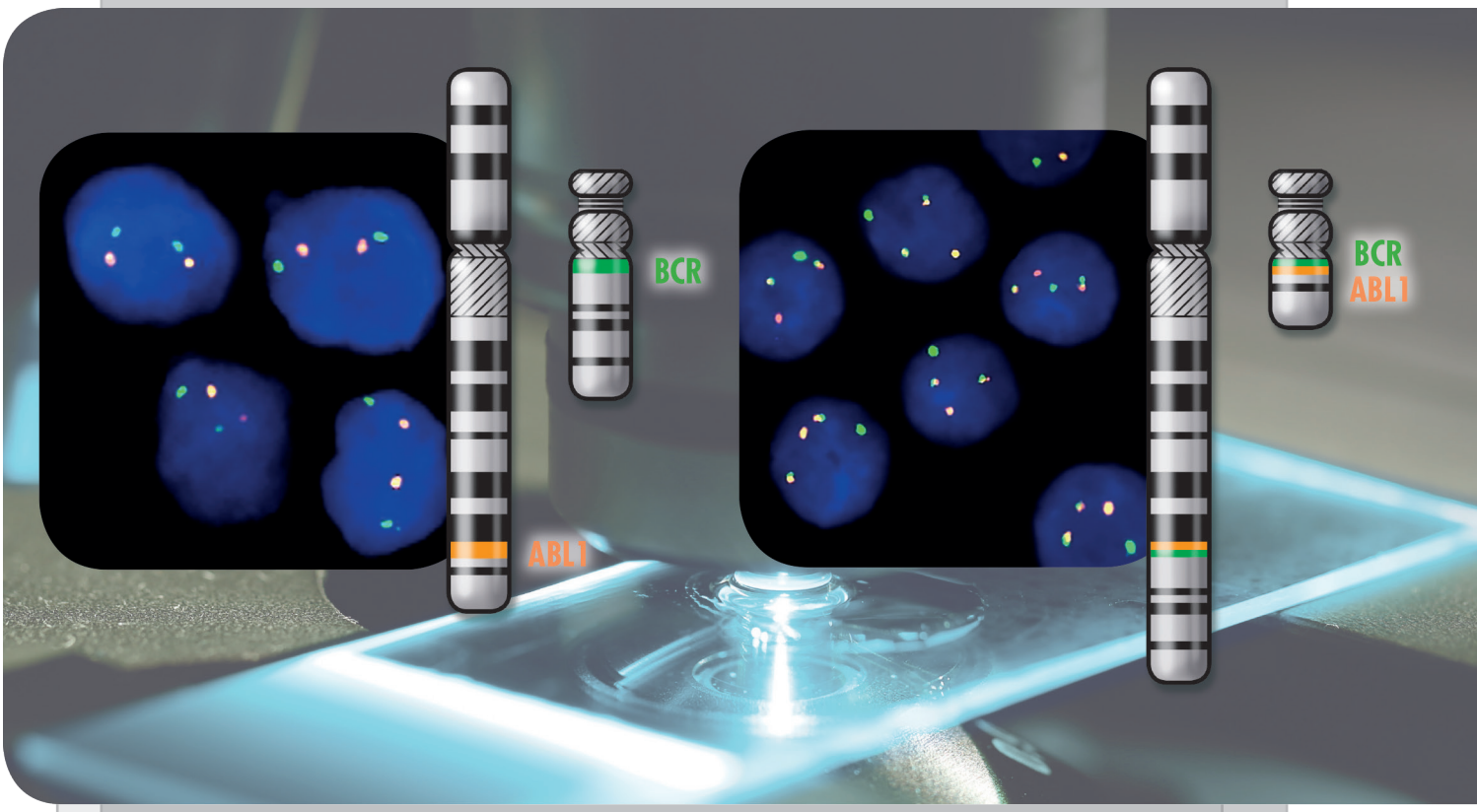
02/15

ZYTONEWS

Detection of BCR/ABL1 Fusion

Comparative Analysis of Six Commercially Available FISH Fusion Probes



Knas T *et al.* (2015) Pathologe 36(4): 372-84.

Dear Readers,
With this new issue of our **ZYTONEWS** we would like to summarize the interesting publication of Knas *et al.* 2015 (available in German language only). By comparing different commercially available probes and protocols the use of the **Zyto*Light*® SPEC BCR/ABL1 Dual Color Dual Fusion Probe** resulted in best hybridization efficiency combined with superior signal quality!

Enjoy reading, Yours ZYTONEWS TEAM

ZYTOVISION
Molecular diagnostics simplified

Aim of the Study

Chronic myeloid leukemia (CML) is diagnostically defined by the reciprocal translocation t(9;22)(q34;q11). This aberration can be detected by the BCR-ABL1 fluorescence *in situ* hybridization (FISH) technique.

This article presents a comparative analysis of different commercially available FISH probes and different FISH protocols in order to optimize this technique on formalin-fixed and paraffin-embedded bone marrow trephine biopsies.

Comparative Analysis of the Following Probes

Manufacturer	Probe
ZytoVision	**ZytoLight® SPEC BCR/ABL1 Dual Color Dual Fusion Probe**
Cytocell DC	Aquarius® BCR/ABL Dual Color Translocation, Dual Fusion Probe
Cytocell TC	Aquarius® BCR/ABL Plus Translocation, Dual Fusion Probe
Kreatech	ON BCR/ABL t(9;22), TC, D-Fusion
MetaSystems	XL BCR/ABL1 plus Translocation Dual Fusion Probe
Abbott	Vysis LSI BCR/ABL, Dual Color, Dual Fusion Translocation Probe Set

Evaluation Criteria

Bone marrow tissue slides are evaluated for the following criteria. 100 cells were counted per slide:

- Intensity of the signals
- Unspecific signals
- Amount of auto fluorescence
- Background ratio

- Positive: 2 fusion, 1 orange, and 1 green signal
- Negative: 2 green and 2 orange signals
- Cut-off level of analyzed tissue samples: 15%

Summary of Conclusions

- Detection of the reciprocal translocation t(9;22) in formalin-fixed, paraffin embedded bone marrow trephine biopsies by fluorescence *in situ* hybridization (FISH) is possible.
- The Ulmer EDTA Protocol has proven to be the best of all tested protocols.
- In direct comparison with 5 different commercially available probes - the use of the **ZytoLight® SPEC BCR/ABL1 Dual Color Dual Fusion Probe** gave the best results.

Results

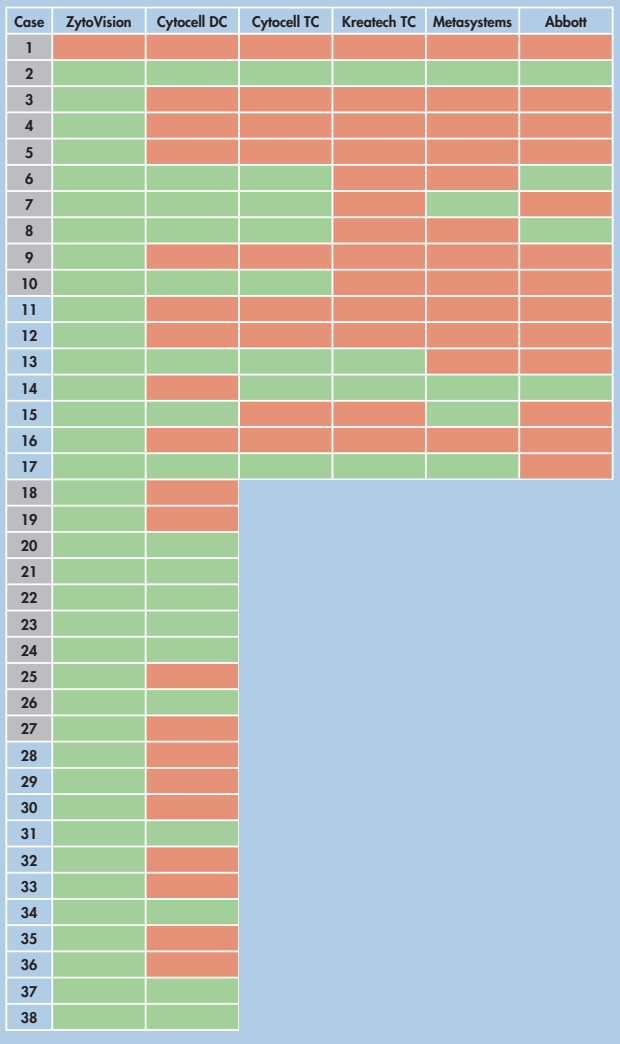
Part I – Comparison of the different FISH probes using the Ulmer Standard Protocol

17 of the 38 cases were evaluated by using all 6 commercially available FISH probes. Due to the convincing results of the ZytoVision and Cytocell probes the remaining 21 cases were evaluated by using only probes of these two manufacturers.

Improved Ulmer Standard Protocol
similar to the ZytoLight FISH Tissue Implementation Kit

- Deparaffinization using xylene and ethanol
- Pretreatment using Citrate-Buffer (10 mM, pH 6), 15 min, 98°C
- Pepsin digestion (25mg/100ml), 25 min, 37°C
- 2 µl probe-mix on marked tissue area
- Probe denaturation, 10 min, 75°C
- Hybridization o/n, 37°C
- Wash with Wash A (incl. formamide, 20x SSC), 3x 5 min, 42°C
- Wash with Wash B (incl. 20x SSC), 3x 5 min, 60°C
- DAPI counterstain
- Mounting with Vectashield®

Tab.1: Improved Ulmer Standard Protocol



Adapted from Knas et al.: Graphical scheme of reciprocal translocation t(9;22)(q34;q11). grey: CML, green: evaluable; red: non-evaluable

Conclusion – Part I

Due to the better hybridization efficiency, the **ZytoLight® SPEC BCR/ABL1 Dual Color Dual Fusion Probe** showed best results related to signal intensity and reliability of the results.

Results

Part II – Evaluation of the hybridization efficiency by comparing different FISH protocols

Three different cases were chosen to test the hybridization efficiency (case No. 3, 8, 11) and hybridized by using all 6 commercially available FISH probes applying the Ulmer Standard Protocol, the Improved Ulmer Standard Protocol, the respective manufacturer protocol, the protocol in relation to Ventura *et al.* 2006, and the Ulmer EDTA Protocol.

Ulmer EDTA Protocol
similar to the Improved Ulmer Standard Protocol

- Deparaffinization using xylene and ethanol
- Pretreatment using EDTA-buffer (1 mM, pH 4.7), 15 min, 98°C
- Pepsin digestion (25mg/100ml), 25 min, 37°C
- 2 µl probe-mix on marked tissue area
- Probe denaturation, 10 min, 75°C
- Hybridization o/n, 37°C
- Wash with Wash A (incl. formamide, 20x SSC), 3x 5 min, 42°C
- Wash with Wash B (incl. 20x SSC), 3x 5 min, 60°C
- DAPI counterstain
- Mounting with Vectashield®

Tab.2: Comparision of Protocols (CML case No. 3)

Protocol	ZytoVision	Cytocell DC	Cytocell TC	Kreatech TC	MetaSystems	Abbott
Ulmer Standard						
Manufacturer						
Improved Ulmer Standard						
Ventura *et al.* 2006						
Ulmer EDTA						

Adapted from Knas et al.: green: evaluable; red: non-evaluable
CML: Chronic myeloid leukemia

Conclusion – Part II

Best hybridization result was achieved by using the Ulmer EDTA Protocol and the **ZytoVision** probe!

Note: A similar excellent hybridization result was achieved by using the ZytoVision probe together with the ZytoLight® FISH-Tissue Implementation Kit.

Results

Part III – Comparison of the Ulmer EDTA Protocol to the Improved Ulmer Standard Protocol

17 cases were selected from those which have been evaluated in the first round with all 6 commercially available FISH probes. These cases were processed again by using all 6 commercially available FISH probes in combination with the Ulmer EDTA Protocol.
The comparative study showed that the first case was not evaluable, independent of probe and protocol applied. Generally, application of the Ulmer EDTA Protocol resulted in a considerably improved hybridization efficiency.

Tab.3: Ulmer EDTA-Protocol

Case	ZytoVision	Cytocell DC	Cytocell TC	Kreatech TC	Metasystems	Abbott
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						

Adapted from Knas et al.: grey: CML, green: evaluable; red: non-evaluable
EDTA: ethylenediaminetetraacetic acid

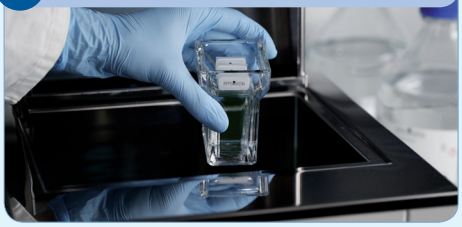
Conclusion – Part III

Application of the Ulmer EDTA Protocol generally led to significantly improved hybridization efficiency.

Protocol Workflow Overview

The protocol of the **ZytoLight® FISH-Tissue Implementation Kit** has been optimized for FFPE tissue (fixed in 10% neutrally buffered formalin) which has been cut in sections between 2-4 µm. Dewaxing with subsequent dehydration should be performed with regular changes of xylene and ethanol solutions!

1 Pretreatment



*Incubate for 15 min in pre-warmed **Heat Pretreatment Solution Citric (PT1)** at 98°C.*

2 Proteolysis

*Apply ready-to-use **Pepsin Solution (ES1)** and incubate for approx. 15 min at 37°C in a humidity chamber.*

3 Probe Application

*Completely air dry section before pipetting 10 µl **ZytoLight Probe (PLXX)** each onto individual samples.*

4 Cover with Coverslip

Cover the samples with a coverslip, avoiding trapped bubbles.

5 Seal with Rubber Cement

Seal the coverslip, e.g., with a layer of rubber cement.

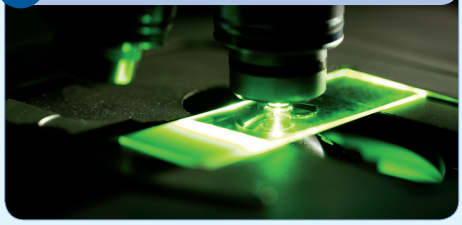
6 Denaturation & Hybridization

Denature the slides at 75°C for 10 min and hybridize overnight at 37°C (in a humid environment).

7 Stringency Wash

*Remove rubber cement and wash using 1x **Wash Buffer A** for 2x 5 min at 37°C.*

8 Evaluation



*Before evaluation, pipette 30 µl **DAPI/ DuraTect™-Solution (MT7)** onto the slides and cover the samples with a coverslip. Use appropriate filter sets for evaluation.*

Our specialists are available to support you whenever adjustments are necessary. Please contact: **helptech@zytovision.com**

Product Information

ZytoLight® Products for FISH analysis

Prod. No.	Product	Label	Tests* (Volume)
Z-2111-50	ZytoLight SPEC BCR/ABL1 Dual Color Dual Fusion Probe CE IVD	●/●	5 (50 µl)
Z-2111-200	ZytoLight SPEC BCR/ABL1 Dual Color Dual Fusion Probe CE IVD	●/●	20 (200 µl)
Related Products			
Z-2028-5	ZytoLight FISH-Tissue Implementation Kit CE IVD Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 150 ml; 25x Wash Buffer A, 50 ml; DAPI/DuraTect-Solution, 0.2 ml		5
Z-2028-20	ZytoLight FISH-Tissue Implementation Kit CE IVD Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; Wash Buffer SSC, 500 ml; 25x Wash Buffer A, 100 ml; DAPI/DuraTect-Solution, 0.8 ml		20
Z-2099-20	ZytoLight FISH-Cytology Implementation Kit CE IVD Incl. Cytology Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 50 ml; 10x MgCl2, 50 ml; 10x PBS, 50 ml; Cytology Stringency Wash Buffer SSC, 500 ml; Cytology Wash Buffer SSC, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		20

** Using 10 µl probe solution per test. CE IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.*

Background

The ZytoLight® SPEC BCR/ABL1 Dual Color Dual Fusion Probe is designed for the detection of the specific translocations involving the chromosomal region 9q34.12 harboring the ABL1 (a.k.a ABL) gene, and the chromosomal region 22q11.23, harboring the BCR (a.k.a. BCR1) gene. Rearrangements involving t(9;22) (q34.1;q11.2) are observed in approx. 90% of patients with chronic myeloid leukemia (CML) and in approx. 25% of adults with acute lymphoblastic leukemia (ALL). The rearrangements are cytogenetically characterized by the presence of the Philadelphia (Ph) chromosome.
The translocation frequently results in the formation of a chimeric BCR/ABL1 fusion gene on the derivative chromosome 22. The gene product is a BCR/ABL1 protein with abnormal tyrosine kinase activity. In normal cells, ABL1 kinase activity is finely regulated in response to growth factors and other stimuli. The BCR/ABL1 fusion protein leads to constitutive activation of down-stream signaling pathways, including Ras, Jak/Stat and PI-3 kinase. In rare cases the BCR/ABL1 fusion gene is located on chromosomal sites other than the Ph chromosome.
Fluorescence *in situ* Hybridization (FISH) allows for the identification of rearrangements that could otherwise not be detected by conventional karyotyping.

References
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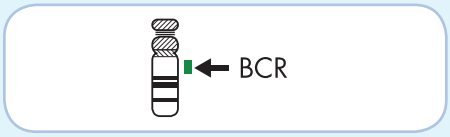
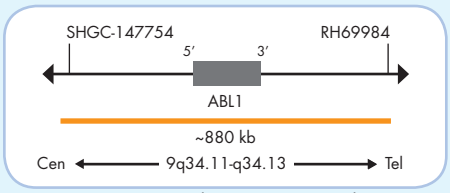
Lim TH, et al. (2005) Ann Acad Med Singapore 34: 533-8.
Primo D, et al. (2003) Leukemia 17: 1124-9.
Rieder H, et al. (1998) Leukemia12: 1473-81.
Sessargeo M, et al. (2000) Haematologica 85: 35-9.
Zheng X, et al. (2009) PLoS One 4: e7661.

Probe Description

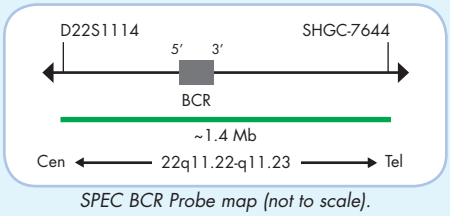
The SPEC BCR/ABL1 Dual Color Dual Fusion Probe is a mixture of a green fluorochrome direct labeled BCR probe spanning the minor and major breakpoint cluster of the BCR gene and an orange fluorochrome direct labeled ABL1 probe spanning the breakpoint region of the ABL1 gene.



Ideograms of chromosomes 22 (above) and 9 (below) indicating the hybridization locations.



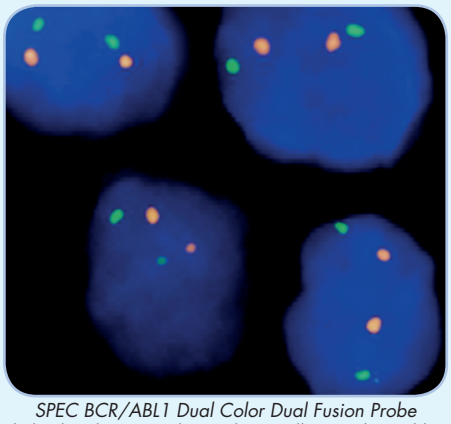
SPEC ABL1 Probe map (not to scale).



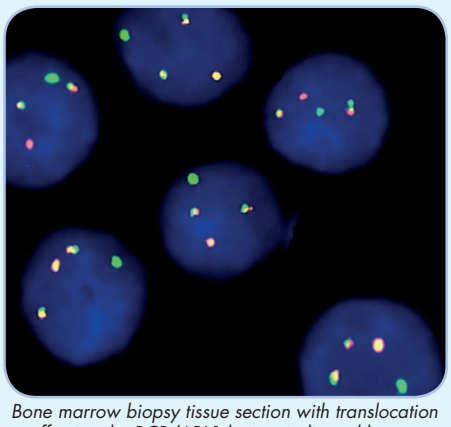
SPEC BCR Probe map (not to scale).

Results

In a normal interphase nucleus, two orange and two green signals are expected. A reciprocal translocation involving two breakpoints splits the two signals and generates a fusion signal on each of the chromosomes involved. The chromosomal regions which are not translocated are indicated by the single orange and green signal.



SPEC BCR/ABL1 Dual Color Dual Fusion Probe hybridized to normal interphase cells as indicated by two orange and two green signals in each nucleus.



Bone marrow biopsy tissue section with translocation affecting the BCR/ABL1 loci as indicated by one separate orange signal, one separate green signal and two orange/green fusion signals.

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