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PERFORMANCE EVALUATION REPORT ON IN VITRO DIAGNOSTICS MEDICAL DEVICE

gb GENETIC APOE

SN_Zpráva_039_APOE_EN
in Hradec Králové

Assessed by:
MSc. Alena Víšková
GENERI BIOTECH s.r.o.

Signature: Víšková
Date: 29. 9. 2017

Approved on behalf of the manufacturer:
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GENERI BIOTECH s.r.o.

Signature:
Date: 9. 10. 2017

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1. Overview

The purpose of the study was to evaluate performance of the real-time PCR kit for the detection of two single-point mutations C112R (allele e4) and R158C (allele e2) in the APOE gene. The performance evaluation of the in vitro diagnostics medical device was carried out in the form of a validation study performed in accordance with the European Standard EN 13612:2002 and the Annex III of the Directive 98/79 EC.

The following validation parameters were assessed in the studies:

1. The precision
    a. The repeatability
    b. The intermediate precision in time
    c. The intermediate precision in different cyclers

    The robustness
2. The limit of detection and the working range
3. The trueness

The kit complied with all the pre-set criteria within all parameters, thus meeting the requirements for performance of in vitro diagnostics medical device.

2. Definitions and abbreviations

Assay	A mix of primers, probes and other reagents, used for detection and quantification of a specific DNA sequence using a real-time PCR.
APOE	Apolipoprotein E (gene ID 348)
C112R	Mutation in the APOE gene (rs429358)
CGB	CGB laboratoř a.s. – Cytogenetic and Cytobioptic Laboratory
Ct	Threshold cycle – a number of PCR cycles when a significant fluorescence increase occurs and the curve intersects the selected limit i.e. threshold.
Cycler	A real-time PCR thermocycler, an instrument that enables to trace a fluorescence signal during the PCR in a real time
DNA	Deoxyribonucleic acid
FAM	Fluorescein – a fluorescent dye
GB	GENERI BIOTECH s.r.o.
Genotype	A particular combination of alleles of the gene of an individual
gDNA	Genomic DNA
HET	Heterozygote (a sample or a standard containing both alleles – a non-mutated and a mutated one)
MUT	Mutant or mutated homozygote (a sample or a standard containing only mutated alleles)
Mutation	An aberration in DNA structure that may cause an inclination to a certain disease or even implicate it; a single-point mutation means a change in the primary DNA structure including a single nucleotide
IVD	In vitro diagnostics
PCR	Polymerase chain reaction
Primer	An oligonucleotide ensuring specific multiplication of the analyzed DNA using a polymerase enzyme
Probe	An oligonucleotide enabling a detection of an analyzed DNA

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qPCR	Quantitative (real-time) polymerase chain reaction
R158C	Mutation in the APOE gene (rs7412)
Run	A concurrent and simultaneous analysis of a specific number of reactions in a PCR cycler
Standard	A positive control sample for a specific genotype
WT	Wild type homozygote (a sample or a standard containing only non-mutated alleles)

3. The identification of the assessed IVD device

The device name:

gb GENETIC APOE
Cat. No.: 3206-025 (25-reaction package)

The principle of the kit:

The kit works on the principle of allelic discrimination, which is a modification of the real-time PCR method. Allelic discrimination enables a specific detection of single-point mutations in gDNA in a real-time PCR cycler. A homozygous wild-type (WT) genotype is detected in FAM channel; a homozygous mutated (MUT) genotype is detected in HEX channel. In case of heterozygous genotype (HET) the signal is present in both channels.

The kit composition:

The validated real-time PCR kit contains 2 qPCR assays (primers and probes specific for non-mutated and mutated gene variants and other necessary reagents – buffer, nucleotides, polymerase), 6 Standards (positive controls) for all 3 genotypes - wild type homozygote (WT), heterozygote (HET) and mutated homozygote (MUT) relevant to the particular polymorphism, and Deionized water.

The intended purpose:

The apolipoprotein E is a significant structure part of cholesterol transporting particles (VLDL, HDL) and is a ligand of cell receptors especially in brain and liver. The gene for apolipoprotein E occurs in three alleles, e2, e3 and e4, that code isoforms E2, E3 and E4 of the protein. The E3 isoform is major in the population and carries cysteine and arginine amino acids on positions 112 and 158. The e4 allele carries a single-point mutation (rs429358) that causes a change from arginine to cysteine on position 112, and e2 allele carries a single-point mutation (rs7412) where cysteine is replaced by arginine on position 158.

The indications for genetic testing of Apolipoprotein E are i.e. hyperlipoproteinemia type III, dyslipidemia in diabetes mellitus type II, premature atherosclerotic process or Alzheimer's disease.

4. The manufacturer identification

GENERI BIOTECH s.r.o.
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5. The identification of participated laboratories and individuals

Partial validations were performed at the following worksites:

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Machkova 587/42
500 11 Hradec Králové 11 – Třebeš
Czech Republic

Phone: +420 495 056 314
Fax: +420 495 056 316

Tests were performed by:

MSc. Alena Víšková, Professional staff
Ilona Svobodová, Laboratory technician

CGB laboratoř, a.s. (a provider of health services)
Kořenského 1210/10
703 00 Ostrava – Vítkovice
Czech Republic

Phone: +420 595 700 170
Fax: +420 595 700 176

Tests were performed by:

MSc. Jarmila Šimová

The final assessment was performed in the laboratories of the manufacturer (GENERI BIOTECH s.r.o.).

Assessed by: MSc. Alena Víšková
Approved by: PharmD. Lucie Krausová, PhD

6. The testing process

6.1. Validation plans

The testing took place in two blocks. For each of them an individual validation plan and a validation report were created. Those are available in the manufacturer's technical documentation.

The testing of the kit performed in the manufacturer's laboratories (GENERI BIOTECH s.r.o.) took place from July 17 to September 27, 2017 (determination of the precision, robustness, limit of detection and working range).

The testing of the kit in CGB laboratories took place from June 20 to July 24, 2017 (determination of the trueness).

6.2. Specifications of samples, standards and reference methods

The manufacturing and QC protocols and specifications of tested batches (listed in validation reports) are available in the manufacturer's technical documentation.

The testing was performed using control samples (Standards) representing all 3 genotypes (WT, MUT and HET) for C112R and R158C mutations in the APOE gene. The genotypes of Standards were validated by

sequencing using an automatic sequencer in GENERI BIOTECH s.r.o. In the precision testing, two concentration levels of Standards were used.

The determination of the limit of detection (LOD) and the working range of the kit was carried out on a serial dilution of two gDNA samples isolated from buccal mucosa skimming using Kit for isolation of DNA from body fluids (GENERI BIOTECH, Cat. No. 3100).

The trueness test was performed using clinical samples of gDNA. In total, 25 samples were examined. The reference method was represented by determination of the genotype by the real-time PCR followed by the reverse hybridization using the CVD StripAssay® (ViennaLab) kit in CGB laboratories.

The detailed specifications of Standards and gDNA samples and their preparation are described in the validation plans and reports.

6.3. Performing and the assessment of the tests

The preparation of reactions and the assessment of a mutation presence were carried out in compliance with the instruction manual relevant to the kit. The distribution of samples in the tests and the acceptance criteria for samples and runs are described in the validation plans, and specific results of the testing are stated in validation reports.

7. The assessment of validation parameters

7.1. The precision

7.1.1. The repeatability

It is a concordance of results within repeated measurements performed by the same person in the same cycler within one day. The measurement was carried out twice in duplicates in the following PCR cyclers:

- CFX96 (Bio-Rad)
- Rotor-Gene 3000 (Corbett Research)
- Rotor-Gene 6000 (Corbett Research)
- ABI 7500 (Applied Biosystems)

Acceptance criteria: Results must be identical in both reactions of the duplicate for both mutations and three types of Standards (WT, MUT and HET) in both concentrations.

Obtained values: The determined genotypes of all Standards in two concentrations were identical in all repetitions and in all used cyclers.

Results: The results meet the required acceptance criteria.

7.1.2. The intermediate precision in time

It is a concordance of results within repeated measurements performed by different people in the same cycler CFX96 (Bio-Rad) within two days (first and seventh day).

Acceptance criteria: Results must be identical in both reactions of the duplicate for both mutations and three types of Standards (WT, MUT, HET) in both concentrations.

Obtained values: The determined genotypes of all Standards in two concentrations were identical in both runs.

Results: The results meet the required acceptance criteria.

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7.1.3. The intermediate precision in different cyclers

It is a concordance of results within measurements performed by different people in different real-time PCR cyclers. Testing was carried out in the following PCR cyclers:

- CFX96 (Bio-Rad)
- Rotor-Gene 3000 (Corbett Research)
- Rotor-Gene 6000 (Corbett Research)
- ABI 7500 (Applied Biosystems)

Acceptance criteria: Results from all cyclers must be identical for both monitored mutations and three types of Standards (WT, MUT and HET) in both concentrations and genotypes must not differ amongst cyclers.

Obtained values: The determined genotypes of all Standards in two concentrations were identical for all used PCR cyclers.

Results: The results meet the required acceptance criteria.

7.2. The robustness

It is a concordance of results within minor intentional deviations from the instruction manual using the CFX96 (Bio-Rad) cycler:

1) An analysis performed 1, 2 and 4 hours after storing the prepared PCR mixture in 2-8°C (i.e. for the limit time interval recommended in the instruction manual and a twice and fourfold prolonged interval, respectively).

2) An analysis performed in different temperature profiles of the signal detection step of the PCR: 1 °C higher and 1 °C lower than stated in the instruction manual.

3) An analysis following a changed pipetted volume of the gDNA sample in two concentrations according to the determined working range (volumes of 3, 5 and 8 µl were used instead of 4 µl required in the instruction manual.

Acceptance criteria: Results of analyses performed under modified conditions must be identical in all runs to the results obtained by the standard procedure following the instruction manual for all three types of Standards (WT, MUT and HET) in all concentrations and volumes.

Obtained values: The determined genotypes for all Standards in two concentrations in all 6 runs obtained after minor intentional deviations from the instructions were identical to the results of analysis performed according to the instruction manual.

Results: The results meet the required acceptance criteria.

7.3. The limit of detection and the working range

The limit of detection (LOD) and the working range were obtained through the analysis of serial dilution of two gDNA samples in the CFX96 cycler (Bio-Rad). The LOD is the lowest amount of DNA in a reaction, where a genotype is detected in specific channels in all multiplicate reactions in concordance with higher concentrations, and where the Ct value is lower than 31. The working range is set by the LOD and the highest amount of DNA in a reaction, where a genotype is detected in specific channels and in all multiplicate reactions in concordance with lower concentrations above the LOD.

Acceptance criteria: The LOD must be 20 ng of gDNA in a reaction or lower, the working range must be at least 20–200 ng of gDNA in a reaction.

Obtained values: The obtained values are stated in the table below. The working range of the kit was set to 20–400 ng of DNA in a reaction (sample input concentration 5–100 ng/µl) in relation to the reliable reserve for genotype assessment on the basis of delta RFU (delta Rn) in some types of cyclers.

Tab. 1 – The highest and the lowest detected amount of gDNA in a reaction

Monitored mutation	Sample ID	Sample genotype	The highest amount (ng)	Limit of detection (ng)
C112R	A	WT	400	1
	B	WT	400	2
R158C	A	WT	400	1
	B	HET	400	2

Results: The results meet the required acceptance criteria.

7.4. The trueness

The detection trueness is a concordance of obtained results with results obtained by a validated reference method. The determination of gDNA clinical samples genotypes was performed using Rotor-Gene 3000A cycler (Corbett Research) in CGB.

Acceptance criteria: Determined genotypes of gDNA samples must be identical to the genotypes obtained by a validated reference method in 100 % of cases in all runs.

Obtained values: The tests were carried out on 25 samples. The determined genotypes are stated in the table below. The determined genotypes of samples were identical with the genotype by a reference method in 100 % of cases.

Tab. 2 – Genomic DNA samples genotypes

Genotype for C112R mutation	Genotype for R158C mutation	Number of samples
WT	MUT	1
WT	HET	6
WT	WT	9
HET	WT	8
MUT	WT	1

Results: The results meet the required acceptance criteria.

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