DNA
DIAGNOSTIC

Instruction For Use

HemaVision®-28Q

Screening test for 28 chromosome translocations and more than 145 breakpoints associated with leukemia

Instruction For Use
Cat No. HV01-28Q
DNA Diagnostic A/S
www.dna-diagnostic.com
Revision 2024.04.08

IVD CE

HemaVision®-28Q

Multiplex RT-PCR test

Screens for 28 leukemia associated translocations

del(1)(p32) (STIL::TAL1)
t(1;11) (p32;q23.3) (KMT2A::EPS15)
t(1;11) (q21;q23.3) (KMT2A::MLLT11)
t(1;19) (q23;p13) (TCF3::PBX1)
t(3;5) (q25;q34) (NPM1::MLF1)
t(3;21) (q26;q22) (RUNX1::MECOM)
t(4;11) (q21;q23.3) (KMT2A::AFF1)
t(5;12) (q33;p13) (ETV6::PDGFRB)
t(5;17) (q35;q21) (NPM1::RARA)
t(6;9) (p23;q34) (DEK::NUP214)
t(6;11) (q27;q23.3) (KMT2A::AFDN)
t(8;21) (q22;q22) (RUNX1::RUNX1T1)
t(9;9) (q34;q34) (SET::NUP214)
t(9;11) (p21.3;q23.3) (KMT2A::MLLT3)
t(9;12) (q34;p13) (ETV6::ABL1)
t(9,22) (q34;q11) (BCR::ABL1)
t(10;11) (p12;q23.3) (KMT2A::MLLT10)
t(11;17) (q23.3;q21) (KMT2A::MLLT6)
t(11;17) (q23;q21) (ZBTB16::RARA)
t(11;19) (q23.3;p13.1) (KMT2A::ELL)
t(11;19) (q23.3;p13.3) (KMT2A::MLLT1)
t(12;21) (p13;q22) (ETV6::RUNX1)
t(12;22) (p13;q12) (ETV6::MN1)
t(15;17) (q24;q21) (PML::RARA)
inv(16) (p13;q22) (CBFB::MYH11)
t(16;21) (p11;q22) (FUS::ERG)
t(17;19) (q22;p13) (TCF3::HLF)
t(X;11) (q13;q23.3) (KMT2A::FOXO4)

Instruction For Use for HemaVision®-28Q
Cat. No. HV01-28Q
12 tests per kit

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1. PURPOSE OF THE TEST (INTENDED USE)

HemaVision®-28Q is a 4-hour CE IVD marked in vitro diagnostic test for qualitative screening of 28 chromosome translocations associated with chronic and acute leukemia.

HemaVision®-28Q is intended for testing total RNA samples from human blood or bone marrow for presence of chromosomal translocations associated with leukemia. The test is qualitative. Each kit contains 12 tests and each test is single use only. Tests should be performed, and results should be analysed by professionals only. The test is intended for use as an adjunct to evaluation of Leukemia in conjunction with other clinicopathological factors (aid to diagnosis). HemaVision®-28Q is not intended for quantitative tests, nor Minimal Residual Disease (MRD) and follow-up tests.

HemaVision®-28Q detects RNA transcripts from fusion genes using a RT-qPCR procedure. Alternative splice variants are also detected.

HemaVision®-28Q detects the following 28 translocations:

del(1)(p32) (STIL::TAL1)
t(1;11) (p32;q23.3) (KMT2A::EPS15)
t(1;11) (q21;q23.3) (KMT2A::MLLT11)
t(1;19) (q23;p13) (TCF3::PBX1)
t(3;5) (q25;q34) (NPM1::MLF1)
t(3;21) (q26;q22) (RUNX1::MECOM)
t(4;11) (q21;q23.3) (KMT2A::AFF1)
t(5;12) (q33;p13) (ETV6::PDGFRB)
t(5;17) (q35;q21) (NPM1::RARA)
t(6;9) (p23;q34) (DEK::NUP214)
t(6;11) (q27;q23.3) (KMT2A::AFDN)
t(8;21) (q22;q22) (RUNX1::RUNX1T1)
t(9;9) (q34;q34) (SET::NUP214)
t(9;11) (p21.3;q23.3) (KMT2A::MLLT3)
t(9;12) (q34;p13) (ETV6::ABL1)
t(9,22) (q34;q11) (BCR::ABL1)
t(10;11) (p12;q23.3) (KMT2A::MLLT10)
t(11;17) (q23.3;q21) (KMT2A::MLLT6)
t(11;17) (q23;q21) (ZBTB16::RARA)
t(11;19) (q23.3;p13.1) (KMT2A::ELL)
t(11;19) (q23.3;p13.3) (KMT2A::MLLT1)
t(12;21) (p13;q22) (ETV6::RUNX1)
t(12;22) (p13;q12) (ETV6::MN1)
t(15;17) (q24;q21) (PML::RARA)
inv(16) (p13;q22) (CBFB::MYH11)
t(16;21) (p11;q22) (FUS::ERG)
t(17;19) (q22;p13) (TCF3::HLF)
t(X;11) (q13;q23.3) (KMT2A::FOXO4)

2. PRINCIPLE OF THE TEST

HemaVision®-28Q is a RT-qPCR based assay for detection of leukemia associated fusion gene transcripts in total RNA from whole blood or bone marrow samples. Included in the kit are ready to use cDNA and qPCR Master Mixes. cDNA is synthesized by adding purified total RNA to the HemaVision®-28Q ready to use cDNA reaction mix. The resulting cDNA is added to 23 ready to use qPCR reaction tubes, which contain specific PCR primers and probes for detection of fusion genes, three reference genes and an internal amplification control (IAC). The qPCR is performed in a real-time qPCR instrument with optical filters for detection of FAM, ROX, and CY5 fluorescence. Amplification plots and Ct (cycle at threshold) values are used for identification of the translocation and fusion gene transcript using an easy-to-use interpretation table.

HemaVision®-28Q detects fusion gene transcripts using specific PCR primers and probes. The translocation specific primers bind to exons in the fusion gene enabling amplification of the region containing the breakpoint. The primers are designed to detect multiple clinically relevant breakpoints and splice variants (Figure 1).

The qPCR master mix probes are dual labeled with a fluorophore and a quencher molecule at each end of the oligoes. During the annealing step of the PCR the probe bind to the PCR products of the previous rounds. Signals are generated in the subsequent elongation step as the 5'->3'exonuclease activity of the Taq polymerase enzyme degrades the hybridized probe. This liberates the fluorophore from the quencher, which thereby increases the fluorescence. The fluorescence is measured at the end of the elongation step of every PCR cycle.

When the fluorescence for a translocation exceeds the threshold level before cycle 35 (Ct <35) the test is positive. The qPCRs are multiplexed by the use of FAM, ROX and CY5 labeled probes. This permits two translocation tests and the IAC to run in the same tube. The identity of a positive translocation test is easily deduced from the Interpretation Table.

As a control for the functionality of the qPCR reaction and for correct transfer of cDNA aliquots to the 23 qPCR reactions, an Internal Amplification Control (IAC) is included in the cDNA reaction mix. A translocation positive control can be ordered from DNA Diagnostic (HemaVision Q Positive Controls, HV05-PCQ).

HemaVision®-28Q also includes primers and probes for detection of the reference gene transcripts ABL-1, B2M and GUS. Detection of the reference genes is a control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.

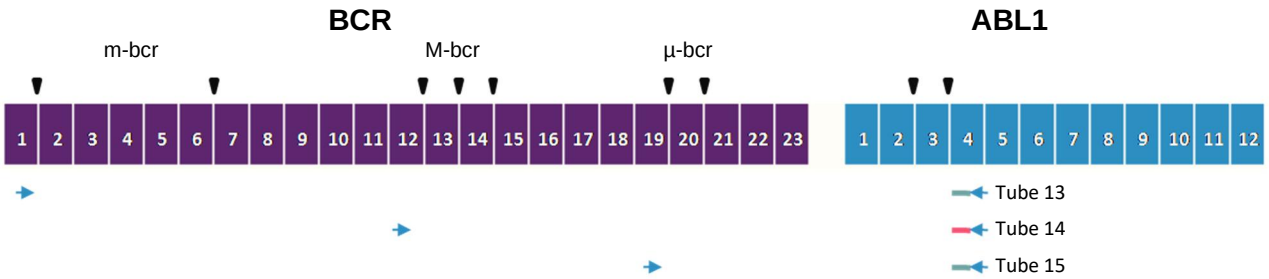


Figure 1. Primers and probes in HemaVision®-28Q are designed to detect multiple clinically relevant breakpoints.
In this example primers and probe for detection of t(9;22) fusion gene transcript BCR::ABL1 are shown. Primers are depicted as blue arrows, FAM and ROX labeled probe as green and red lines and breakpoints as black triangles. Exons are numbered for the fusion genes BCR and ABL1. The three breakpoint regions (m-bcr, M-bcr , µ-bcr) of BCR are detected with the primer and probe combination of qPCR tube 13, 14 and 15, respectively.

3. KIT COMPONENTS AND STORAGE

HemaVision®-28Q contains reagents for 12 tests.

Included in HemaVision®-28Q kit is the following components:

- 12 cDNA tubes with 42 µL reaction mix (0.65 mL tubes with yellow screw cap)
- 12 blocks of 24 qPCR tubes containing 23 µL qPCR reaction mix.
- 12 extra set of optical caps for the qPCR tubes.
- Two tubes with RNase free H_2O (0.65 mL tubes with blue screw cap)
- One user manual

HemaVision®-28Q is produced in 3 formats to suit various qPCR apparatus. The qPCR mix can be supplied in 0.1 mL white low profile (WLP), 0.2 mL white regular profile (WRP), 0.1 mL Clear Low Profile (CLP) or 0.2 mL frosted regular profile (FRP) PCR tubes. Adaptor plates can be supplied with the kit if the qPCR instrument requires a 96-tube plate format (e.g. Roche LightCycler 480 and some ABI models).

The cDNA reaction tubes contain reverse transcriptase, nucleotides, buffer, cDNA primers and IAC oligo template.

The qPCR tubes contain hot start Taq DNA polymerase, nucleotides, buffer and primers/probes.

Tube no. 1-6, 9-15, 17-23 contain primers and probes for both fusion genes and the IAC template.

Tube no. 7, 8, 16, contain primers and probes for a reference gene and the IAC template.

Tube no. 24 is empty.

To confirm the orientation of the PCR strips after qPCR analysis check for FAM amplification signals in tube no. 7, 8, 16, and the absence of Cy5 amplification signal in tube no. 24.

The kit must be stored at -20°C.

Avoid thawing and freezing of the kit.

The qPCR tubes must be protected from strong light to avoid bleaching of the probes.

4. REQUIRED EQUIPMENT

- Centrifuge for 96 well plates or PCR 8-tube strips.
- Thermal Heating Block, or Thermocycler (for cDNA synthesis).
- A qPCR instrument with filters for FAM (Abs 495 nm, Em 520 nm), ROX (Abs 585 nm, Em 605 nm) and CY5 (Abs 635 nm, Em 665 nm).
- Pipettes and sterile RNase free filtered tips.
- Gloves

5. PRECAUTIONS

- The quality and concentration of the RNA sample greatly affects the results of this test. To minimize the risk of degradation by ribonucleases, we strongly recommend purification of total RNA immediately after blood or bone marrow extraction. Optionally, mononuclear cells can be purified prior to RNA extraction using Ficoll Hypaque. Do not freeze Ficoll purified cells without adding a denaturing solution [e.g. containing guanidinium isothiocyanate (GTC)] immediately after isolation and before freezing.
- Blood samples can be stabilized in PAXgene Blood RNA tubes, Qiagen cat. no. 762165 and bone marrow samples can be stabilized in PAXgene Bone marrow RNA tubes, Qiagen cat. no. 764114.
- Do NOT use Heparin for stabilization of blood samples.
- Always store cell samples and aqueous RNA solutions at -80°C. Even an overnight storage at -20°C may result in RNA degradation.
- When working with RNA always use gloves, as hands are a major source of ribonuclease contamination.
- The integrity and purity of RNA is important for the efficiency of the cDNA synthesis and thus detection of translocations. The quality of RNA can be checked by OD 260/280 nm measurements, agarose gel electrophoresis, or using RIN number from the Agilent Bioanalyzer or the TapeStation.
- For more guidance on specimen collection, RNA isolation and storage please refer to "ISO 20186:2019 Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 1: isolated cellular RNA" and CLSI guidance MM13-A "Collection, Transport, Preparation and Storage of Specimens for Molecular Methods; Approved guideline.
- RT-qPCR is a very sensitive technique. Therefore, precautions must be taken to avoid false positive results caused by contamination with RNA, cDNA or PCR products from other samples.
- Dedicate four separate rooms/areas to the following activities:

  1) RNA extraction

  2) cDNA synthesis

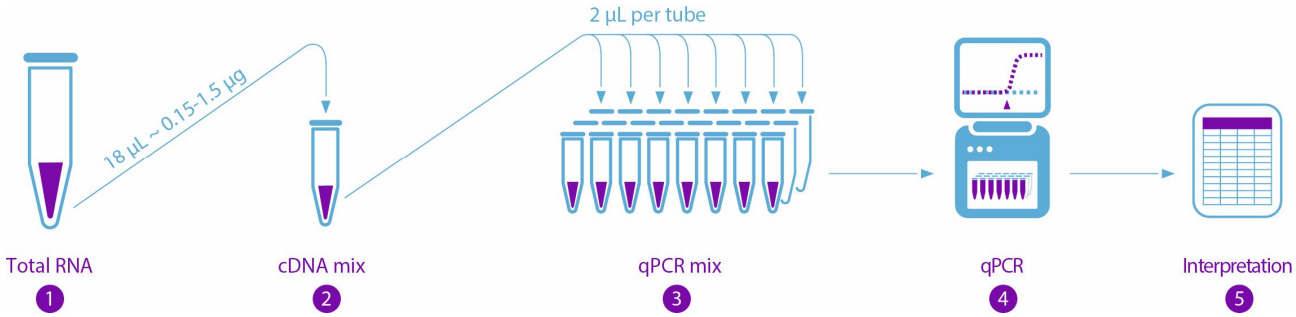
  3) Addition of cDNA to qPCR master mix

  4) qPCR
- A set of micropipettes, aerosol barrier pipette tips, disposable gloves and clean lab coats should be kept in each of the four rooms. The work must be organized so that mixes and reaction products only moves in the direction from 1-4. NEVER move mixes or reaction products in the opposite direction.
- Laboratory workbenches, pipettes, and lab coats must be cleaned on a regular basis.
- Use of aerosol barrier pipette tips is **highly recommended** during the entire procedure. It is essential to change gloves very often when handling tubes containing RNA or cDNA. To minimize contamination avoid opening PCR tubes after amplification as they contain very high copy numbers of PCR amplicons.
- The detection of translocations can be checked with our HemaVision Q Positive Controls (HV05-PCQ).
- For more general guidance on best practice in qPCR testing please refer to the CLSI guidance document MM01: Molecular Methods for Clinical Genetics and Oncology Testing, 3rd Edition.

Safety

- Read and understand the procedure before starting.
- Normal laboratory aseptic technique should be followed at all times.
- Treat each sample as if it is infectious.
- Wear eye protection and disposable gloves during all steps of the assay.
- The products can be discarded as normal laboratory waste.
- Check the safety data sheet for the product on our website for further information or ask for a copy of it.

6. PROTOCOL AT A GLANCE



1: RNA denaturation	2: cDNA synthesis	3: Add cDNA to qPCR tubes	4: Program qPCR Instrument	5: qPCR analysis
18 µL total RNA (0.15-1.5 ug) ▼ 65°C / 5 min ▼ 0°C / 1 min ▼ Spin / 1 min	Transfer to one cDNA tube ▼ Mix and spin / 1 min ▼ 42°C / 60 min ▼ 95°C / 5 min ▼ 0°C / 1 min ▼ Spin / 1 min	Spin 3x8-tube block 10 seconds ▼ Remove and discard lids ▼ Add 2 µL cDNA to each of the 23 qPCR mixes ▼ Close tubes with new optical lids ▼ Spin / 1 min	1 cycle: 95°C / 15 min ▼ 40 cycles: 95°C / 30 sec 60°C / 50 sec 72°C / 80 sec* *read FAM, ROX, CY5	Generate amplification plots and use Interpretation Table

Figure 2. Overview of HemaVision®-28Q protocol.

7. DETAILED PROTOCOL

7.1 RNA Preparation

Total RNA is extracted from blood or bone marrow cells. A minimum of 0.15 µg total RNA with a minimum concentration of 8 ng/µL RNA is needed for the subsequent steps.

Due to the inherent instability of RNA use patient samples as fresh as possible.

RNA can be extracted from whole blood with QIAmp RNA blood Mini Kit, Qiagen cat. no. 52304.

For blood collected in PAXgene tubes, RNA can be extracted with the PAXgene Blood RNA Kit, Qiagen cat. no. 762164.

For bone marrow collected in PAXgene tubes, RNA can be extracted with PAXgene Bone marrow RNA Kit, Qiagen cat. no. 764133.

Other total RNA extraction methods can be used, as long as RNA integrity is maintained, and inhibiting agents removed.

Total RNA should be stored at -80°C.

7.2 cDNA Synthesis

- Incubate total RNA at 65°C for 5 minutes and cool immediately on ice. Spin tube for 1 minute to collect condensate.
- Take one yellow capped tube with cDNA master mix from the HemaVision®-28Q kit stored at -20°C. Add 18 µL (0.15-1.5 µg) of denatured total RNA to the cDNA master mix tube (tube with yellow cap). If necessary, the RNA sample can be diluted with the provided RNase free H_2O (tube with blue cap). Mix and spin for 1 minute.
- Incubate the cDNA reaction at 42°C for 60 minutes.
  Note: Incubation can be performed in the yellow capped cDNA mix tube (in thermal heating block), or by transferring mix to a 0.2mL tube and incubating 42°C for 60 minutes and 95°C for 5 minutes in a thermocycler. If transferring to other tube, a second centrifugation step of the yellow capped tube can aid in maximum recovery of sample and cDNA mix.
- Inactivate Reverse Transcriptase enzyme by heating to 95°C for 5 minutes.
- Cool the cDNA reaction tube on ice for 1 minute and spin it again for 1 minute to collect condensate.

7.3 qPCR

- For each cDNA sample use one block of 3x8-tube qPCR master mix tubes. Spin the 3x8 tubes for 10 second to collect qPCR master mix at the bottom of tubes. If you are using a Rotor-Gene instrument you need to transfer the qPCR mix to Rotor-Gene tubes and spin again.
- Remove and discard the foil from one block of 3x8-tubes. Be careful not to separate the three 8-tube strips from each other. Also, be careful to avoid cross contamination and spill of reaction mixes while removing the foil.
- Add 2 µL cDNA reaction to each of the 23 tubes containing 23 µL qPCR master mix (tube 1-23). Tube number 24 is empty and is used for orientation of the 3x8 tube block, see figure 3.

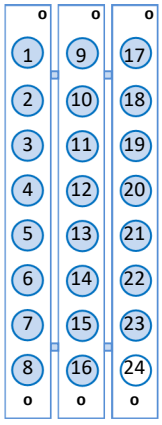


Figure 3. Positions of the 23 qPCR master mixes in each block of 3 x 8 tubes. Tube number 24 is empty and can be used for orientation of the 24 tube block. The hole in the upper right corner of each 8-tube strip (depicted as °) marks the beginning of the strip and the centered hole marks the end of the strip. The tube numbering refers to the tube numbering in the Interpretation table.

- Close the 24 tubes with a fresh set of optical caps (supplied with the kit).
- Mix contents by vortexing the tubes shortly (e.g. 5 seconds), or by flicking the tubes, and spin tubes briefly to collect liquid at the bottom.
- Place the 3x8-tube block into the qPCR instrument.
- Run the qPCR reaction with the following program:

Step	Time/Temperature	Cycles	Comment
1	15 minutes / 95°C	1	Activation of Taq Polymerase
2	30 seconds / 95°C 50 seconds / 60°C 80 seconds / 72°C *	40	DNA amplification * Read fluorescence for FAM, ROX and CY5 at the end of each extension step

7.3.1 Important notes for ABI 7500, ViiA7 and QuantStudio users

- Ramp speed: Use standard ramp speed (not fast mode).
- Targets: While defining the 3 targets FAM, ROX and CY5 choose for all targets “None” as Quencher.
- Passive reference dye: Select “None” instead of “ROX” as passive reference dye.

7.3.2 Important notes for Roche LightCycler 480 users

A Color Compensation (CC) file should be generated before the first run on the Light Cycler 480 and if the lamp has been replaced. A CC-kit with FAM, ROX and CY5 dye is available from DNA Diagnostic.

Recommended qPCR software setup:

- Melt factor: 1, Quant factor: 5, Max integration time: 2 seconds.
- Set the ramp speed to 2.2 C/sec for annealing step and 4.4 C/sec for the remaining steps.
- For the amplification step of the PCR program, under Analysis Mode choose Quantification and Acquisition Mode: Single.
- Filter choice: Either choose 4 color hydrolysis probe or manually choose FAM, Red 610 and Cy5.
- **NB. For advanced LightCycler 480 software and analysis settings please contact DNA Diagnostic for a separate technical note.**

7.3.3 Important notes for Qiagen Rotor-Gene Q users

The recommended gain setting for Rotor-Gene Q is using the Autogain function.

7.3.4 Important notes for Agilent MX3005p and MX3000 users

- Targets: Define the three targets: FAM, ROX and CY5.
- Read fluorescence at end of each annealing/elongation step.
- Set “gain settings” to ROX=1 and FAM=2 and CY5=1.

7.4 Analysis and Interpretation

Generate amplification plots showing:

- Thermal cycles on the X-axis.
- Background subtracted fluorescence on the Y-axis. The exact name on the Y-axis differs among qPCR instruments. See table 1.

Instrument	Y-axis on amplification plot with background subtracted fluorescence
ABI: 7500, ViiA7, QuantStudio	ΔRn
BioRad: CFX96, CFX96 Touch	RFU
Roche: Light Cyclers 480	Fluorescence (using Analysis option: Abs Quant / 2nd Derivative Max for All Samples)
Qiagen: Rotor-Gene	Norm. Fluoro.
Agilent: Mx3000P / Mx3005P	dR
Agilent: AriaMx	ΔR
Bio Molecular Systems: Mic qPCR cycler	Normalized Fluorescence

Table 1. Examples of instrument nomenclature for the Y-axis when the amplification plot shows background subtracted fluorescence.

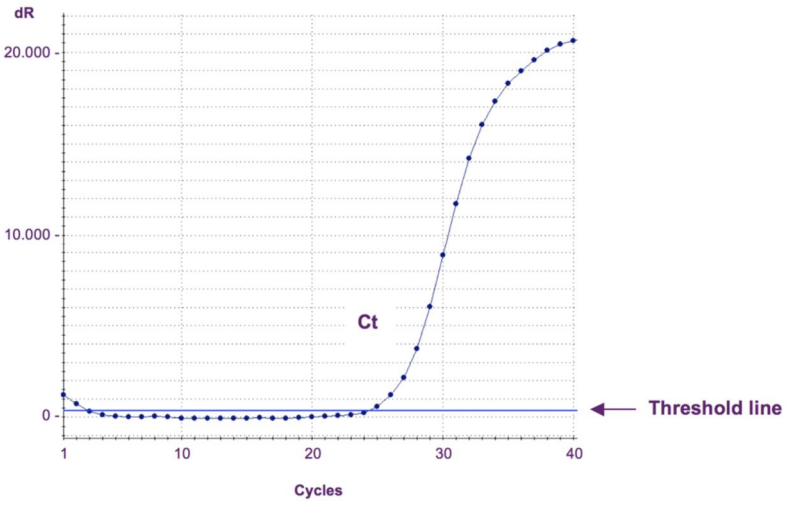


Figure 4. Amplification plot from Agilent Mx3005P with X-axis showing cycle number and Y-axis showing background subtracted fluorescence.

The threshold line is used to find the Ct value.

Ct is the intersection between the amplification curve and the threshold line.

The threshold line is set just above the base line in this example resulting in a Ct value of 24.4.

Ct values are calculated automatically by the instrument. The algorithms used to calculate Ct values differ between qPCR instrument software packages. The threshold line in the amplification plot should be set manually to allow accurate Ct determination. The threshold line should be set just above the background fluorescence, so that the threshold line intersects the amplification curves when the fluorescence starts to increase due to PCR amplification. The threshold level should however be set sufficiently high to allow for fluctuations in background fluorescence and background

fluorescence drift (a linear increase in fluorescence). The threshold can be set at a fixed level in each run for better comparison between runs. A fixed threshold is model and machine specific and therefore need to be determined locally at the user. The threshold level can be set manually using the following the steps:

ABI; 7500, 7500 Fast, ViiA 7: In the Analysis window, choose "Plot Type: deltaRn vs Cycle", and "Graph Type: Linear". Unmark "Auto Threshold" and "Auto Baseline". Then click "Show Threshold" and with the mouse move the threshold line above the baseline.

ABI; QuantStudio: In the Results screen use the menu marked with an eye; choose graph type "linear". Unmark "Auto Threshold" and "Auto Baseline". Then click "Show Threshold" and with the mouse move the threshold line above the baseline.

Bio-Rad CFX96 and CFX96 Touch: Under "Settings" and "Baseline Threshold" choose auto calculated baseline cycles. Click the "Settings" tab, choose "Baseline Setting" and "Baseline Subtracted Curve Fit". Using the mouse adjust the threshold level above baseline.

Qiagen Rotor-Gene Q: Click "Analysis", choose the fluorescence to analyze, click "Linear Scale", "Slope Correct" and "Auto-Scale". In the right side panel, under "CT Calculation", click the button next to "Threshold" and mark the threshold level on the amplification plot. During analysis of the Rotor-Gene Q curves, it is recommended to try with- and without the features 'Dynamic Tube' and 'Slope Correct', to obtain optimal curves. If there are baseline fluctuations (noise) in the first cycles, use the 'Ignore First' function for the cycles where fluctuations occur.

Roche LightCycler480: Choose "Absolut Quantification" and "Fit Points". Then click the tab "Noise Band" and click the "Noiseband" drop down button and choose "Noiseband (Fluoresc)". Use the mouse to set the Noise Band.

Agilent MxPro: The automatic threshold level can be used. Choose "Adaptive Baseline Correction" and "Background-based Threshold" using cycle 6-11 and a sigma multiplier of 10.

Agilent AriaMx: Use default baseline correction (adaptive) and use "Background Based Threshold" using cycle 6-11 and a sigma multiplier of 10.

Bio Molecular Systems Mic qPCR cycler: Use default analysis settings.

Note! The Ct values cannot be used for exact quantification of the fusion transcripts level since the fusion gene amplicons differ in length, resulting in different PCR efficiencies.

- Check that a CY5 signal is present from the Internal Amplification Control (IAC) in tubes no. 1-23.

  The IAC CY5 fluorescence should yield Ct values between 29 and 34. This is a control for functionality of the qPCR reaction.

  If no CY5 signals are detected the test has failed either due to no addition of cDNA to the qPCR tube(s), incorrect instrument settings or evaporation from the qPCR tube(s). Repeat the test.

  Due to competition for PCR constituents CY5 Ct value above 34 may occur when the tube is also positive for FAM or ROX fluorescence.

  Check the Ct values for GUS, B2M and ABL1. The Ct values should be below: 28 in tube 7 (GUS), 25 in tube 8 (B2M), and 29 in tube 16 (ABL1). This is a control for RNA extraction and cDNA synthesis have been functional.

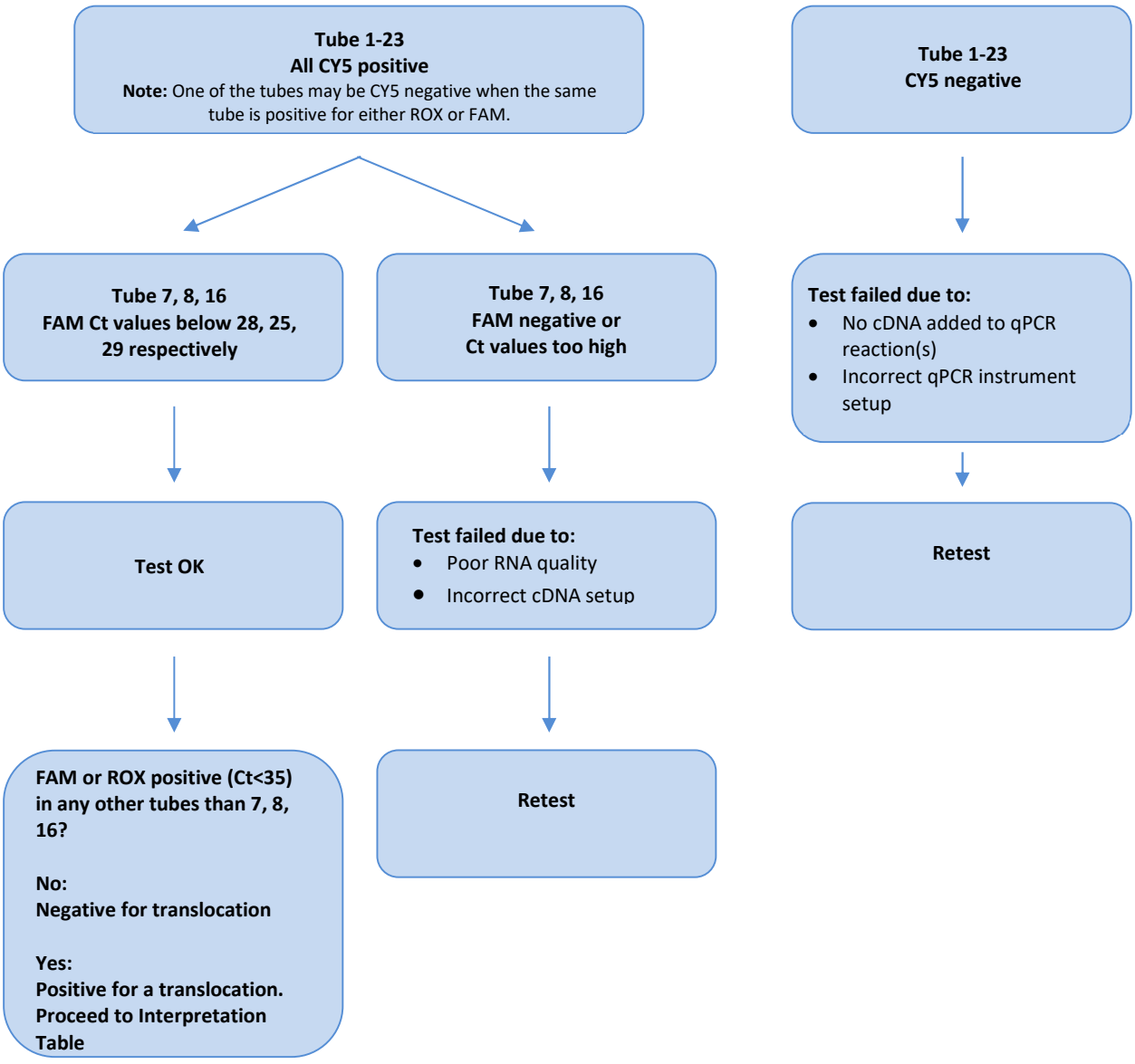
  GUS, B2M and ABL1 Ct values higher than the above mentioned values indicates that the quality of the RNA may be too low to generate a valid test result. Repeat test with fresh RNA.

- Check for a FAM or ROX signal with a Ct value below 35 in the “translocation” tubes 1-6, 9-15, 17-23.

- Translocation tests with Ct values below 35 for FAM and ROX signals and amplification curves with exponential growth can be considered as true positive. Use the Interpretation Table to identify the specific translocation.

- Ct values above cycle 35 for FAM and ROX signals may be the result of unspecific amplification (false positive). Repeat the test to confirm or reject the result. When the second test is negative, the sample is negative for the translocation observed in the first test. When the second test is also positive, the sample may be positive for the corresponding translocation. However, we highly recommend using other diagnostic techniques to confirm the result from positive tests having Ct values above 35.

Flowchart for data interpretation:



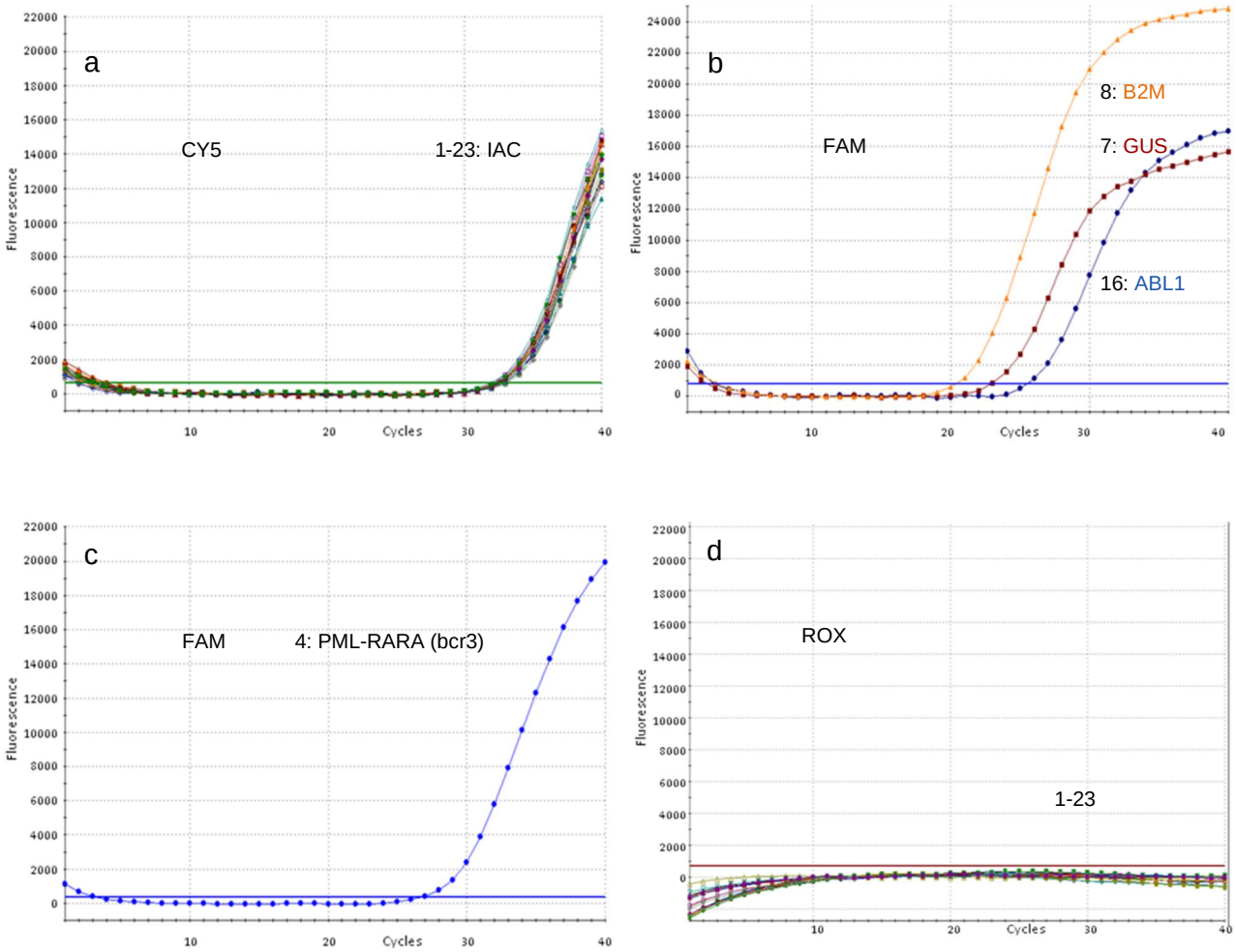


Figure 5. Example of amplification plots from a HemaVision®-28Q test.

a) The CY5 amplification plot shows all 23 IAC control curves are present and have Ct values near 32. This serves as a positive control for correct transfer of 2 µL cDNA to all 23 qPCR tubes and functionality of the qPCR reactions.

b) The FAM amplification plot for the reference genes B2M, GUS, and ABL1 in tubes 8, 7, 16 all show curves with Ct below 25, 28, and 29 respectively. This is a positive control for both the RNA quality and the RT-qPCR reactions were functional.

c) The FAM amplification plot for tube 4 shows an amplification curve with a Ct value below 35. Therefore, the test is positive for t(15;17)(q24;q21)(PML::RARA, bcr3, S-form). See Interpretation Table.

d) The ROX amplification plot shows no amplification for the tubes 1-23. Therefore, the test is negative for “ROX amplicons”.

8. INTERPRETATION TABLE

Tube	Translocation	Fusion Gene	Fw primer :: Rev primer	Fluorochrome	
1	t(15;17)(q24;q21)	PML::RARA (bcr2, V)	PML ex5::RARA ex3	FAM	CY5
	inv(16)(p13;q22)	CBFB::MYH11	CBFB ex3::MYH11 ex30	ROX	CY5
2	inv(16)(p13;q22)	CBFB::MYH11	CBFB ex4::MYH11 ex34	FAM	CY5
	t(8;21)(q22;q22)	RUNX1::RUNX1T1	RUNX1 ex6::RUNX1T1 ex3	ROX	CY5
3	t(15;17)(q24;q21)	PML::RARA (bcr1, L)	PML ex6a::RARA ex3	FAM	CY5
	t(9;11)(p21.3;q23.3)	KMT2A::MLLT3	KMT2A ex8::MLLT3 ex7	ROX	CY5
4	t(15;17)(q24;q21)	PML::RARA (bcr3, S)	PML ex3::RARA ex3	FAM	CY5
	t(9;11)(p21.3;q23.3)	KMT2A::MLLT3	KMT2A ex7::MLLT3 ex11	ROX	CY5
5	t(11;19)(q23.3;p13.1)	KMT2A::ELL	KMT2A ex7::ELL ex3	FAM	CY5
	t(16;21)(p11;q22)	FUS::ERG	FUS ex6::ERG ex12	ROX	CY5
6	t(12;22)(p13;q12)	ETV6::MN1	ETV6 ex2::MN1 ex2	FAM	CY5
	t(6;9)(p23;q34)	DEK::NUP214	DEK ex9::NUP214 ex19	ROX	CY5
7	Reference gene	GUS	GUS ex11::GUS ex12	FAM	CY5
8	Reference gene	B2M	B2M ex2::B2M ex4	FAM	CY5
9	t(1;11)(p32;q23.3)	KMT2A::EPS15	KMT2A ex8+9::EPS15 ex3	FAM	CY5
	t(6;11)(q27;q23.3)	KMT2A::AFDN	KMT2A ex8+9::AFDN ex2	ROX	CY5
10	t(1;19)(q23;p13)	TCF3::PBX1	TCF3 ex16::PBX1 ex3	FAM	CY5
	t(12;21)(p13;q22)	ETV6::RUNX1	ETV6 ex5::RUNX1 ex4b	ROX	CY5
11	t(11;19)(q23.3;p13.3)	KMT2A::MLLT1	KMT2A ex8+9::MLLT1 ex2	FAM	CY5
	t(4;11)(q21;q23.3)	KMT2A::AFF1	KMT2A ex8+9::AFF1 ex9	ROX	CY5
12	t(17;19)(q22;p13)	TCF3::HLF	TCF3 ex14::HLF ex4	FAM	CY5
	del(1)(p32)	STIL::TAL1	STIL ex1::TAL1 ex2	ROX	CY5
13	t(9;22)(q34;q11)	BCR::ABL1 (m-bcr, P190)	BCR ex1::ABL1 ex3	FAM	CY5
	t(9;9)(q34;q34)	SET::NUP214	SET ex9::NUP214 ex19	ROX	CY5
14	t(11;19)(q23.3;p13.3)	KMT2A::MLLT1	KMT2A ex7::MLLT1 ex9	FAM	CY5
	t(9;22)(q34;q11)	BCR::ABL1 (M-bcr, P210)	BCR ex12::ABL1 ex3	ROX	CY5
15	t(9;22)(q34;q11)	BCR::ABL1 (µ-bcr, P230)	BCR ex19::ABL1 ex3	FAM	CY5
	t(11;17)(q23;q21)	ZBTB16::RARA	ZBTB16 ex3::RARA ex3	ROX	CY5
16	Reference gene	ABL1	ABL1 ex2::ABL1 ex3	FAM	CY5
17	t(9;12)(q34;p13)	ETV6::ABL1	ETV6 ex2+5::ABL1 ex3	FAM	CY5
	t(5;12)(q33;p13)	ETV6::PDGFRB	ETV6 ex2+5::PDGFRB ex12	ROX	CY5
18	t(10;11)(p12;q23.3)	KMT2A::MLLT10	KMT2A ex8+9::MLLT10 ex17	FAM	CY5
	t(1;11)(q21;q23.3)	KMT2A::MLLT11	KMT2A ex8+9::MLLT11 ex2	ROX	CY5
19	t(X;11)(q13;q23.3)	KMT2A::FOXO4	KMT2A ex7::FOXO4 ex2	FAM	CY5
	t(11;17)(q23.3;q21)	KMT2A::MLLT6	KMT2A ex7::MLLT6 ex12	ROX	CY5
20	t(3;21)(q26;q22)	RUNX1::MECOM	RUNX1 ex6::MECOM ex2	FAM	CY5
	t(10;11)(p12;q23.3)	KMT2A::MLLT10	KMT2A ex7::MLLT10 ex7	ROX	CY5
21	t(5;17)(q35;q21)	NPM1::RARA	NPM1 ex4::RARA ex3	FAM	CY5
	t(3;5)(q25;q34)	NPM1::MLF1	NPM1 ex4::MLF1 ex2	ROX	CY5
22	t(10;11)(p12;q23.3)	KMT2A::MLLT10	KMT2A ex7::MLLT10 ex11	FAM	CY5
	t(3;21)(q26;q22)	RUNX1::MECOM	RUNX1 ex6::MECOM ex6	ROX	CY5
23	t(10;11)(p12;q23.3)	KMT2A::MLLT10	KMT2A ex8::MLLT10 ex10	ROX	CY5
24	-	-	-	-	-

When more than one of the tubes 1-6, 8-15, 17-23 are positive for FAM or ROX interpret as follows:

Positive tubes	Interpret as tube
1 (ROX) 2 (FAM)	1 (ROX): Inv(16)(CBFB::MYH11)
1 (FAM) 3 (FAM) 4 (FAM)	3 (FAM): t(15;17)(PML::RARA), bcr1, L-form
1 (FAM) 3 (FAM)	3 (FAM): t(15;17)(PML::RARA), bcr1, L-form
3 (FAM) 4 (FAM)	3 (FAM): t(15;17)(PML::RARA), bcr1, L-form
1 (FAM) 4 (FAM)	1 (FAM): t(15;17)(PML::RARA), bcr2, V-form
3 (ROX) 4 (ROX)	3 (ROX): t(9;11)(KMT2A::MLLT3)
11 (FAM) 14 (FAM)	14 (FAM): t(11;19)(KMT2A::MLLT1)
13 (FAM) 14 (ROX)	14 (ROX): t(9;22)(BCR::ABL1), M-bcr, P210
20 (ROX) 22 (FAM)	20 (ROX): t(10;11)(KMT2A::MLLT10)
22 (FAM) 23 (ROX)	23 (ROX): t(10;11)(KMT2A::MLLT10)

Fusion genes targeted with more than one pair of amplification primers may result in positive signals in more than one tube. Use table above in combination with Interpretation Table to identify the breakpoint.

9. HGNC GENE NAMES AND NCBI ACCESSION NUMBERS

Old Abbreviation	HGNC Abbreviation	Chromosome	HGNC ID	NCBI Accession
ABL	ABL1	9q34.1	HGNC:76	NG_012034.1
AF10	MLLT10	10p12	HGNC:16063	NM_001195626.3
AF17	MLLT6	17q21	HGNC:7138	NM_005937.3
AF1p	EPS15	1p32	HGNC:3419	NM_001981.3
AF1q	MLLT11	1q21	HGNC:16997	NM_006818.3
AF4	AFF1	4q21.3	HGNC:7135	NM_001166693.2
AF6 or MLLT4	AFDN	6q27	HGNC:7137	NM_001207008.1
AF9	MLLT3	9p21.3	HGNC:7136	NM_004529.4
AFX1	FOXO4	Xq13.1	HGNC:7139	NM_005938.4
AML1	RUNX1	21q22.3	HGNC:10471	NG_011402.2
BCR	BCR	22q11	HGNC:1014	NG_009244.2
CAN	NUP214	9q34	HGNC:8064	NG_023371.1
CBFβ	CBFB	16q22.1	HGNC:1539	NG_009281.1
DEK	DEK	6p23	HGNC:2768	NM_003472.4
E2A	TCF3	19p13.3	HGNC:11633	NG_029953.2
ELL	ELL	19p13.1	HGNC:23114	NM_006532.4
ENL	MLLT1	19p13.3	HGNC:7134	NM_005934.4
ERG	ERG	21q22.3	HGNC:3446	NG_029732.1
ETO	RUNX1T1	8q22	HGNC:1535	NG_023272.3
FUS	FUS	16p11.2	HGNC:4010	NG_012889.2
HLF	HLF	17q22	HGNC:4977	NM_002126.5
MDS1-EVI1	MECOM	3q26	HGNC:3498	NG_028279.2
MLF1	MLF1	3q25	HGNC:7125	NM_001369783.1
MLL	KMT2A	11q23.3	HGNC:7132	NG_027813.1
MN1	MN1	22q12.1	HGNC:7180	NG_023258.1
MYH11	MYH11	16p13.11	HGNC:7569	NG_009299.1
NPM	NPM1	5q35	HGNC:7910	NG_016018.1
PBX1	PBX1	1q23.3	HGNC:8632	NG_028246.2
PDGFRβ	PDGFRB	5q31-q32	HGNC:8804	NG_023367.1
PLZF	ZBTB16	11q23	HGNC:12930	NG_012140.2
PML	PML	15q24	HGNC:9113	NG_029036.1
RARα	RARA	17q21	HGNC:9864	NM_000964.4
SET	SET	9q34	HGNC:10760	NG_030356.1
SIL1	STIL	1p32	HGNC:10879	NG_012126.1
Tal1	TAL1	1p32	HGNC:11556	NM_003189.5
TEL	ETV6	12p13	HGNC:3495	NG_011443.1
TLS	FUS	16p11.2	HGNC:4010	NG_012889.2

The HUGO Gene Nomenclature Committee (HGNC) approves a unique and meaningful name for every known human gene. The table contains a list of all relevant genes for the HemaVision®-28Q kit, with the old gene name abbreviation and the corresponding HGNC abbreviation. Furthermore, the table contains the NCBI (www.ncbi.nlm.nih.gov/nucleotide) accession numbers for the gene or transcript sequences targeted by HemaVision®-28Q primers and probes.

10. REVISION HISTORY

REVISION NUMBER	SECTION CHANGED	DATE
11	Section 1: Purpose of the test; clarification of the text. Section 5: Precautions; added more description and reference to guidances on specimen, RNA and PCR. Updated table 9 with most recent NCBI accession numbers.	2019.07.22
12	Added more detail to section 5: Precautions and update 7.1 RNA extraction with more guidance and detail on recommended methods.	2019.08.26
13	Added one more combination for PML-RARA bcr1 in table on pg 16: FAM positive tube 3 and 4.	2020.05.15
14	Changed from User Manual to Instruction For Use	2021.04.23
15	Section 4: 'Thermal Heating Block' to 'Thermal Heating Block, or Thermocycler (for cDNA synthesis)' Section 7.2: Note added to cDNA step: 'Note: Incubation can be performed in the yellow capped cDNA mix tube (in thermal heating block), or by transferring mix to a 0.2mL tube and incubating 42°C for 60 minutes and 95°C for 5 minutes in a thermocycler. If transferring to other tube, a second centrifugation step of the yellow capped tube can aid in maximum recovery of sample and cDNA mix.' Section 7.3.2: 'Ask us' changed to 'contact DNA Diagnostic' Section 7.3.3: The recommended gain setting for Rotor-Gene Q is changed from 'Green (FAM): 8, Orange (ROX): 8, Red (Cy5): 3' to 'using the Autogain function.' Section 7.4: Following added: 'During analysis of the Rotor-Gene Q curves, it is recommended to try with- and without the features 'Dynamic Tube' and 'Slope Correct', to obtain optimal curves. If there are baseline fluctuations (noise) in the first cycles, use the 'Ignore First' function for the cycles where fluctuations occur.' Section 7.4: 'QuantStudio 5' changed to 'QuantStudio' Section 7.4: 'standard analysis settings' changed 'default analysis settings' 'Trade mark' corrected to 'Trademark'	2023.05.03
16	Translocations 'SYMBOL-SYMBOL' replaced with 'SYMBOL::SYMBOL' according to 'HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions' | Leukemia (https://www.nature.com/articles/s41375-021-01436-6). 't(3;5)(q25.1;q35)' to 't(3;5)(q25;q34)' and 't(12;22)(p13;q11-12)'/'(p13;q11)' to 't(12;22)(p13;q12)'. Section 7.3: A brief mixing/vortex step of the tubes added to the protocol. Section 8: tube 3 "KMT2A ex7::MLLT3 ex7" changed to "KMT2A ex8::MLLT3 ex7" and : tube 4 "KMT2A ex8::MLLT3 ex11" changed to "KMT2A ex7::MLLT3 ex11" Section 9: MLLT10 NCBI accession updated from NG_027818.1 to NM_001195626.3, and hence the section 8, tube 18 changed from KMT2A ex8+9::MLLT10 ex18 to KMT2A ex8+9::MLLT10 ex17, and MLF1 NCBI accession updated from NG_027720.1 to NM_001369783.1 and hence tube 21 NPM1 ex4::MLF1 ex3 changed to NPM1 ex4::MLF1 ex2. Section 1: Added 'HemaVision®-28Q is not intended for quantitative tests, nor Minimal Residual Disease (MRD) and follow-up tests'. Section 7.1, Added: 'Other total RNA extraction methods can be used, as long as RNA integrity is maintained, and inhibiting agents removed.' CLP tube format added. Minor changes in wording and punctuations.	2024.04.08

DNA
DIAGNOSTIC

Availability / questions

Our team and distributors are always at hand
to answer all your questions.
Contact us to find your nearest HemaVision® partner.

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*DNA Diagnostic A/S was established in 1992.
DNA Diagnostic A/S is an ISO 13485 certified
developer, manufacturer, and worldwide supplier
of PCR based CE IVD marked in vitro diagnostic kits.*