 (32%)	193	94	83 (43%)	79 (41%)	70 (74%)
Group 3	> ±1.2-fold	any	2 (10.5%)	87	25	22 (25%)	17 (20%)	15 (60%)

## Subproject Molecular Monitoring

→ Nomination of 28 national reference laboratories

**Aim:** to cover Europe with competence in standardized BCR-ABL monitoring

EUTOS for CML



*European Treatment and Outcome Study*

# How to transmit the `International Scale` in Europe?

- Lab A (central lab, approved CF)  
Lab B (participating lab)
- Two major ways to align results between two labs:
  - 1) Send `reference material` from lab A to lab B  
which has been adequately characterized in lab A  
→ align results
  - 2) Send aliquots of patient samples from lab B to lab A  
→ align results

# Standardization of BCR-ABL quantification in Europe - 2002



 Countries with a ref lab with validated CF





## Status of CF validation in national reference laboratories (n=28)

Country	validated	Town	Responsible
(Nordic) Denmark	yes	Aarhus	Dr. Charlotte Nyvold / Lykke Grubach
(Nordic) Finland	yes	Turku	Dr. Veli Kairisto, Dr. Vesa Juvonen
(Nordic) Norway	yes	Tromso/Oslo	Dr. Franz Gruber / Aleksandra Silye
(Nordic) Sweden	yes	Uppsala	Dr. Hans Boström
Austria	yes	Vienna	Prof. Gerlinde Mitterbauer-Hohendanner
Belgium (French part)	yes	Brussels	Prof. Philippe Martiat / Dr. Hakim El Housni
Belgium (Dutch part)	yes	Leuven	Dr. Nancy Boeckx
Croatia, +Bosnia, Serbia, Albania, Macedonia	yes	KBC Rebro, Zagreb	Prof. Renata Zadro
Czech Republic	yes	Prague	Dr. Katerina Machova / Dr. Jana Rulcova
for the Slovak republic	yes	Olomouc	Prof. Indrak/ Dr. Rohon
France	yes	Paris	Dr. Jean-Michel Cayuela
Germany	yes	Mannheim	Prof. Andreas Hochhaus, Dr. Martin Müller
Greece, + Cyprus	yes	Athens	Dr. Katerina Zoi
Hungary	yes	Budapest	Dr. Hajnalka Andrikovics
Israel	yes	Tel-Hashomer	Prof. Arnon Nagler
Italy	yes	Turin	Prof. Giuseppe Saglio / Dr. Enrico Gottardi
Lithuania, for Baltic states	no	Vilnius	Prof. Laimonas Griskevicius / Dr. Mindaugas Stoskus
Netherlands, + Luxembourg	yes	Rotterdam	Prof. Cornelissen / Dr. Peter Valk
Poland	yes	Krakow	Dr. Tomasz Sacha
Portugal	yes	Lissabon	Dr. Maria Gomes Silva
Romania	yes	Bucharest	Dr. Daniel Coriu / Dr. Rodica Talmaci
Russia	no	St. Petersburg	Prof. Zaritzky / Dr. Michael Dubina
Russia	yes	Moscow	Prof. Anna Turkina / Dr. Andrey Misyurin
Slovenia	yes	Ljubljana	Dr. Tadej Pajic
Spain	yes	Barcelona	Dr. Dolors Colomer
Switzerland	yes	Bern	Dr. Elisabeth Oppliger-Leibundgut
Turkey	yes	Istanbul	Prof. Ugur Özbek
UK, + Ireland	yes	Southampton	Prof. Nick Cross / Dr. Helen White

## Centers with validated CF (no reference laboratories, n=23)

Country	validated	Town	Responsible
Austria	yes	Vienna	Prof. Thomas Lion
Czech Republic	yes	Brno	Prof. Jiri Mayer
France	yes	Bordeaux	Prof. Francois-Xavier Mahon
France	yes	Lille	Prof. Claude Preudhomme
France	yes	Lyon	Dr. Sandrine Hayette
France	yes	Marseille	Prof. Jean Gabert
Germany	yes	Dresden	Prof. Christian Thiede
Germany	yes	Düsseldorf	Dr. Frank Neumann
Germany	yes	Frankfurt	Dr. Heike Pfeifer
Germany	yes	Hamburg	Dr. Philippe Schafhausen
Germany	yes	Hannover	Dr. Nils von Neuhoff
Germany	yes	Kiel	Dr. Christiane Pott
Germany	yes	Jena	Dr. Martin Roskos
Germany	yes	Leipzig	Dr. Thoralf Lange
Germany	yes	München	Dr. Susanne Schnittger
Germany	yes	Münster	Dr. Utz Krug
Germany	yes	Ulm	Prof. Konstanze Döhner
Italy	yes	Bologna	Prof. Giovanni Martinelli
Italy	yes	Naples	Prof. Fabrizio Pane
Spain	yes	Barcelona	Dr. Josep Nomdedeu
The Netherlands	yes	Amsterdam	Dr. Jeroen Janssen
The Netherlands	yes	Rotterdam	Dr. Vincent van der Velden
UK	yes	London	Prof. Letizia Foroni