ViennaLab®

α-Globin StripAssay®

Instructions For Use

REF	Σ
4-160	10 tests
4-160-A	24 tests
4-160-TRIAL	5 tests

2°C – 8°C

IVD

Version: rev 1.3 / English
eIFU and other languages available at
www.viennalab.com

CE 0123

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REVISION HISTORY:

version	date	description
rev 1.1	2022-02	CE mark with identification number of notified body; link to SSP; statement of sample source and manual/semi-automated use (I); specification of kit components (IV); clinical performance data (VIII)
rev 1.2	2022-05	Date of issue, reference to electronic IFU (eIFU), Iran (II) included
rev 1.3	2022-11	Improved layout and resolution of figures

Summary of Safety and Performance (SSP) of the StripAssay® is retrievable from the European Database on Medical Devices (EUDAMED): https://ec.europa.eu/tools/eudamed or from the manufacturer.

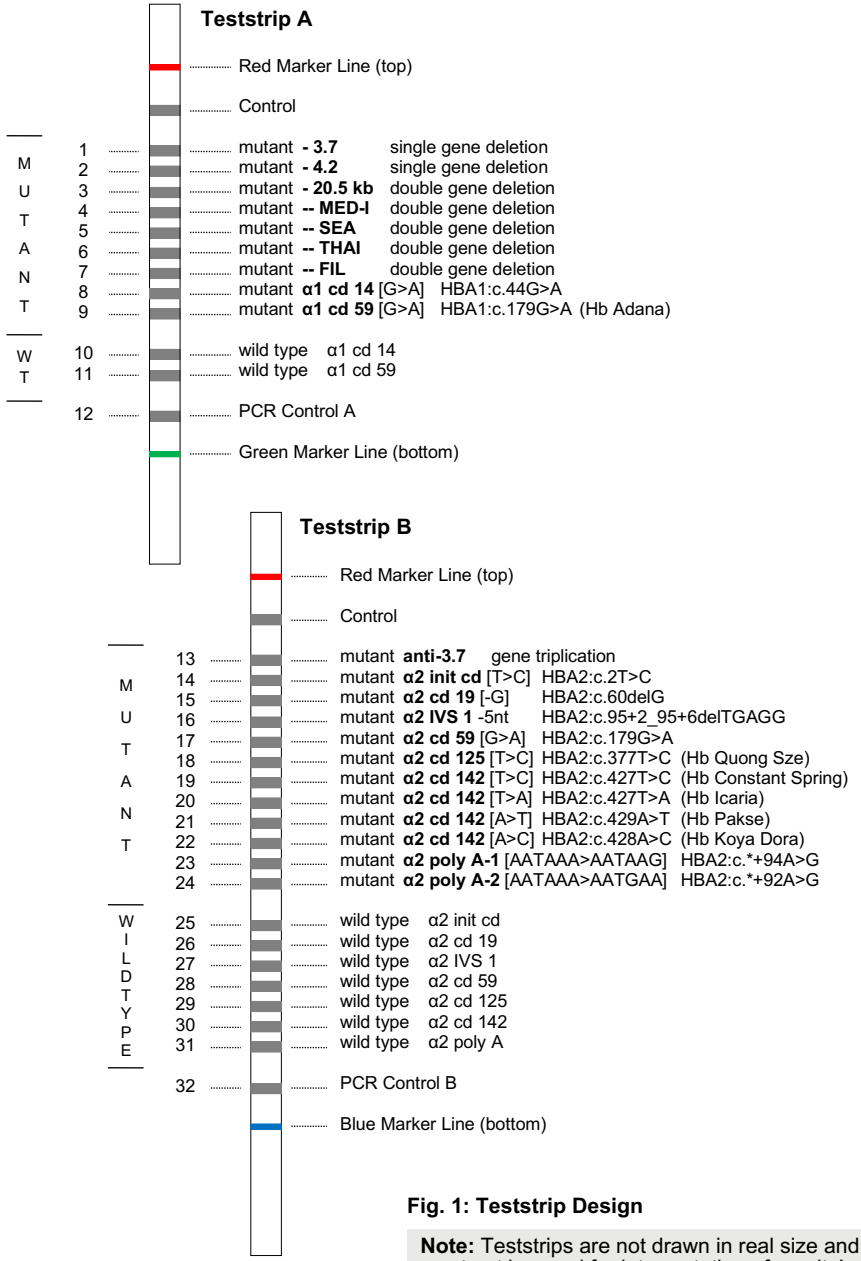

Fig. 1: Teststrip Design

Note: Teststrips are not drawn in real size and must not be used for interpretation of results!

I. INTENDED PURPOSE

The α-Globin StripAssay® is a qualitative genetic test for the targeted analysis of 21 common large deletions and point mutations of the *hemoglobin subunit alpha 1* (*HBA1*) and *alpha 2* (*HBA2*) genes in DNA isolated from human peripheral blood. The test is used as an aid to genetically confirm a suspected diagnosis of alpha-thalassemia (alpha-thal). Furthermore, the assay can be used for screening the thalassemia-carrier status in the patient's relatives and the general population. The StripAssay® can be carried out either manually or semi-automated.

For human *in vitro* diagnostic use.

II. BACKGROUND

Mutations in the *alpha-globin* genes are the genetic cause of alpha-thalassemia, an autosomal recessive inherited disorder, which is characterized by insufficient or absent alpha-globin chain production leading to a variable clinical picture depending on the number of affected alleles.

Patients with characteristic hematological values of microcytic anemia and corresponding hemoglobin patterns, family members of an affected patient, prospective parents, as well as individuals from high-risk populations (e.g. Mediterranean region, Africa, Arabian Peninsula, Iran, India and Southeast Asia), who are at risk to be carriers of alpha-thalassemia should be tested.

III. METHODOLOGY

The α-Globin StripAssay® is based on polymerase chain reaction (PCR) and reverse-hybridization. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to a Teststrip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines (Fig. 1). Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

The α-Globin StripAssay® detects the following mutations in the *alpha-globin* gene locus:

	legacy name	HGVS nomenclature	RefSNP
1	-3.7 kb	NG_000006.1:g.34164_37967del3804	--
2	-4.2 kb	n.a.	--
3	--20.5 kb	NG_000006.1:g.15164_37864del22701	--
4	--MED-I	NG_000006.1:g.24664_41064del16401	--
5	--SEA	NG_000006.1:g.26264_45564del19301	--
6	--THAI	NG_000006.1:g.10664_44164del33501	--
7	--FIL	NG_000006.1:g.11684_43534del31851	--
8	anti-3.7 gene triplication	n.a.	--
9	cd 14 [G>A]	HBA1:c.44G>A	rs63750090
10	cd 59 [G>A] Hb Adana	HBA1:c.179G>A	rs28928878
11	initiation cd [T>C]	HBA2:c.2T>C	rs111033603
12	cd 19 [–G]	HBA2:c.60delG	rs886041399
13	IVS1-5nt	HBA2:c.95+2_95+6delTGAGG	rs41474145
14	cd 59 [G>A]	HBA2:c.179G>A	rs281864846
15	cd 125 [T>C] Hb Quong Sze	HBA2:c.377T>C	rs41397847
16	cd 142 [T>C] Hb Constant Spring	HBA2:c.427T>C	rs41464951
17	cd 142 [T>A] Hb Icaria	HBA2:c.427T>A	rs41464951
18	cd 142 [A>T] Hb Pakse	HBA2:c.429A>T	rs41412046
19	cd 142 [A>C] Hb Koya Dora	HBA2:c.428A>C	rs41321345
20	polyA-1 [AATAAA>AATAAG]	HBA2:c.*+94A>G	rs63751269
21	polyA-2 [AATAAA>AATGAA]	HBA2:c.*+92A>G	rs63750067

Reference Sequence (RefSeq):

NG_000006.1
NM_000558.3 (*HBA1*)
NM_000517.4 (*HBA2*)

The test can be carried out manually or semi-automated using instruments designed for automation of Teststrip processing (see section VI. 3.4).

IV. KIT COMPONENTS

REF

	4-160	4-160-A	4-160 -TRIAL
1a. **Amplification Mix A1** *(yellow cap)*	250 µl	2x 250 µl	250 µl
1b. **Amplification Mix A2** *(white cap)*	250 µl	2x 250 µl	250 µl
1c. **Amplification Mix B** *(green cap)*	250 µl	2x 250 µl	250 µl
2. **Taq Dilution Buffer** *(transparent cap)*	500 µl	500 µl	500 µl
3. **HS-Taq DNA Polymerase (5 U/µl)** *(red cap)*	125 U	175 U	125 U
4. **DNAT** *(blue cap)*	1.5 ml	1.5 ml	1.5 ml
Warning: DNAT contains 1.6 % NaOH H315: Causes skin irritation H319: Causes serious eye irritation P280: Wear protective gloves/protective clothing/eye protection/face protection P337 + P313: If eye irritation persists: Get medical advice/attention			
5. **Typing Trays**	3	---	2
6a. **Teststrips A** *(black cap)*	10	24	5
6b. **Teststrips B** *(white cap)*	10	24	5
7. **Hybridization Buffer** *(white cap)*	25 ml	65 ml	25 ml
8. **Wash Solution A** *(white cap)*	80 ml	200 ml	80 ml
9. **Conjugate Solution** *(transparent cap)*	25 ml	65 ml	25 ml
10. **Wash Solution B** *(transparent cap)*	80 ml	200 ml	80 ml
11. **Color Developer** *(brown cap)*	25 ml	65 ml	25 ml
Warning: Color Developer contains ≤0.4% maleic acid H317: May cause an allergic skin reaction P280: Wear protective gloves/protective clothing/eye protection/face protection P302 + P352: If on skin: wash with plenty of water P333 + P313: If skin irritation or rash occurs: get medical advice /attention			
12. **Instructions For Use**	1	1	1
13. **Collector™ Sheet**	1	3	1

Note: Store all reagents at 2°C to 8°C when not in use!

name of component	composition
Amplification Mix A1/A2/B	sequence-specific 5'-biotin labelled oligonucleotides, an equimolar mixture of deoxy ribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), $MgCl_2$, ammonium sulfate buffer, betaine, 0.05% sodium azide
Taq Dilution Buffer	buffer for HS-Taq DNA Polymerase, including KCl, $(NH_4)_2SO_4$ and $MgCl_2$, 0.05% sodium azide
HS-Taq DNA Polymerase (5 U/µl)	hot-start-Taq DNA polymerase at a concentration of 5U/µl
DNAT	basic solution containing 1.6 % sodium hydroxide and a blue dye indicating a change of pH
Typing Trays	plastic tray with eight wells

name of component	composition
Teststrips A/B	allele-specific oligonucleotide probes, control for positive PCR reaction and a hybridization control immobilized as an array of parallel lines on a polyester-supported membrane framed by a red line on the top and a green (Teststrip A) or blue (Teststrip B) line on the bottom
Hybridization Buffer	phosphate buffer with <2% detergent
Wash Solution A	citrate buffer with <1% detergent
Conjugate Solution	streptavidin conjugated alkaline phosphatase diluted in a saline based buffer with 0.05% sodium azide
Wash Solution B	tris buffer containing <2% detergent and 0.05% sodium azide
Color Developer	color substrate for the alkaline phosphatase contains nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)
Instructions For Use	printed paper
Collector™ Sheet	printed paper

V. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Spin Micro DNA Extraction Kit (REF 2-020, ViennaLab)
- Thermocycler with heated lid (for specification of ramp rates see section VIII)
- Waterbath with shaking platform, lid and adjustable temperature (45°C ± 1°C)
- Shaker (rocker or orbital shaker)

Optional:

- Vacuum aspiration apparatus
- Thermoshaker for microtiter plate format with lid and adjustable temperature (45°C ± 1°C), e.g. PST-60 HL (Biosan) or equivalent device
- Instrument for automated hybridization, adjustable to the time-temperature profile as described in section VI. 3.4, e.g. DYNABLOT Heat (Dynex) or equivalent device
- Agarose gel electrophoresis equipment (for control of amplification products)

VI. ASSAY PROCEDURE

1. Sample Preparation

Specimen: Use fresh or frozen blood with EDTA anticoagulant. Blood containing heparin or citrate has not been tested. Do not store blood for more than 3 days at ambient temperature or more than 1 week at 2°C to 8°C before use. Blood that has been kept frozen for more than one year, or gone through more than three freeze-thaw cycles shall not be used. For specimen collection and transportation follow the instructions for use of the EDTA-blood collection tube and general recommendations for blood sampling.

DNA Extraction: Bring blood samples to room temperature. Mix well by carefully inverting blood collection tubes several times. Repeat mixing each time before withdrawing an aliquot of blood. It is recommended to use the **Spin Micro DNA Extraction Kit** (REF 2-020, ViennaLab) for DNA isolation from whole blood. Use of other DNA isolation methods with the α-Globin StripAssay® has not been validated. In case other DNA extraction systems are used, concentration and purity of DNA should be within a range of 2 to 10 ng/µl and an $OD_{A260/280}$ ratio of 1.7 to 2.0, respectively. Higher DNA concentrations have to be diluted to the recommended range prior to PCR input.

Note: DNA containing PCR inhibitors and/or magnetic-particles derived from bead-based extraction system may be refractory to amplification and should be diluted to 2 ng/µl using PCR grade water.

Extracted DNA shall be stored at 2°C to 8°C (up to one week) or at -30°C to -15°C (for long term) until analysis is carried out.

2. In Vitro Amplification (PCR) – 3 separate reactions per sample

Important: Keep all PCR reagents and DNA templates refrigerated throughout.

- Freshly prepare each time an appropriate amount of working solution (1:15, final conc. 0.33 U/µl) of **HS-Taq DNA Polymerase** (5 U/µl, red cap) in **Taq Dilution Buffer** (transparent cap) for the number of samples to be analyzed, plus the **no-template control (NTC)**.

component	per reaction	e.g. 10 reactions
HS-Taq DNA Polymerase (5 U/µl)	0.33 µl	3.3 µl
Taq Dilution Buffer	4.67 µl	46.7 µl
working solution	5 µl	50 µl

- Prepare three reaction tubes for each sample to be amplified. Place tubes on ice.
- For each sample prepare 3 final PCR reaction mixes (A1, A2, B) on ice:

A1: **15 µl Amplification Mix A1** (yellow cap)
5 µl diluted HS-Taq DNA Polymerase (1.66 U)
5 µl DNA template

A2: **15 µl Amplification Mix A2** (white cap)
5 µl diluted HS-Taq DNA Polymerase (1.66 U)
5 µl DNA template

B: **15 µl Amplification Mix B** (green cap)
5 µl diluted HS-Taq DNA Polymerase (1.66 U)
5 µl DNA template

Note: It is recommended to prepare a mastermix for all samples containing Ampification Mix and diluted HS-Taq DNA Polymerase. First pipette 20 µl of the mastermix into each PCR tube, and then add DNA template. Include a no-template control in each run by using PCR grade water instead of DNA (or preferably the negative control of your DNA extraction).

Generally, prepare working solutions / mastermix with a 10% excess volume to compensate for pipetting inaccuracies.

- Cap tubes tightly. Preheat the thermocycler to 95°C.
- Insert reaction tubes and run the following thermocycling program:

pre-PCR: **95°C/5 min.**
thermocycling: **97°C/40 sec. - 64°C/40 sec. - 72°C/1:30 min. (3 cycles)**
97°C/40 sec. - 58°C/40 sec. - 72°C/1:30 min. (37 cycles)
final extension: **72°C/5 min.**

- Store amplification products on ice or at 2°C to 8°C for further use.

Optional: Analyze amplification products by gel electrophoresis (e.g. 3% agarose gel).

Fragment lengths: 881 bp; deletions: 1783 bp (A1)
296 bp; deletions: 250, 329, 373, 474, 578, 1025 bp (A2)
302, 864 bp; deletions: 1772 bp (B)

3. Processing of Teststrips

3.1. Hybridization (manual) – 2 Teststrips per sample (45°C, shaking waterbath)

Important: Adjust the water level of the waterbath to approx. ½ of the height of the Typing Tray. Heat the waterbath to exactly 45°C. Check water temperature with a calibrated thermometer. Prewarm Hybridization Buffer and Wash Solution A to 45°C. Take care that all precipitates formed at 2°C to 8°C become completely dissolved. Allow Teststrips, DNAT, Conjugate Solution, Wash Solution B and Color Developer to reach room temperature. Prepare Typing Tray(s).

Remove one Teststrip A and one Teststrip B for each sample using clean tweezers. Touch Teststrips with unpowdered gloves only! Label Teststrips outside of the marker lines with a pencil (no ballpoint pens, markers, etc.).

For all **Teststrips A** (one lane per sample):

- Pipette **20 µl DNAT** (blue cap) into the lower corner of each lane to be used in the Typing Trays.
- Add **10 µl amplification product A1** into the corresponding drop of DNAT.
- Add **10 µl amplification product A2** into the same drop.
- Mix thoroughly with a pipette. (The solution will remain blue.)

For all **Teststrips B** (one lane per sample):

- Pipette **10 µl DNAT** (blue cap) into the lower corner of each lane to be used in the Typing Trays.
- Add **10 µl amplification product B** into the corresponding drop of DNAT.
- Mix thoroughly with a pipette. (The solution will remain blue.)

- Let stand for **5 min.** at room temperature.
- Add **1 ml Hybridization Buffer** (prewarmed to 45°C) into each lane. Gently agitate tray. (The blue color will disappear.)
- Insert **Teststrip A** or **Teststrip B** with marked side up (lines visible!) into the respective lanes. Submerge completely.
- Incubate for **30 min.** at **45°C** on the shaking platform of the waterbath.

Set moderate shaking frequency (approx. 50 rpm) to avoid spilling. Keep the cover of the waterbath closed to avoid variations in temperature.

- At the end of incubation remove hybridization solutions by vacuum aspiration or pipetting.

Proceed immediately. Do not allow Teststrips to run dry during the entire procedure.

3.2. Stringent Wash (45°C, shaking waterbath)

- Add **1 ml Wash Solution A** (prewarmed to 45°C). Rinse briefly (10 sec.). Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath. Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath. Remove liquids by vacuum aspiration or pipetting.

3.3. Colorimetric Detection (room temperature, 22°C ± 3°C)

- Add **1 ml Conjugate Solution**.
- Incubate for **15 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**. Rinse briefly (10 sec.).
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Color Developer**.
- Incubate for **15 min.** at **room temperature** in the dark on a rocker or orbital shaker.
  A purple staining will appear upon positive reaction.
- Wash Teststrips several times with distilled water.
  Let strips dry in the dark on absorbent paper.

Do not expose Teststrips to intense light after Color Development.

3.4. Hybridization (automated) - optional instead of waterbath and shaker

An instrument for the automated processing of Teststrips shall meet the following requirements:

- Programmable temperature and time profile according to section 3.1 to 3.3 of the StripAssay® procedure.
- Integrated preheating station for Hybridization Buffer and Wash Solution A.
- Temperature control of trays during Hybridization and Stringent Wash steps at 45°C ± 1°C.
- Active cooling system for the tray to ensure rapid temperature decrease for Colorimetric Detection steps at room temperature.
- Shaking capability for tray.
- Heated lid for the tray to avoid evaporation of reagents during incubation.
- Dispensation of defined reagent volumes.
- Aspiration of reagents.
- Depending on the instrument used and the number of samples processed in one run, additional reagents may be required. Separate StripAssay® Detection Reagents are available for 20 tests (REF CS-012) and 48 tests (REF CS-017).

VII. INTERPRETATION OF RESULTS

The genotype of a sample is determined from corresponding Teststrips A and B using the enclosed Collector™ sheet. Place both processed Teststrips into the designated fields, align them to the schematic drawing using the red marker line (top) and the green or blue marker line (bottom), and fix them with adhesive tape.

A positive reaction of the uppermost Control line indicates the correct function of Conjugate Solution and Color Developer. This line should always stain positive.

A positive reaction of the PCR Control A and PCR Control B lines indicates the presence of the correct amplification products. These lines must always stain positive, except for the no-template control, which contains water in place of DNA template (see example H, page 18).

Absence of PCR Controls on Teststrips could indicate false hybridization of Mix A1/A2 amplification products to Teststrip B and Mix B amplification products to Teststrip A. Repeat testing.

For each polymorphic position, one of the following staining patterns (Fig. 2) should be obtained:

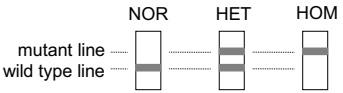

Fig. 2: Genotypes – staining patterns on the Teststrip

	wild type line	mutant line	genotype
NOR	**positive**	negative	normal
HET	**positive**	**positive**	heterozygous
HOM	negative	**positive**	homozygous mutant

Note: Staining intensities of positive lines may vary. This is of no significance for the result.

See examples of StripAssay® results on page 18 (Fig. 3).

Some of the point mutations covered by the α-Globin StripAssay® are located within a few nucleotides on the *α-globin* gene. On the Teststrips these are represented by a common wild type probe, so that the 21 mutations are covered by 9 wild type probes only:

line	wild type probe	mutation
10	α1 cd 14	α1 cd 14 [G>A]
11	α1 cd 59	Hb Adana
25	α2 init cd	α2 init cd [T>C]
26	α2 cd 19	α2 cd 19 [-G]
27	α2 IVS 1	α2 IVS 1 -5nt
28	α2 cd 59	α2 cd 59 [G>A]
29	α2 cd 125	Hb Quong Sze
30	α2 cd 142	Hb Constant Spring, Hb Icaria, Hb Pakse, Hb Koya Dora
31	α2 poly A	α2 poly A-1, α2 poly A-2

Samples that are compound heterozygous for two of these mutations (e.g. Hb Constant Spring + Hb Pakse) will be lacking the common wild type signal (see example E, page 18).

Samples that are compound heterozygous for one of the α1/α2 mutations, and a single or double gene deletion, will in most cases be lacking the respective wild type signal (see example D, page 18).

For single and double gene deletions, several wild type probes discriminate between the heterozygous and the homozygous mutant state (see examples B and C, page 18):

deletion	heterozygous	homozygous mutant
- 3.7	all WT signals present	WT signals 25-31 absent
- 4.2	all WT signals present	WT signals 25-31 absent
- 20.5 kb	all WT signals present	WT signals 10 and 25-31 absent
-- MED-I	all WT signals present	all WT signals absent
-- SEA	all WT signals present	all WT signals absent
-- THAI	all WT signals present	all WT signals absent
-- FIL	all WT signals present	all WT signals absent

As with any diagnostic test, results of the α-Globin StripAssay® shall be interpreted in the context of the patient's overall clinical phenotype and other medical investigations available to the physician. ViennaLab Diagnostics GmbH is not responsible for any clinical decisions that are taken.

VIII. PERFORMANCE EVALUATION

Accuracy of the α-Globin StripAssay® was determined by analyzing 330 pretyped genomic DNA samples. Apart from one sample results were concordant with the reference method (Sanger sequencing, gap-PCR, ARMS-PCR, reverse dot-blot analysis, EQA derived samples). A heterozygous polyA-2 sample, emerged from a rare recombination of the 3'-ends of the *alpha-2* and *pseudo-alpha* genes, was typed false negative (wild type) by the StripAssay®. The assay correctly detected 359 mutant alleles (= 99.7% Positive Percent Agreement) and 300 wild type alleles (= 100% Negative Percent Agreement).

Precision of the α-Globin StripAssay® was assessed as variability between replicates, operators, days, thermocyclers and hybridization devices. In a total of 62 tests carried out under the investigated parameters, 61 showed the expected genotyping results, and one sample failed due to inaccuracy of pipetting template DNA. Only negligible differences in staining intensity of Teststrips were visible, and no background staining was observed. The α-Globin StripAssay® was validated on the AB GeneAmp® PCR System 2700, MJ Research PTC-200 and Eppendorf Mastercycler X50s, which represent a heating and cooling rate in the range of 1.7 to 6.3°C/sec and 1.4 to 3.7°C/sec, respectively.

Use of other thermocyclers must be verified by the user.

Analytical Specificity is first and foremost ensured by the selection of the gene-specific primers and the allele-specific capture probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene databases by sequence comparison analysis. Thereby, the detectability of all relevant genotypes has been ensured. Potential cross-reactivity between capture probes was verified by synthetic DNA harboring the respective gene fragment. No cross-reactivity was observed.

Clinical Performance: In a multi-center comparison study (Puehringer et al. 2007), a total of 272 patient samples from the catchment area of eight different thalassemia centers worldwide was tested with the α-Globin StripAssay® and the reference methods routinely used in these laboratories. Of the 544 wild-type or mutant α-globin alleles in the patient cohort, the results for 523 (96.14%) were completely concordant between the StripAssay® and in-house methods.

IX. INTERFERING SUBSTANCES

Five interfering substances (hemoglobin, immunoglobin G, traces of blood, ethanol and EDTA) potentially being present in EDTA-blood derived DNA preparations have been tested. Their effects on PCR were evaluated in three purified DNA samples spiked with various concentrations of substances and compared to their controls without addition of any interfering substances. All samples were analyzed in triplicate.

A final concentration of <10 µM hemoglobin, 0.1 µM immunoglobulin G, <1% peripheral blood, 1.25% ethanol or 0.1 mM EDTA in the reaction did not interfere with StripAssay® performance.

X. LIMITATIONS OF THE ASSAY

The α-Globin StripAssay® is exclusively designed for the diagnosis of 21 known mutations as listed in section III, which are represented by allele-specific capture probes on the Teststrips. Other alpha-globin deletions, point mutations or recombinations that may be present in a patient's sample cannot be detected. At best, a disregarded point mutation located within the sequence spanned by a capture probe can be indicated by the loss of wild type signal on the Teststrip when it is concomitantly present with a single or double gene deletion or in the homozygous state.

Rare or private variants within primers and probes binding sites, as well as gene conversions may lead to amplification failure and missing signals on Teststrips.

The α-Globin StripAssay® does not allow to distinguish between the heterozygous and the homozygous mutant state of the anti-3.7 gene triplication (anti-3.7/αα and anti-3.7/anti-3.7).

In the presence of large gene deletions not detectable by the assay, single gene deletions (-3.7 or -4.2) and point mutations appear as homozygous.

The α-Globin StripAssay® must not be used for the purpose of prenatal diagnosis or preimplantation genetic diagnosis. The assay has not been validated on specimens derived from chorionic villus sampling, amniotic fluid or umbilical cord blood.

The α-Globin StripAssay® is intended for laboratory professional use only.

XI. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, as well as standard laboratory techniques and appropriate equipment are required to obtain reliable results.
- Do not use StripAssay® kits beyond their expiration date.
- After first opening of the primary container, StripAssay® reagents are stable until the expiry date printed on the outer label of the kit when stored properly at 2°C to 8°C.
- Use sterile disposable pipette tips with filters to avoid microbial contamination and cross-contamination of reagents or samples. Do not interchange bottle caps.
- Single use only.

XII. SAFETY

- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Avoid contact of DNAT and Color Developer with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spilled, dilute with water before wiping dry.
- Adhere to all local and federal safety and environmental regulations which may apply.

XIII. TECHNICAL SUPPORT

Technical support may be obtained by:

- the local ViennaLab Diagnostics distributor (www.viennalab.com/distribution)
- Video Tutorials (www.viennalab.com/support)
- the StripAssay® Manual (www.viennalab.com/support)
- the StripAssay® Troubleshooting Guide (www.viennalab.com/support)
- contacting techhelp@viennalab.com

XIV. REFERENCES

- OMIM Online Mendelian Inheritance in Man (www.omim.org)
- HbVar database (http://globin.cse.psu.edu/hbvar/menu.html)
- Thalassemia International Federation (www.thalassaemia.org.cy)
- Ithanet (www.ithanet.eu)
- Puehringer et al. Clin Chem Lab Med 2007;45(5):605-10, DOI:10.1515/CCLM.2007.125

XV. FEEDBACK TO THE MANUFACTURER

Any serious incident that has occurred in relation to the StripAssay® must be reported to the competent authority of the country and to the manufacturer.

XVI. SYMBOLS

REF	Catalog number
LOT	Batch code
IVD	*In vitro* diagnostic medical device
CE 0123	Compliant with European IVD Regulation 2017/746 Identification number of notified body
	Sufficient for <n> tests
	Storage temperature limits
	Use by
	Caution
	Manufacturer
	Date of manufacture
	Consult Instructions For Use

XVII. EXAMPLES OF TEST RESULTS

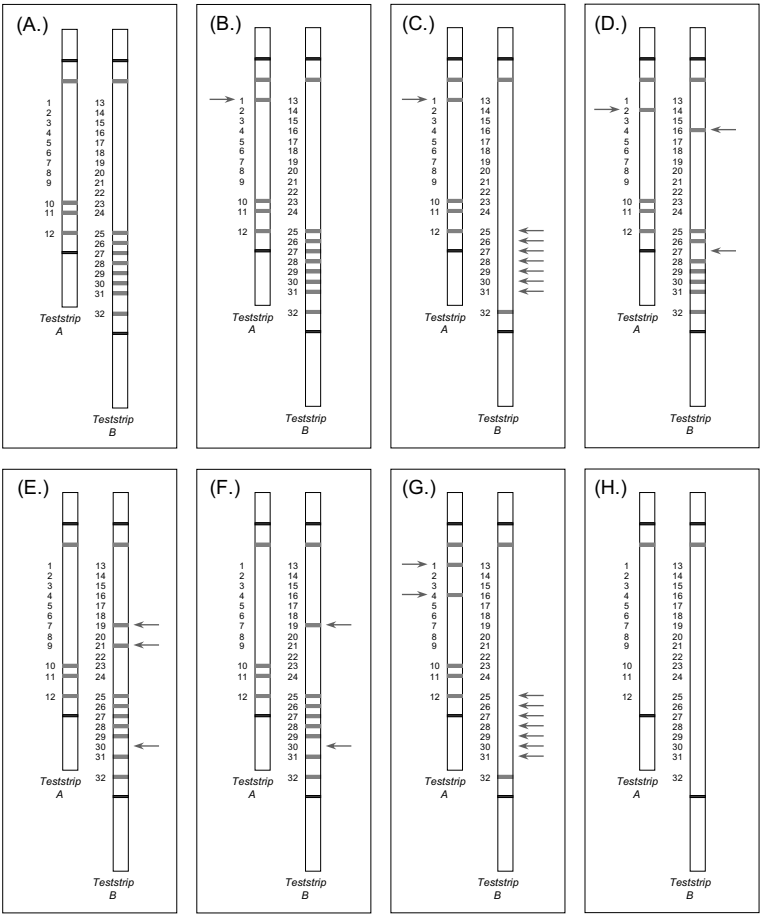

Fig. 3: Examples of results obtained with the α-Globin StripAssay®

(A.) normal (αα/αα)
(B.) -3.7 heterozygous (-3.7/αα)
(C.) -3.7 homozygous (-3.7/-3.7)
(D.) -4.2 + IVS1-5nt heterozygous (-4.2/IVS1-5nt)
(E.) Hb Constant Spring + Hb Pakse heterozygous (HbCS/HbPak)
(F.) Hb Constant Spring homozygous (HbCS/HbCS)
(G.) -3.7 + --MED-I heterozygous (-3.7/--MED-I)
(H.) negative control or PCR failure

NOTES

XVIII. RELATED PRODUCTS

REF		Σ
4-125	β-Globin StripAssay® AZE1	20 tests
4-126	β-Globin StripAssay® AZE2	20 tests
4-130	β-Globin StripAssay® MED	20 tests
4-140	β-Globin StripAssay® IME	20 tests
4-150	β-Globin StripAssay® SEA	20 tests
4-160	α-Globin StripAssay®	10 tests
4-170	β-Thal Modifier StripAssay®	20 tests
CS-012	StripAssay® Detection Reagents	20 tests
CS-017	StripAssay® Detection Reagents 48	48 tests
2-014	GEN^{X}TRACT™ Blood DNA Extraction System	100 extractions
2-020	Spin Micro DNA Extraction Kit	20 extractions
6-080	Typing Trays	5

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