BIOTYPE

Mentype® **AMLplex^{QS}**
PCR Amplification Kit

Instructions for Use

Detection of chromosomal aberrations in acute myeloid leukemia

For in vitro diagnostic use

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Made in Germany

Biotype GmbH develops, produces, and distributes PCR-based applications for medical diagnostics.

Our Mentype® test kits guarantee highest quality standards.

We are at your disposal for further information and suggestions.

Contact us or visit our website www.biotype.de

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Mentype® **AMLplex^{QS}**

1. Intended use

Mentype® **AMLplex^{QS}** is intended as a test for qualitative detection of 34 variants of gene transcripts that can arise in certain subtypes of acute myeloid leukemia (AML) through chromosomal translocations (somatic mutations) involving 11 different gene fusions.

Mentype® **AMLplex^{QS}** applications are intended exclusively for professional use in specialized laboratories. Personnel should be trained in PCR techniques and in the use of in-vitro diagnostic medical devices (IVDD).

2. Background information

The verification of specific chromosomal aberrations has high prognostic value in nearly all types of acute leukemia. Molecular biological evidence of chromosomal aberrations (translocations) represents an important diagnostic completion. Detecting specific translocations enables the subtype classification of leukemic diseases and provides essential information for the risk-directed therapy of patients.

Mentype® **AMLplex^{QS}** facilitates the detection of the most common chromosomal aberrations observed so far in AML and represents a simple-to-use, routine-fit, and reliable screening tool.

3. Product description of Mentype® AMLplex^{QS}

Mentype® **AMLplex^{QS}** contains optimized reagents for the high-resolution detection of 11 fusion gene transcripts (RUNX1-RUNX1T1, BCR-ABL, PICALM-MLLT10, CBFB-MYH11, DEK-NUP214, KMT2A-MLLT4, KMT2A-MLLT3, KMT2A-ELL, KMT2A-PTD, NPM1-MLF1 and PML-RARA) with 34 transcript variants in total (see Table 1).

The test kit includes an internal PCR control (Quality Sensor “QS-Control”) and a “cDNA Control” (ABL-Control), providing helpful information about PCR efficiency, the quality of applied cDNA templates, and the presence of PCR-inhibitors.

The test is performed by fragment analysis using capillary gel electrophoresis. One primer for each transcript is fluorescence-labelled with **6-FAM, BTG** or **BTY**.

Table 1 Gene fusions and transcript variants detectable with Mentype® **AMLplex^QS^**

Gene-Fusions	Chromosomal Aberration	Variants
RUNX1-RUNX1T1 (AML1-ETO)	t(8;21) (q22;q22)	-
BCR-ABL	t(9;22) (q34;q11)	e1a3 e1a2 b3a2 b3a3 b2a2 b2a3
PICALM-MLLT10 (CALM-AF10)	t(10;11) (p13;q14)	MLLT10_240-PICALM_1987 MLLT10_240-PICALM_2092
CBFB-MYH11	inv(16) (p13;q22)	Type A Type B Type C Type D Type E Type F Type G Type H Type I Type J
DEK-NUP214 (DEK-CAN)	t(6;9) (p23;q34)	-
KMT2A-MLLT4 (MLL-AF6)	t(6;11) (q27;q23)	-
KMT2A-MLLT3 (MLL-AF9)	t(9;11) (p22;q23)	6A_(THP-1) 7A_(10A) 8A_(MM6) 6B_(9B)
KMT2A-ELL (MLL-ELL)	t(11;19) (q23;p13.1)	e10e2 e10e3
KMT2A-PTD (MLL-PTD)	Partial Tandem Duplication	e9e3 e10e3 e11e3
NPM1-MLF1	t(3;5) (q25.1;q34)	-
PML-RARA	t(15;17) (q22;q21)	bcr1 (PR-L) bcr2 (PR-V) bcr3 (PR-S)

3.1 Instruments

Mentype® **AMLplex^{QS}** has been validated and evaluated on following PCR cyclers:

- GeneAmp® 9700 Silver Thermocycler (Applied Biosystems™)
- Eppendorf Mastercycler ep-S (Eppendorf AG)
- Biometra T1 (Analytik Jena AG)

The following capillary gel electrophoresis systems running with POP-4™ (Applied Biosystems™) and 36 cm capillary length have been evaluated and validated. Additionally, the application of POP-7™ has been evaluated:

- ABI PRISM® 310 Genetic Analyzer (Applied Biosystems™)
- ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems™)
- ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems™)

Data analysis has been been performed with the following software versions:

- GeneMapper™ ID 3.2 (Applied Biosystems™)
- GeneMapper™ ID-X 1.4 (Applied Biosystems™)

The use of Mentype® **AMLplex^{QS}** with instruments or software other than those mentioned above must be verified and validated by the user.

3.2 Sample type

The Mentype® **AMLplex^{QS}** assay has been validated with cDNA transcribed from RNA isolated from citrated whole blood.

The product Mentype® **AMLplex^{QS}** is validated for an RNA input of 1 µg in a 20 µL transcription reaction. From transcribed cDNA, generally, 1 µL shall be used in the PCR reaction. The use of larger amounts of cDNA must be validated by the user.

4. Warnings and safety instructions

Please refer to the material safety data sheet (MSDS) of Biotype products, which will be sent on request on when writing to support@biotype.de. For SDS of reagents not included in the test kit, please contact the respective manufacturer.

Read the instructions carefully before using the product.

Upon arrival, please check the product and its components for number, type and filling (see Chapter 5.1 Kit content), correct labelling, frozen reagent status, and integrity of reagent packs.

When using the assays, wear gloves, a lab coat, and, if necessary, eye protection.

Avoid nuclease (DNase/RNase) contamination of the samples by using DNase-/RNase-free disposable pipette tips with aerosol-tight filters.

Use separate workspaces and equipment for sample preparation (pre-PCR), master mix preparation, and sample post-processing and analysis (post-PCR). Ensure that the positive controls are stored spatially separated from the other kit components.

Additional controls may be necessary according to the guidelines or requirements of country-specific medical associations, authorities, or accreditation organizations.

Do not use components of the kit that have exceeded their expiration date.

Discard sample and test waste according to local safety regulations.

Until kit lot LEUK01095 (Reaction Mix Alot CH2001597):

The following potentially hazardous substance is contained in this kit:

Table 2. Potentially hazardous substance contained in Mentype® **AMLplex^QS** assays

Kit component	Reagent	Danger
Reaction Mix A	Sodium azide NaN	Toxic if swallowed, toxic upon contact with acids

4.1 Quality assurance

All contents of the test kit have been subjected to intensive quality assurance by Biotype GmbH. The quality of the test kits is continuously checked to ensure unrestricted usability. Please contact us at support@biotype.de for questions regarding quality assurance.

5. Material provided

5.1 Kit content

The Mentype® **AMLplex**^{QS} kit contains the following components, which can be used to perform 25, 100, or 400 reactions:

Table 3. Packaging sizes and included components of Mentype® **AMLplex**^{QS} kit

Component	Volume per Packaging Size		
	25 reactions	**100 reactions**	**400 reactions**
Nuclease-Free Water	1.5 mL	2 x 1.5 mL	6 x 1.5 mL
Reaction Mix A	250 µL	500 µL	2 x 1.0 mL
Mentype® **AMLplex**^{QS} Primer Mix	63 µL	250 µL	4 x 250 µL
Multi Taq 2 DNA Polymerase	10 µL	40 µL	160 µL
Mentype® **AMLplex**^{QS} Control cDNA Kasumi 1	10 µL	10 µL	10 µL
Mentype® **AMLplex**^{QS} Allelic Ladder	25 µL	25 µL	4 x 25 µL
DNA Size Standard 550 (BTO)	13 µL	50 µL	200 µL

Be aware that the kit components of different kit lots must not be mixed. An overview of the lot numbers can be found on the label which is situated on the inside of the box flap. Aliquoting the kit components into other reaction vessels is not permitted.

5.2 Order information

Please order Mentype® **AMLplex**^{QS} kits via mail to sales@biotype.de, including the order numbers from Table 4 or Table 16 (page 27), respectively.

Note: Please note that the packaging size 10 reactions is no longer sold.

Table 4. Ordering numbers for Mentype® **AMLplex**^{QS} kits

Product	Packaging Size	Cat. No.
Mentype® **AMLplex**^{QS}	25 reactions	45-31220-0025
Mentype® **AMLplex**^{QS}	100 reactions	45-31220-0100
Mentype® **AMLplex**^{QS}	400 reactions	45-31220-0400

5.3 Additionally required reagents and equipment not included in the kit

The following reagents provided by Biotype GmbH are required for the initial spectral calibration of the capillary gel electrophoresis device before applying the Mentype® **AMLplex^{QS}** kit:

Table 5 Additionally required reagents provided by Biotype GmbH

Reagent	Application	Packaging Size	Cat. No.
Matrix Standard BT5 single	Spectral calibration for single capillary gel electrophoresis systems	5 x 25 µL	00-10411-0025
Matrix Standard BT5 multi	Spectral calibration for multi capillary gel electrophoresis systems	25 µL	00-10421-0025
Matrix Standard BT5 multi	Spectral calibration for multi capillary gel electrophoresis systems	2 x 25 µL	00-10421-0050

The following general materials and instruments are required for PCR amplification:

- A table centrifuge with a rotor for 2 mL reaction tubes
- 96-well reaction plates or 0.2 mL reaction tubes, for usage with 96-well reaction plates, appropriate lids or sealing film, and a centrifuge with a rotor for microliter plates
- A laboratory shaker (e. g. Vortex) suitable for 96-well reaction plates or reaction tubes
- Pipettes and disposable aerosol-tight filter pipette tips
- Disposable powder-free gloves
- Suitable RNA purification kit (see Chapter 7.1.1, RNA isolation)
- Suitable instrument for the quantitative measurement of RNA concentration after purification (see Chapter 7.1.1, RNA isolation)
- Suitable kit for transcription of RNA into cDNA (see Chapter 7.1.2, Transcription of RNA into cDNA)
- Suitable PCR instrument (see Chapter 3.1, Instruments)
- Hi-Di™ Formamide (Thermo Fisher Scientific Inc., Waltham, US)
- Consumables and reagents for capillary gel electrophoresis systems
- Suitable capillary gel electrophoresis instrument (see Chapter 3.1, Instruments)

Note: Make sure that all devices are installed, maintained, and calibrated according to the manufacturers' instructions. Make sure that all reagents required for operating the respective PCR and capillary gel electrophoresis devices are present (for use, see instructions of the respective device manufacturer).

6. Storage

Mentype® **AMLplex^QS^** kits are delivered on dry ice. The ingredients of the assays are frozen. If one or more components are not frozen after reception or if the tubes have been damaged during transportation, please contact Biotype GmbH for further assistance (support@biotype.de).

The components must be stored at a temperature between -25 °C and -15 °C. The Control cDNA samples and post-PCR reagents (Allelic Ladder and DNA Size Standard 550) should be stored separately from PCR reagents.

Frequent thawing and freezing should be avoided; a maximum of 20 freeze-thaw cycles should not be exceeded.

The Mentype® **AMLplex^QS^** kits must be stored protected from light.

The shelf life of the test kit is indicated on the packaging label.

7. Working Procedures Mentype® AMLplex^QS^

7.1 Sample preparation and cDNA insert volume

7.1.1 RNA isolation

The quality of the isolated DNA has an important influence on the performance and quality of the entire test system. It must be ensured that nucleic acid isolation methods that are employed are compatible with PCR technology.

The following kits have been tested and found suitable for nucleic acid isolation:

- RNeasy Mini Kit (Qiagen GmbH, Hilden, DE)
- RNeasy Plus Mini Kit (Qiagen GmbH, Hilden, DE)

The usage of alternative RNA isolation kits must be validated by the user.

Note: For accurate results, RNA quantification is required (e. g. RNA quantification by UV/VIS-spectroscopy at A260 nm and quality determination by A260/A280 ratio, which should be between 1.7 and 2.0).

7.1.2 Transcription of RNA into cDNA

After isolation and quantification of the RNA, the RNA sample needs to be transcribed into cDNA. An RNA input of 1 µg should be used in a final reaction volume of 20 µL for the transcription.

The following kits have been tested and found suitable for the transcription reaction:

- High Capacity cDNA Transcription Kit (Thermo Fisher Scientific Inc., Waltham, US)
- QuantiTect Reverse Transcription Kit (Qiagen GmbH, Hilden, DE)

The usage of alternative transcription kits must be validated by the user.

7.1.3 Template cDNA

The Mentype® **AMLplex^{QS}** kit is optimized for the use of 1 µL of transcribed cDNA, as shown in chapter 7.1.2.

The amount of applied template cDNA can be increased for critical clinical samples. The maximum template amount should not exceed 1/10th of the RT-reaction volume. This procedure must be validated by the user.

7.2 Applying the master mix

All reagents should be mixed thoroughly (vortex) and centrifuged briefly (approx. 10 seconds) before applying the master mix. The total volume of the PCR mixture should always be 25 µL.

Consider the positive and negative controls for the number of PCR reactions to be applied. Add one or two additional reactions to the total number to compensate for pipetting errors.

Table 6 shows the volumes of kit components used with 1 µL sample volume (template cDNA) and a total reaction volume of 25 µL.

Table 6. Master mix set-up for one reaction Mentype® **AMLplex^QS^** using 1 µL cDNA

Component	Volume per PCR Reaction
Nuclease-Free Water	16.1 µL
Reaction Mix A*	5.0 µL
Mentype® **AMLplex^QS^** Primer Mix	2.5 µL
Multi Taq 2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µL
Total volume of Master Mix	**24.0 µL**
Template cDNA	1.0 µL

* contains Mg^{2+}, dNTPs, BSA

7.2.1 Positive control

First, make a one in two dilution of the Control cDNA Kasumi 1, which is included in the kit, with nuclease-free water (final concentratuion of 250 ng/µL, e.g. 1 µL Control cDNA Kasumi 1 + 1 µL Nuclease-Free Water).

When using Control cDNA Kasumi 1 as a positive control, use 1 µL of the diluted Control cDNA Kasumi 1 instead of template cDNA.

Table 7 shows the volumes of the kit components used with 1 µl of the Control cDNA and a reaction volume of 25 µL.

Table 7. Master Mix set-up for Mentype® **AMLplex^QS^** kit using 1 µL Positive Control Sample

Component	Volume per qPCR Reaction
Nuclease-Free Water	16.1 µL
Reaction Mix A*	5.0 µL
Mentype® **AMLplex^QS^** Primer Mix	2.5 µL
Multi Taq 2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µL
Total volume of Master Mix	**24.0 µL**
Diluted Control cDNA Kasumi 1	1.0 µL

* contains Mg^{2+}, dNTPs, BSA

7.2.2 Negative control

As a negative control, pipette 1 µL of nuclease-free water instead of template-DNA into the reaction vessels containing the PCR master mix.

Table 8 shows the volumes of the kit components used with 1 µL of nuclease-free water and a reaction volume of 25 µL.

Table 8. Master Mix set-up for Negative Control of Mentype® **AMLplex^{QS}** kit using 1 µL nuclease-free Water

Component	Volume per PCR Reaction
Nuclease-Free Water	16.1 µL
Reaction Mix A*	5.0 µL
Mentype® **AMLplex^{QS}** Primer Mix	2.5 µL
Multi Taq2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µL
Total volume of Master Mix	**24.0 µL**
Negative Control in form of nuclease-free water	1.0 µL

* contains Mg^{2+}, dNTPs, BSA

In addition, an already known cDNA that has tested negative for the detectable gene fusions and translocations may be included as a negative control sample. This sample is to be processed like a normal sample and used in the PCR as an extra reaction.

7.3 Reaction volume

Pipette 24 µL of the PCR Master Mix (without template cDNA) into the reaction tubes or the multi-well plate . Then add 1 µL of the cDNA and 1 µL of positive or negative control.

The reaction vessels or the multi-well plates should be tightly sealed (caps, sealings) after pipetting. Mix and centrifuge the reaction tubes briefly and place them in the PCR cycler for amplification.

8. PCR program and amplification

Perform a “hot start” PCR in order to activate the Multi Taq 2 DNA Polymerase and to prevent the formation of unspecific amplification products.

The internal ABL-Control may serve as a point of reference to evaluate the optimal number of required PCR cycles. The optimal range of the internal ABL-Control should not exceed the specified measuring range of the instrument used herein (for example 500-5 000 RFU on the ABI 3130)

Very small amounts of cDNA may result in statistical dropouts and imbalances of the peaks. Increasing the number of PCR cycles raises the risk of cross-contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

Note: To provide an optimal kit balance, the ramping rate of the thermal cycler should be adjusted to 4 °C/s.

Table 9. PCR amplification parameter to be used for Mentype® **AMLplex^QS^**

Temperature	Time	Cycles
96 °C	4 min	1 x (hot start to activate Multi Taq2 DNA Polymerase)
96 °C	30 s	
61 °C	**120 s**	22-28 x
72 °C	75 s	
68 °C	10 min*	1 x
10 °C	∞	hold

* If a higher amount of minus -Adenine peaks (-1 bp) is observed, extension up to 60 min is possible.

The number of PCR cycles depends on the amount of cDNA applied and the expression levels of the transcript variant to be detected. PCR cycles between 22 and 28 have previously been tested. For reference samples from cell cultures (high expression levels) a reduction to 22 PCR cycles is recommended.

To achieve the highest analytical sensitivity (11.1 Detection limit), the maximal cycle number of 28 cycles is recommended.

9. Capillary gel electrophoresis

9.1 Preparation of the PCR products

After the completion of the PCR, remove the samples from the cycler and centrifuge briefly. Thaw the reagents Hi-Di™ Formamide (not included in the kit) and DNA Size Standard 550 (BTO), mix and centrifuge the tubes briefly. Prepare the approach described in Table 10 consisting of Hi-Di™ Formamide and the DNA Size Standard 550 (BTO), add one or two reactions to the approach to compensate for pipetting variations.

Table 10. Approach of the denaturation mixture containing Hi-Di™ Formamide and DNA Size Standard 550 (BTO)

Componente	Volume per reaction
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL

Pipette 12 µL of the denaturation mixture of formamide and BTO in the appropriate number of wells in a PCR plate (suitable for use in the Genetic Analyzer). Then add either 1 µL PCR reaction or 1 µL Mentype® **AMLplex^QS^** Allelic Ladder provided with the kit to each well. Seal the PCR plate with a suitable foil, vortex and centrifuge the plate briefly.

Note: The allelic ladder is used to correctly determine the fragments analyzed during data analysis. In each fragment length analysis run, the allelic ladder must be analyzed at least once to ensure successful data analysis.

Note: The capillaries of the gel electrophoresis device should never run dry. If the samples do not occupy all capillary positions, fill the additional wells of the plate with 12 µL Hi-Di™ Formamide according to the capillary number

Denature the prepared PCR products on a PCR cycler for 3 minutes at 95 °C, cool the samples to 4 °C in the cycler. Centrifuge the samples briefly before fragment length analysis.

9.2 Fragment length analysis

After the spectral calibration of the capillary gel electrophoresis device with the reagent Matrix Standard BT5 (Biotype GmbH) has been successfully run, create a specific run module (ABI 310, ABI 3130) or instrument protocol (ABI 3500) with the following parameters:

Table 11. Specific parameters for the run module resp. instrument protocol of the capillary gel electrophoresis device

	ABI 310	ABI 3130	ABI 3500
Injections Voltage [kV]	15.0	3.0	3.0
Run Time	28 min	1560 s	1560 s
Injection Time [s]	5	10	8

Differing from the values given in Table 11, the run time can be adjusted to analyze all fragments (60-550 bp) of DNA Size Standard 550 (BTO).

Note: Follow the instructions for use of the manufacturer of the capillary gel electrophoresis device to set the specific running parameters.

Note: Also refer to the additional information leaflets available for calibration and application of the Mentype® products on the capillary gel electrophoresis instruments. These are supplied on request via support@biotype.de by Biotype GmbH.

10. Data Analysis

10.1 Software and analysis templates

The data analysis is carried out with the software GeneMapper™ ID or GeneMapper™ ID-X (Applied Biosystems™).

For easy data analysis, Biotype GmbH offers at www.biotype.de/en ready-made settings (Table 12) that can be imported into the corresponding GeneMapper™ version and replace the manual creation of the analysis parameters.

Note: The import and allele assignment with the help of the offered evaluation templates can only be guaranteed for the GeneMapper™ ID/ID-X software. If you are using GeneMapper™, problems may occur when importing the evaluation templates.

Note: The available Bins and Panel Set templates define the length of the individual fragments. Slight differences in the performance of different capillary gel electrophoresis devices may cause slight deviations between the devices. For a specific adaptation of the bins and panels by Biotype GmbH, please contact us via support@biotype.de.

Table 12 Overview over available import templates fpr GeneMapper™ ID/ID-X

Template	Name	
Panels	AMLplex_Panels_v2/v2x	Or higher version
BinSets	AMLplex_Bins_v2/v2x	Or higher version
Size Standard	SST-BTO_60-550bp	
Analysis Method	AMLplex_HID_310_200RFU	Recommended
	AMLplex_HID_3130_200RFU	Recommended
Plot Settings	PlotsBT5_4dyes	
Table Setting	Table for 10 Alleles	
	Table for 22 Alleles	

If the Analysis Method is created manually, the following parameters need to be selected (Tabel 13):

Table 13 Parameter to be selected when creating the Analysis Method manually at GeneMapper™ ID/ID-X

Parameter	Setting
Peak Detection Algorythm	Advanced
Allele	No specific stutter ratio, set all to 0.0 Amelogenin cut off: 0.0
Ranges	Analysis: Full Range Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B: 200; Y: 200; G: 200; R: 200; O: 50 Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts* Slope Thresholds: 0.0
Peak Quality	Heterozygote Balance: 0.0 Max expected alleles: 22

* If necessary, the Peak Window Size can be deceased to 11 pts

10.2 Procedure for data evaluation

10.2.1 General minimum criteria for data evaluation

The fsa files of the capillary gel electrophoresis are qualitatively evaluated, meaning that they are checked for the presence of amplicon peaks. In this case, a peak must reach at least 200 RFU height and should be at least three times as large as the background noise of the baseline. These criteria apply to control peaks (QS and ABL control) as well as to peaks of the gene fusions.

The only exception is the DNA Size Standard 550 (BTO), for which a minimum peak height of 50 RFU needs to be achieved.

10.2.2 Checking the DNA Size Standard 550 (BTO)

The determination of the exact lengths of the amplified products depends on the DNA Size Standard that is applied. Consequently a size standard with evenly distributed references

should be preferentially used. Therefore, the DNA Size Standard 550 (BTO, Figure 1) shall be used with the following lengths of fragments: 60, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 425, 450, 475, 500, 525, and 550 bp.

Check the electropherogram (orange channel) of all samples to ensure that all alleles of the size standard are present, have a sufficient peak height of at least 50 RFU, and have been correctly assigned (see Figure 1).

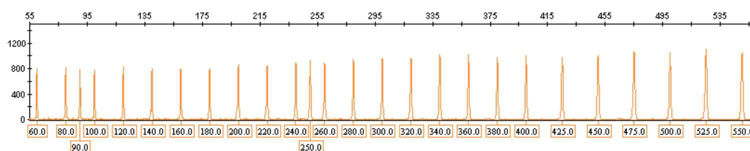


Figure 1 Electropherogram of the DNA Size Standard 550 (BTO), analyzed on ABI 3500, software GeneMapper™ ID-X 1.4, template files v3x, y axis 55-600 bp, x axis 0-1 500 RFU

Note: If not all fragments of the size standard are analyzed in the samples, an inadequate evaluation of the allelic ladders and samples may occur. Always check the size standard for successful analysis.

10.2.3 Checking the Allelic Ladder

The Allelic Ladder contains all fragments detectable with the Mentype® **AMLplex^QS** (see Table 1 and Figure 2). Accordingly, these fragments must be present in the Allelic Ladder and must be detected with at least 200 RFU. The fusion genes and the associated transcript variants are always in one color channel.

Table 14 Overview over the distribution of the gene fusion in the three relevant color channels

Blue Channel	Green Channel	Yellow Channel
CBFB-MYH11	DEK-NUP214 (DEK-CAN)	PML-RARA
BCR-ABL	KMT2A-MLLT4 (MLL-AF6)	
PICALM-MLLT10 (CALM-AF10)	KMT2A-MLLT3 (MLL-AF9)	
RUNX1-RUNX1T1 (AML1-ETO)	KMT2A-ELL (MLL-ELL)	
NPM1-MLF1	KMT2A-PTD (MLL-PTD)	
QS and ABL Kontrolle		

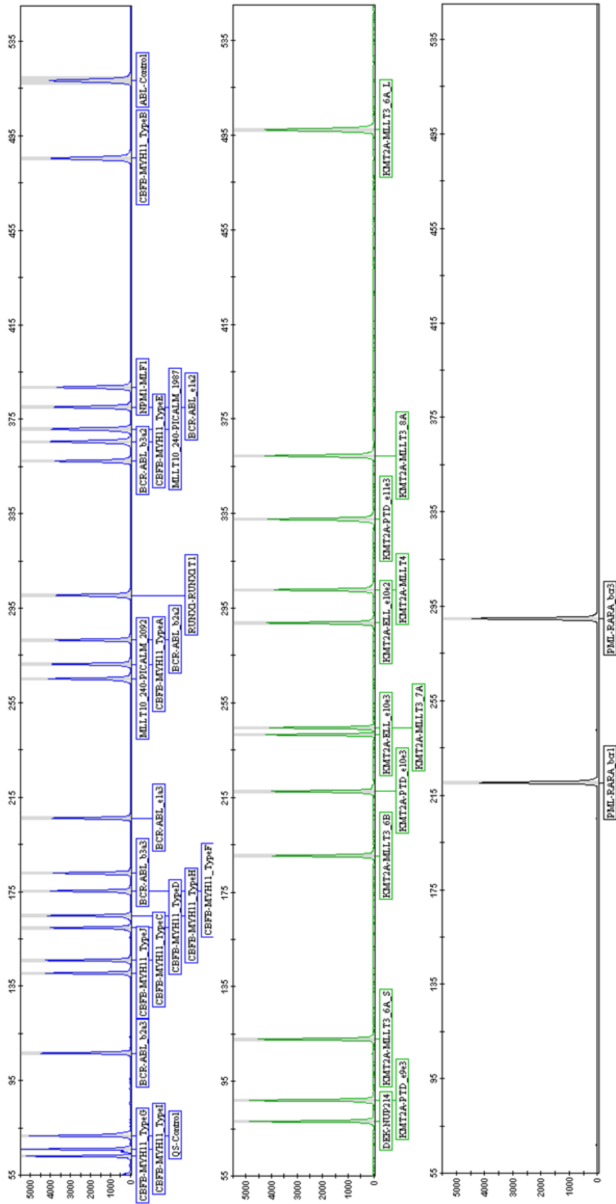


Figure 2 Electropherogram of the Mentype® **AMLplex^{QS}** Allelic Ladder, analyzed on ABI 3500, software GeneMapper^{TM} ID-X 1.4, template files v3x, y axis 55-500 bp, x axis 0-5 500 RFU

Note: If not all fragments of the Allelic Ladder are named automatically when using the evaluation templates (especially of the Bins and Panels Set), please contact support@biotype.de, as it may be necessary to adopt the templates to your setting. In such a case, a missing assignment of the peaks in the samples is possible.

10.2.4 Checking the control cDNA Kasumi 1

The kit contains the Mentype® **AMLplex^{QS}** Positive Control cDNA Kasumi 1*, which is positive for the gene fusion RUNX1-RUNX1T1 (AML1-ETO).

Check that the control peaks QS-Control and ABL-Control have sufficient height. Check that the peak for the translocation RUNX1-RUNX1T1 appears with sufficient height in the electropherogram (Figure 3). Check that no unexpected by-products appear in the electropherogram.

* The cell culture for the preparation of the cDNA was obtained from: DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany. The use of this cDNA is intended exclusively for the Mentype® **AMLplex^{QS}**.

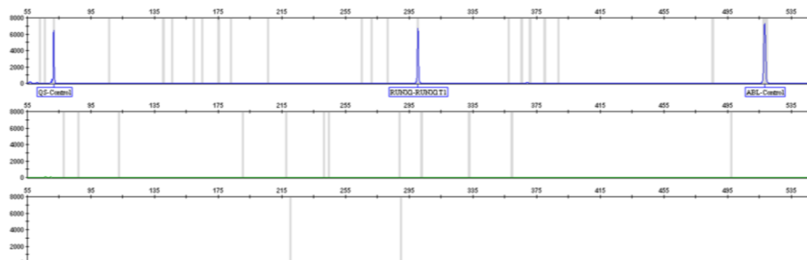


Figure 3 Electropherogram of Control cDNA Kasumi 1, analyzed on ABI 3500, GeneMapper™ ID-X 1.4, template files v3x, y axis 0-8 000 RFU, x axis 55-550 bp

10.2.5 Checking the negative control

Check that no translocation-specific peaks greater than 200 RFU appear in the electropherogram (channels blue, green, yellow).

No-Template Control: Check that only the control peak QS-Control with sufficient altitude appears, but not the ABL-Control Peak (Figure 4).

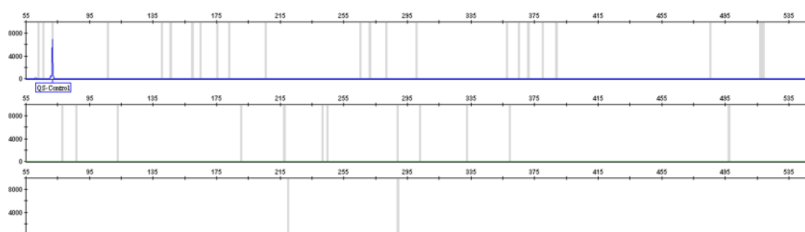


Figure 4 Electropherogram of a No-Template Control, analyzed on ABI 3500, GeneMapper™ ID-X 1.4, template files v3x, y axis 0-10 000 RFU, x axis 55-550 bp

Negative control sample (not included in the kit): Check that a known cDNA, which is negative for the detectable gene fusions and translocations, shows control peaks QS-Control and ABL-Control of sufficient height (Figure 5).

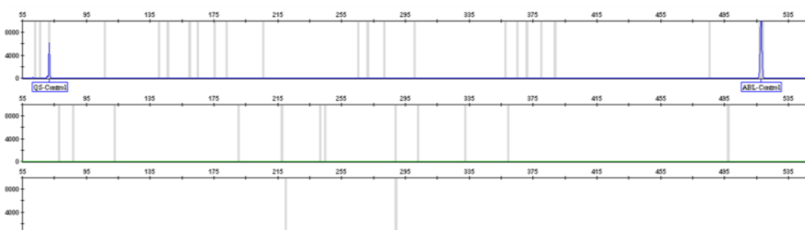


Figure 5 Electropherogram of a negative control sample, analyzed on ABI 3500, GeneMapper™ ID-X 1.4, template files v3x, y axis 0-10 000 RFU, x axis 55-550 bp

10.2.6 Sample data analysis

After checking the DNA Size Standards, Allelic Ladders, and the control samples, the sample data have to be evaluated.

Note: The Mentype® **AMLplex^QS^** is an exclusively qualitative test. A quantitative evaluation, e. g. in the context of an MRD determination, is explicitly not possible.

When using the evaluation templates of Biotype GmbH and successful evaluation of the Allelic Ladder of the run, the detected PCR fragments are named automatically. An overview of the fragment length of the PCR products can be found in Table 15.

Note: Mentype® **AMLplex^QS^** has been validated and certified on POP-4™. The use of another polymer (e. g. POP-7™ or POP-6™) can alter the running behavior of specific PCR products. The adaptation of the Biotype templates (Panels and BinSet) may be necessary. Please contact our technical support (support@biotype.de) for this purpose. In addition,

increased background noise has been observed due to altered behavior of free fluorescent dye residues.

Table 15 Overview over the fragment lengths of the translocations in a Allelic Ladder of Mentype® **AMLplex^QS^**, determined using POP-4™; ‡ Two amplicons for variant MLL-AF9_6A; * Although this variant is detectable with Mentype® **AMLplex^QS^** primers, the varying length of the amplicon (apprx. 173 bp) prevents automated allocation.

Panel/Translocation	Heigth [bp]	Panel/Translocation	Heigth [bp]
Blue Channel		**Green Channel**	
CBFB-MYH11_TypeG	63	DEK-NUP214	78
CBFB-MYH11_TypeI	66	KMT2A-PTD_e9e3	87
QS-Control	72	KMT2A-MLLT3_6A_S‡	113
BCR-ABL_b2a3	107	KMT2A-MLLT3_6B	191
CBFB-MYH11_TypeJ	141	KMT2A-PTD_e10e3	218
CBFB-MYH11_TypeC	146	KMT2A-ELL_e10e3	242
CBFB-MYH11_TypeD	160	KMT2A-MLLT3_7A	245
CBFB_MYH11_TypeH	165	KMT2A-ELL_e10e2	289
CBFB_MYH11_TypeF	175	KMT2A-MLLT4	303
BCR-ABL_b3a3	183	KMT2A-PTD_e11e3	333
BCR-ABL_e1a3	206	KMT2A-MLLT3_8A	360
MLLT10_240-PICALM_2092	265	KMT2A-MLLT3_6A_L‡	498
CBFB-MYH11_TypeA	271	**Yellow Channel**	
BCR-ABL_b2a2	282	PML-RARA_bcr1	220
RUNX1-RUNX1T1	301	PML-RARA_bcr3	288
BCR-ABL_b3a2	358	PML_RARA_bcr2*	
CBFB-MYH11_TypeE	365		
MLLT10_240-PICALM_1987	371		
BCR-ABL_e1a2	380		
NPM1-MLF1	389		
CBFB-MYH11_TypeB	486		
ABL-Control	518		

11. Troubleshooting

As mentioned above, post-PCR analysis and automatic allele assignment with suitable analysis software ensure the precise and reliable discrimination of fusion gene transcripts and variants. Please check for correct allelic ladder assignment within each run.

11.1 Detection limit

Using plasmids and 25 amplification cycles, a detection limit of ≤ 1 000 copies could be determined for 32 of 34 transcript variants. The transcript variants CBFB-MYH11_Type C and KMT2A-MLLT4 (MLL-AF6) deviate from these findings. With 28 amplification cycles, a detection limit of 1 000 copies for CBFB-MYH11_Type C and of 10 000 copies for KMT2A-MLLT4 (MLL-AF6) could be established. When the afore mentioned copy numbers were present, peaks of > 200 RFU were detected.

Please note that Mentype® **AMLplex^{QS}** has been designed, validated, and certified as a screening tool for the subtype classification of AML. This application is not suited to quantify copy numbers or monitor Minimal Residual Disease (MRD).

11.2 Pull-up peaks

Pull-up peaks may occur if peak heights of the PCR product are outside the linear detection range of the instrument, or if an incorrect matrix has been applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities.

Note: If necessary, please dilute the PCR product prior to capillary gel electrophoresis to confirm results. If pull-up effects persist despite optimal peak heights, a new matrix run should be performed.

11.3 Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq 2 DNA Polymerase tends to add an adenosine radical at the 3’-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All Biotype primers are designed to minimize these artefacts. Artefact formation is further reduced through the final extension step of the PCR protocol at 68 °C for 10 minutes. Peak height of the artefact correlates with the amount of cDNA. Laboratories should define their individual limits for analysis of the peaks.

11.4 Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, due to which shoulder peaks or split peaks may occur. Furthermore, automated assignment could be influenced in some cases. In such cases, we recommend injecting the sample again at higher room temperature and maybe using more than one allelic ladder sample per run. Pay attention to keep ambient conditions as recommended by the instrument manufacturer. Optimal settings were reported to be > 22 °C room temperature.

11.5 Influence of polymer types

The Mentype® **AMLplex^QS^** kit has been validated and certified for analysis on POP-4™ polymer. The use of other polymers (e. g. POP-7™ or POP-6™) might influence the run behavior of specific PCR products. In certain cases, Biotype Templates (Panels and BinSet) may have to be adjusted. In such cases, please contact our technical support (support@biotype.de). Furthermore, background noise might increase due to different behavior of free fluorescent dyes.

12. Order information

Table 16. Ordering information for Mentype® **AMLplex^{QS}** kits

Kit	Packaging Size	Order Number
Mentype® **AMLplex^{QS}**	25 reactions	45-31220-0025
Mentype® **AMLplex^{QS}**	100 reactions	45-31220-0100
Mentype® **AMLplex^{QS}**	400 reactions	45-31220-0400

13. References

Asou H, Tashiro S, Hamamoto K, Otsuji A, Kita K, Kamada N (1991)
Establishment of a human acute myeloid leukemia cell line (Kasumi-1) with 8;21 chromosome translocation. *Blood 77(9): 2031-2036*.

Beillard E, Pallisgaard N, van der Velden VHJ, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJM, Hokland P, Gabert J (2003)
Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using ‚real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR)- a Europe against cancer program. *Leukemia 17:2474-2486.*

Van Dongen JJM, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, DOtti G, Griesinger F, Parreira A, Gameiro P, Gonzalez Diaz M, Malec M, Langerak AW, San Miguel JF, Biondi A (1999)
Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease - Report of the BIOMED-1 Concerted Action: Investigation of minimal residual disease in acute leukemia. *Leukemia 13:1901-1928.*

14. Trademarks and disclaimers

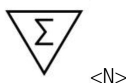
Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law: Biotype®, Mentype® (Biotype GmbH); ABI PRISM®, GeneMapper™, Hi-Di™ Formamide, POP-4™, POP-6™, POP-7™, Applied Biosystems™ (Thermo Fisher Scientific Inc.); FAM™ (Life Technologies Ltd.).

Mentype® **AMLplex^{QS}** kits are CE-marked according to the Directive 98/79/EC of the European Parliament on in vitro diagnostic devices. The kits are not available as in-vitro diagnostic devices outside this regulatory area.

15. Symbols

Manufacturer

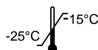
Lot number



Contains sufficient reagents for <N> tests

Reference to eIFU

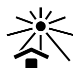
Use by



Temperature limit

Catalogue number

For in vitro diagnostic use



Protect from light

Keep dry

Verification and validation of Mentype® AMLplex^{QS} PCR amplifications kits

A Analytical performance data (verification)

A a) Determination of the standard reaction and batch specific tolerance

Objective: Determination of the standard reaction and batch-specific tolerances of the multiplex PCR with respect to the absolute signal heights (RFU), the balance of the signal heights, and the base line. In addition, the test-specific device settings for genotyping using capillary electrophoresis (bins and panels) are determined through the evaluation templates of the DNA-sequencing machines.

Methods: A control cDNA of the cell line KASUMI-1 (ACC220, Leibniz Institute DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig) is included in the test kit and contains the gene fusion RUNX1-RUNX1T1 (AML1-ETO) with the chromosomal aberration t (8; 21) (q22, q22) [7]. Additionally, an artificial equimolar template mixture of plasmids containing 33 of the 34 detectable variants was applied. The standard reaction was performed with the control cDNA at the nominal concentration of 250 ng per PCR amplification with 25 PCR cycles. The template mixture was adjusted so that signal heights fell into the linear measuring range of the analyzers (5 000 RFU max.) when using 25 PCR cycles. A four-fold determination with additionally four blank values (no template control, NTC) without DNA was carried out.

Results: The following specifications were determined for the batch-specific mixing of the PCR primers when template-mixes are applied: Using an ABI PRISM® 310 Genetic Analyzer, signal heights of 1 000-4 000 RFU and using an ABI PRISM® 3130 Genetic Analyzer were signal levels of 1 000-5 000 RFU. Specific signals are detected from a signal amplitude of 200 RFU. In the scaling range, no unspecific signals (free dyes, artefacts) > 200 RFU were detected for the blank values (baseline).

A b) Accuracy of the measurement

Objective: Information of the accuracy of the method of measurement, and a sufficiently detailed summary of the data to assess whether the means of ascertaining the accuracy are appropriate. Accuracy measurements for quantitative and qualitative tests can only be issued if a reference standard or method is available.

Methods: The kit is validated through regular participation in ring trails with regard to the correct qualitative statement (diagnostics). Biotype has been participating regularly since June 3, 2013 in the United Kindom National Quality Assessment Scheme (UKNEQAS, www.ukneqas.org.uk) for the detection of BCR-ABL and AML translocations. Trails are carried out regularly (2 x) per year and are evaluated by the authority. The results (performance status) in comparison with other participants are also published.

Results: Biotypes performance status is "Green". The product is demonstrably suitable for the detection of BCR-ABL variants and AML translocations and

achieves the correct results (qualitative) in comparison with other molecular genetic methods.

A c) Analytical specificity

A c) a) Analytical specificity of negatively pre-typed cDNA

Objective: The purpose of this study is to exclude false-positive results due to interference and cross-reactivity with selected cDNAs from negatively pre-typed samples (patients and healthy donors).

Methods: 22 cDNAs negatively pre-typed for the translocation variants detectable with the test kit were tested. The amounts of cDNA used should cover the range of concentrations expected in clinical practice and ranged from 145 ng to max. 934 ng per PCR batch run with 25 cycles.

Results: No cross-reactivity (> 200 RFU) was found in the allele range determined by the bins and panels. The measurement signal for the internal cDNA control (ABL gene) was > 200 RFU for 21 cDNAs - in one case approximately 50 RFU. The automatic allele allocation limit was set to 200 RFUs.

A c) b) Analytical specificity of positively pre-typed cDNA

Objective: The investigations served to exclude false-negative results due to interference and cross-reactivity of the primers with selected cDNAs from positively pre-typed patients.

Methods: 20 cDNAs positively pre-typed for the translocation variants detectable with the test kit were tested. The amount of cDNA was adjusted to 250 ng per PCR amplification. The PCR program was performed with 25 cycles.

Results: All somatic mutations were clearly identified. No cross-reactivity (> 50 RFU) was observed in the allele area. The measurement signals for the somatic mutations and the internal cDNA control (ABL gene) were > 200 RFU in 16/20 samples and > 50 RFU for 3/20 samples. One sample reached values below 50 RFU, but the fusion gene variant was still detectable. The automatic allele allocation limit was set to 200 RFUs.

A d) Analytical sensitivity

Objective: The analytical detection limit of the test should be determined (sensitivity).

Methods: A dilution series with 1 µg to 31.25 ng of reference cDNA (Kasumi-1) was tested in quadruplicates. The PCR program was performed with 25 cycles. In addition, for each transcription variant to be detected, the dilution series of an artificially prepared reference template (plasmide encoded gene fusion inserts, GeneArt/Life Technologies, Regensburg, DE)) with a fixed number of copies were tested in duplicates.

Results: Up to a cDNA concentration of 62.5 ng, signal intensities > 200 RFU could be achieved for the specific translocations and for the ABL control. At

31.25 ng, the observed signal intensity values of the specific variant were > 200 RFU, and the ABL control reached > 50 RFU. Optimal values with respect to the measuring range of the capillary sequencer were achieved in the range of 150-250 ng. The measurements of the plasmid dilutions showed a detection limit > 200 RFU and 100-1000 copies that can be achieved for all translocation variants.

A e) Assay performance with different PCR thermocyclers

Objective: PCR thermocyclers from different manufacturers differ in their specifications. In particular, different heating and cooling rates as well as different temperature control techniques can be present.

Methods: The standard reactions with control cDNA in the nominal concentration of 250 ng were carried out with the following thermocyclers in four-fold determination with the same mastermix and two blank samples (without DNA): Thermocycler Eppendorf Mastercycler ep-S (Eppendorf AG, Hamburg), Applied Biosystems® GeneAmp 9700 with silver block (Life Technology GmbH, Darmstadt), Applied Biosystems® GeneAmp 9700 with aluminum block (Life Technology GmbH, Darmstadt).

Results: Correct assignments of all amplificates was observed on all types of thermocycler. All fragments of the template mixture were successfully amplified.

A f) PCR annealing temperatures

Objective: To determine the robustness of the PCRs, temperature fluctuations were simulated for the primer annealing step in the multiplex PCR. This temperature step is critical for the sensitivity and specificity of the PCRs.

Methods: The kit-specific annealing temperature of 60 °C for the standard reaction with control cDNA in the nominal concentration of 250 ng was varied by ± 1 °C and ± 2 °C. A three-fold determination with the same master mix was applied.

Results: The kit proved to be stable at ± 1 °C by the specified annealing temperature. Optimum signal heights of all systems are reached with the annealing temperature of 61 °C.

A g) Fluctuations of PCR buffer batches

Objective: The concentration ratios of the ingredients of the PCR buffer REM A (dNTPs, ion concentrations, in particular Mg^{2+}) are critical for the sensitivity, specificity and balance of the signals in the multiplex PCR. Therefore the robustness of the test is analyzed for batch fluctuations of the supplied PCR buffer.

Methods: Three independent REM-A batches were tested in the standard reaction with control DNA of the nominal concentration of 250 ng, as well as the allelic-ladder and a cDNA line with weak expression (KMT2A-MLLT4 (MLL-AF6)).

Results: Each newly produced batch REM A will be tested with the Mentype® **AMLplex^{QS}** amplification kit. Approval of respective REM A batch will be provided only if the results obtained with the Mentype® **AMLplex^{QS}** are within the specification.

A h) In-use stability

Objective: The stability of the reagents of the PCR kit was tested after repeated freezing and thawing.

Methods: The kit reagents were subjected to a 20-fold freezing and thawing cycle. The freezing was carried out for at least 1 h at -20 °C. The mixture was thawed at room temperature and the reagents were homogenized by shaking before use. Subsequently, a standard reaction with control DNA of the nominal concentration of 250 ng and additional blank values without DNA was performed in triplicate determinations. The evaluation was carried out in comparison to a standard reaction without any freezing and thawing cycle.

Results: The deviation of averaged peak heights compared to the standard reaction was a maximum of 20 % (in particular signal loss). No additional peaks > 50 RFU were found within the scaling range for the blanc values.

B Clinical performance data

B a) Study design, ethics and regulatory aspects

A clinical performance study was conducted according to §§ 20 - 24 of German Medizinproduktegesetz. The protocol was approved by the National Competent Authority BfArM according to § 7 German Verordnung über klinische Prüfungen von Medizinprodukten and by the institutional ethics committee. All participants (10 volunteers, 297 patients) have given written informed consent.

B b) Reference methods

The primary target is the determination of diagnostic sensitivities and specificities in comparison to the reference methods. Standardized cytogenetic methods (karyotyping, FISH analyzes) were available for a selection of the translocations [8]. Validated and established monoplex-nested PCR assays were used for translocations that could not be addressed cytologically [9, 10].

B c) DNA extraction and purification

Mononuclear cells (MNC) were collected from heparinized whole blood through density gradient centrifugation. Subsequently, total mRNA was isolated using commercially available mRNA extraction kits (RNeasy Mini Kit, Qiagen GmbH, Hilden, DE). Reverse transcription to cDNA was performed with commercially available kits. The quality of the cDNA was analyzed through real-time PCR (QuantiTect Reverse Transcription Kit, Qiagen GmbH, Hilden, DE). Validated in-house single PCR assays served to confirm the fusion genes and mutations.

B d) Results

The Mentype® **AMLplex**^{QS} PCR amplification kit showed no false positive results by the analysis of the cDNA from 10 healthy volunteers. In total, 297 patient samples were examined. Out of these, five samples could not be evaluated (control signal for ABL below the recommended threshold). Of the remaining 292 samples, 199 were found to be correctly negative as compared to karyotyping, FISH and/or control PCR. Of the correctly negative samples, 56 in the karyogram showed genetic changes (chromosomal anomalies), which could not be recognized by the test kit. This can be explained by the fact that the Mentype® **AMLplex**^{QS} PCR amplification kit, although containing the most frequent translocations, only covers approximately 37 % of the genetic abnormalities frequently observed in AML [8]. The individual results of the comparison tests are summarized in Table 17.

Overall, a diagnostic sensitivity of 94 % and a diagnostic specificity of 99.5 % were achieved. All cytogenetic findings verified by control PCR [9, 10] were clearly confirmed.

Table 17 Conclusion of the clinical performance data, *prevalence data was cited from Grimwade et al. 2010

Biomarker			Evaluation of Clinical Performance Testing (n=292)						
Gene Fusion	Chromo. Aberration	Variant	Prev. [%] *	Correct Positive	Correct Negative	False Positive	False Negative	Diagnostic Sensitivity [%]	Diagnostic Specificity [%]
RUNX1-RUNX1T1	t(8;21) (q22;q22)	NA	7	14	277	0	1	93.3	100.0
BCR-ABL	t(9;22) (q34;q11)	e1a3 e1a2 b3a2 b3a3 b2a2 b2a3	1	1	291	0	0	100.0	100.0
PICALM-MLLT10	t(10;11) (p13;q14)	MLLT10_240-PICALM_1987 MLLT10_240-PICALM_2092	1	0	262	0	0	NA	100.0
CBFB-MYH11	inv(16) (p13;q22)	Type A Type B Type C Type D Type E Type F Type G Type H Type I Type J	5	25	264	1	2	92.6	99.6
DEK-NUP214	t(6;9) (p23;q34)	NA	1	3	289	0	0	100.0	100.0
KMT2A-MLLT4	t(6;11) (q27;q23)	NA	< 0.5	0	292	0	0	NA	100.0
KMT2A-MLLT3	t(9;11) (p22;q23)	6A_(THP-1) 7A_(10A) 8A_(MM6) 6B_(9B)	1	4	287	0	1	80.0	100.0
KMT2A-ELL	t(11;19) (q23;p13.1)	e10e2 e10e3	1	0	291	0	1	0	100.0
KMT2A-PTD	Partial Tandem Duplication	e9e3 e10e3 e11e3	5-7	23	269	0	0	100.0	100.0
NPM1-MLF1	t(3;5) (q25.1;q34)	NA	< 0.5	1	291	0	0	100.0	100.0
PML-RARA	t(15;17) (q22;q21)	bcr1 (PR-L) bcr2 (PR-V) bcr3 (PR-S)	13	8	284	0	0	100.0	100.0
Total			**37**	**79**	**207**	**1**	**5**	**94.0**	**99.5**

B e) References

Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. *Am J Pharmacogenomics 2004; 4: 177-87.*

Mohr B, Koch R, Thiede C, Kroschinsky F, Ehninger G, Bornhäuser M. CD34+ cell dose, conditioning regimen and prior chemotherapy: factors with significant impact on the early kinetics of donor chimerism after allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant 2004; 34: 949-54.*

Bacher U, Haferlach T, Kern W, Haferlach C, Schnittger S. A comparative study of molecular mutations in 381 patients with myelodysplastic syndrome and in 4130 patients with acute myeloid leukemia. *Haematologica. 2007 Jun;92(6):744-52. PubMed PMID: 17550846.*

Huret JL, Dessen P, Bernheim A. Atlas of Genetics and Cytogenetics in Oncology and Haematology, year 2003. *Nucleic Acids Res. 2003 Jan 1;31(1):272-4. PubMed PMID: 12520000; PubMed Central PMCID: PMC165573.*

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood. 2009 Jul 30;114(5):937-51. Epub 2009 Apr 8. Review. PubMed PMID: 19357394.*

References: AML1-ETO, t(8;21)(q22;q22):

Licht JD. AML1 and the AML1-ETO fusion protein in the pathogenesis of t(8;21) AML. *Oncogene. 2001 Sep 10;20(40):5660-79. Review. PubMed PMID: 11607817.*

Lo Coco F, Pisegna S, Diverio D. The AML1 gene: a transcription factor involved in the pathogenesis of myeloid and lymphoid leukemias. *Haematologica. 1997 May-Jun;82(3):364-70. Review. PubMed PMID: 9234595.*

Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. *Blood. 1995 Jul 1;86(1):1-14. Review. PubMed PMID: 7795214.*

Richkind K, Hromas R, Lytle C, Crenshaw D, Velasco J, Roherty S, Srinivasiah J, Varella-Garcia M. Identification of two new translocations that disrupt the AML1 gene. *Cancer Genet Cytogenet. 2000 Oct 15;122(2):141-3. Review. PubMed PMID: 11106827.*

Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. *Leukemia. 2003 Jan;17(1):9-16. Review. PubMed PMID: 12529654.*

References: BCR-ABL, t(9;22)(q34;q11):

Burmeister T, Reinhardt R. A multiplex PCR for improved detection of typical and atypical BCR-ABL fusion transcripts. *Leuk Res. 2008 Apr;32(4):579-85. Epub 2007 Oct 24. PubMed PMID: 17928051.*

Emilia G, Luppi M, Ferrari MG, Temperani P, Marasca R, Giacobbi F, Vaccari P, Bandieri E, Di Donato C, Carapezzi C, Torelli G. Chronic myeloid leukemia with thrombocythemic onset may be associated with different BCR/ABL variant transcripts. *Cancer Genet Cytogenet. 1998 Feb;101(1):75-7. PubMed PMID: 9460506.*

Jones D, Luthra R, Cortes J, Thomas D, O'Brien S, Bueso-Ramos C, Hai S, Ravandi F, de Lima M, Kantarjian H, Jorgensen JL. BCR-ABL fusion transcript types and levels and their interaction with secondary genetic changes in determining the phenotype of Philadelphia chromosome-positive leukemias. *Blood. 2008 Dec 15;112(13):5190-2. Epub 2008 Sep 22. PubMed PMID: 18809762; PubMed Central PMCID: PMC2597614.*

Kim TD, Türkmen S, Schwarz M, Koca G, Nogai H, Bommer C, Dörken B, Daniel P, le Coutre P. Impact of additional chromosomal aberrations and BCR-ABL kinase domain mutations on the response to nilotinib in Philadelphia chromosome-positive chronic myeloid leukemia. *Haematologica. 2010 Apr;95(4):582-8. Epub 2009 Dec 16. PubMed PMID: 20015884; PubMed Central PMCID: PMC2857187.*

Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood. 2009 Feb 19;113(8):1619-30. Epub 2008 Sep 30. Review. PubMed PMID: 18827185.*

References: DEK-CAN, t(6;9)(p23;q34):

Soekarman D, von Lindern M, Daenen S, de Jong B, Fonatsch C, Heinze B, Bartram C, Hagemeijer A, Grosveld G. The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. *Blood. 1992 Jun 1;79(11):2990-7. PubMed PMID: 1586743.*

Soekarman D, von Lindern M, van der Plas DC, Selleri L, Bartram CR, Martiat P, Culligan D, Padua RA, Hasper-Voogt KP, Hagemeijer A, et al. Dek-can rearrangement in translocation (6;9)(p23;q34). *Leukemia. 1992 Jun;6(6):489-94. PubMed PMID: 1602786.*

References: CALM-AF10, t(10;11)(p13;q22):

Jones LK, Chaplin T, Shankar A, Neat M, Patel N, Samuel DP, Hill AS, Debernardi S, Bassini A, Young BD, Saha V. Identification and molecular

characterisation of a CALM-AF10 fusion in acute megakaryoblastic leukaemia. *Leukemia. 2001 Jun;15(6):910-4. PubMed PMID: 11417476*

References: CBFB-MYH11, inv(16) (p13;q22):

Corbacioglu A, Scholl C, Schlenk RF, Eiwen K, Du J, Bullinger L, Fröhling S, Reimer P, Rummel M, Derigs HG, Nachbaur D, Krauter J, Ganser A, Döhner H, Döhner K. Prognostic impact of minimal residual disease in CBFB-MYH11-positive acute myeloid leukemia. *J Clin Oncol. 2010 Aug 10;28(23):3724-9. Epub 2010 Jul 12. PubMed PMID: 20625124.*

Mühlematter D, Lafage-Pochitaloff M, Gabert J, Reiffers J, Bilhou-Nabera C, van Ommen GJ, Hagemeijer A, Breuning MH. Genomic acute myeloid leukemia-associated inv(16)(p13q22) breakpoints are tightly clustered. *Oncogene. 1999 Jan 14;18(2):543-50. PubMed PMID: 9927211.*

Roth CG, Contis L, Gupta S, Agha M, Safyan E. De novo acute myeloid leukemia with Philadelphia chromosome (BCR-ABL) and inversion 16 (CBFB-MYH11): report of two cases and review of the literature. *Leuk Lymphoma. 2011 Mar;52(3):531-5. Epub 2011 Feb 1. Review. PubMed PMID: 21281226*

References: MLL-AF6, t(6;11)(q27;q23):

Prasad R, Gu Y, Alder H, Nakamura T, Canaani O, Saito H, Huebner K, Gale RP, Nowell PC, Kuriyama K, et al. Cloning of the ALL-1 fusion partner, the AF-6 gene, involved in acute myeloid leukemias with the t(6;11) chromosome translocation. *Cancer Res. 1993 Dec 1;53(23):5624-8. PubMed PMID: 8242616.*

Welborn JL, Jenks HM, Hagemeijer A. Unique clinical features and prognostic significance of the translocation (6;11) in acute leukemia. *Cancer Genet Cytogenet. 1993 Feb;65(2):125-9. Review. PubMed PMID: 8453597.*

References: MLL-AF9, t(9;11)(q22;q23):

Giugliano E, Rege-Cambrin G, Scaravaglio P, Serra A, Wlodarska I, Emanuel B, Saglio G, Hagemeijer A. MLL-AF6 fusion resulting from a new three-way translocation t(6;11;7) in a patient with acute myeloid leukemia. *Leukemia. 2001 Oct;15(10):1674-6. PubMed PMID: 11587234.*

Mitterbauer G, Zimmer C, Pirc-Danoewinata H, Haas OA, Hojas S, Schwarzinger I, Greinix H, Jäger U, Lechner K, Mannhalter C. Monitoring of minimal residual disease in patients with MLL-AF6-positive acute myeloid leukaemia by reverse transcriptase polymerase chain reaction. *Br J Haematol. 2000 Jun;109(3):622-8. PubMed PMID: 10886213.*

Strehl S, König M, Mann G, Haas OA. Multiplex reverse transcriptase-polymerase chain reaction screening in childhood acute myeloblastic leukemia. *Blood. 2001 Feb 1;97(3):805-8. PubMed PMID: 11157501.*

Tanabe S, Zeleznik-Le NJ, Kobayashi H, Vignon C, Espinosa R 3rd, LeBeau MM, Thirman MJ, Rowley JD. Analysis of the t(6;11)(q27;q23) in leukemia shows a consistent breakpoint in AF6 in three patients and in the ML-2 cell line. *Genes Chromosomes Cancer. 1996 Apr;15(4):206-16. PubMed PMID: 8703846.*

References: MLL-ELL, t(11;19)(q23;p13.1):

Huret JL, Brizard A, Slater R, Charrin C, Bertheas MF, Guilhot F, Hählen K, Kroes W, van Leeuwen E, Schoot EV, et al. Cytogenetic heterogeneity in t(11;19) acute leukemia: clinical, hematological and cytogenetic analyses of 48 patients--updated published cases and 16 new observations. *Leukemia. 1993 Feb;7(2):152-60. Review. PubMed PMID: 8426468.*

Mitani K, Kanda Y, Ogawa S, Tanaka T, Inazawa J, Yazaki Y, Hirai H. Cloning of several species of MLL/MEN chimeric cDNAs in myeloid leukemia with t(11;19)(q23;p13.1) translocation. *Blood. 1995 Apr 15;85(8):2017-24. PubMed PMID: 7718874.*

References: MLL-PTD, Partial Tandem Duplication:

Basecke J, Whelan JT, Griesinger F, Bertrand FE. The MLL partial tandem duplication in acute myeloid leukaemia. *Br J Haematol. 2006 Nov;135(4):438-49. Epub 2006 Sep 11. Review. PubMed PMID: 16965385.*

Pajuelo-Gámez JC, Cervera J, García-Casado Z, Mena-Durán AV, Valencia A, Barragán E, Such E, Bolufer P, Sanz MA. MLL amplification in acute myeloid leukemia. *Cancer Genet Cytogenet. 2007 Apr 15;174(2):127-31. PubMed PMID: 17452254.*

Shih LY, Liang DC, Fu JF, Wu JH, Wang PN, Lin TL, Dunn P, Kuo MC, Tang TC, Lin TH, Lai CL. Characterization of fusion partner genes in 114 patients with de novo acute myeloid leukemia and MLL rearrangement. *Leukemia. 2006 Feb;20(2):218-23. PubMed PMID: 16341046.*

Whitman SP, Ruppert AS, Marcucci G, Mrózek K, Paschka P, Langer C, Baldus CD, Wen J, Vukosavljevic T, Powell BL, Carroll AJ, Kolitz JE, Larson RA, Caligiuri MA, Bloomfield CD. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and MLL partial tandem duplication: a Cancer and Leukemia Group B study. *Blood. 2007 Jun 15;109(12):5164-7. Epub 2007 Mar 6. PubMed PMID: 17341662; PubMed Central PMCID: PMC1890839.*

References: NPM1-MLF1, t(3;5)(q25.1;q34):

Arber DA, Chang KL, Lyda MH, Bedell V, Spielberger R, Slovak ML. Detection of NPM/MLF1 fusion in t(3;5)-positive acute myeloid leukemia and myelodysplasia. *Hum Pathol. 2003 Aug;34(8):809-13. PubMed PMID: 14506644.*

Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ, Carroll AJ, Morris SW. The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. *Oncogene. 1996 Jan 18;12(2):265-75. PubMed PMID: 8570204.*

References: PML-RARA, t(15;17)(q22;q21):

Alcalay M, Zangrilli D, Pandolfi PP, Longo L, Mencarelli A, Giacomucci A, Rocchi M, Biondi A, Rambaldi A, Lo Coco F, et al. Translocation breakpoint of acute promyelocytic leukemia lies within the retinoic acid receptor alpha locus. *Proc Natl Acad Sci U S A. 1991 Mar 1;88(5):1977-81. PubMed PMID: 1848017; PubMed Central PMCID: PMC51149.*

Pandolfi PP, Alcalay M, Fagioli M, Zangrilli D, Mencarelli A, Diverio D, Biondi A, Lo Coco F, Rambaldi A, Grignani F, et al. Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode aberrant PML proteins and PML/RAR alpha isoforms in acute promyelocytic leukaemia. *EMBO J. 1992 Apr;11(4):1397-407. PubMed PMID: 1314166; PubMed Central PMCID: PMC556589.*

Other References

Wenz H, Robertson JM, Menchen S, Oaks F, Demorest DM, Scheibler D, Rosenblum BB, Wike C, Gilbert DA, Efcavitch JW. High-precision genotyping by denaturing capillary electrophoresis. *Genome Res 1998; 8: 69-80.*

Sgueglia JB, Geiger S, Davis J. Precision studies using the ABI prism 3100 genetic analyzer for forensic DNA analysis. *Anal Bioanal Chem 2003; 376: 1247-54.*

Gilder JR, Doom TE, Inman K, Krane DE. Run-specific limits of detection and quantitation for STR-based DNA testing. *J Forensic Sci 2007; 52: 97-101.*

Bacher U, Haferlach T, Kern W, Haferlach C, Schnittger S. A comparative study of molecular mutations in 381 patients with myelodysplastic syndrome and in 4130 patients with acute myeloid leukemia. *Haematologica 2007; 92: 744-52.*

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood. 2009 Jul 30;114(5):937-51.*

Huret JL, Ahmad M, Arsaban M, Bernheim A, Cigna J, Desangles F, Guignard JC, Jacquemot-Perbal MC, Labarussias M, Leberre V, Malo A, Morel-Pair C, Mossafa H, Potier JC, Texier G, Viguié F, Yau Chun Wan-Senon S, Zasadzinski A, Dessen P. Atlas of genetics and cytogenetics in oncology and haematology in 2013. *Nucleic Acids Res 2013; 41(Database issue): D920-4.*

Kozu T, Miyoshi H, Shimizu K, Maseki N, Kaneko Y, Asou H, Kamada N, Ohki M. Junctions of the AML1/MTG8(ETO) fusion are constant in t(8;21) acute myeloid leukemia detected by reverse transcription polymerase chain reaction. *Blood 1993; 82: 1270-6.*

Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood 2010; 116: 354-65.*

Steudel C, Wermke M, Schaich M, Schäkel U, Illmer T, Ehninger G, Thiede C. Comparative analysis of MLL partial tandem duplication and FLT3 internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia. *Genes Chromosomes Cancer 2003; 37: 237-51.*

van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, Dotti G, Griesinger F, Parreira A, Gameiro P, Diáz MG, Malec M, Langerak AW, San Miguel JF, Biondi A. Standardized RTPCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia 1999; 13: 1901-28.*

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