CF StripAssay® EXT

Instructions For Use

REF	Σ
4-440	10 tests
4-440-A	24 tests
4-440-TRIAL	5 tests

2°C – 8°C

IVD

CE 0123

Version: rev 1.0 / English
eIFU and other languages available at
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REVISION HISTORY:

version	date	description
rev 1.0	2022-11	Addition of IVDR-related contents to version 2020-01.

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.

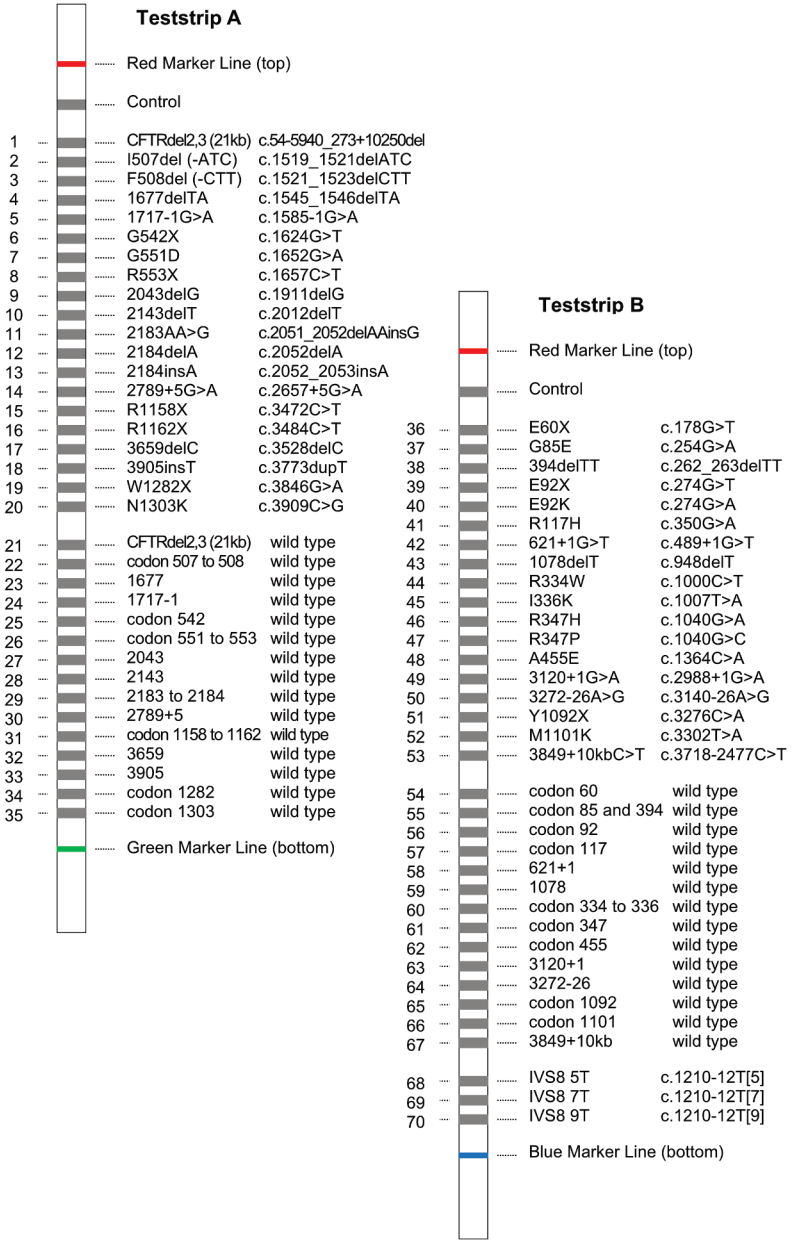

Fig. 1: Teststrip Design

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

I. INTENDED PURPOSE

The CF StripAssay® is a qualitative assay for the targeted analysis of the most common mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)* gene. Mutation analysis is performed on DNA extracted from peripheral blood samples or from dried blood spots. The test is used as an aid to genetically confirm the presence of two CFTR mutations in patients with suspected diagnosis of cystic fibrosis (CF) and as a second or third tier diagnostic test in newborn screening programs. The CF StripAssay® can be used for screening the carrier status of CFTR mutations in relatives of an affected patient or in adults of reproductive age as well as in the general population. The CF StripAssay® EXT covers 38 most common CFTR mutations plus the IVS8 polyT variants occurring in the Caucasian population. The StripAssay® can be carried out either manually or semi-automated.

For human *in vitro* diagnostic use.

II. BACKGROUND

Cystic fibrosis (CF) is a complex multi-organ disorder inherited by mutations in the *CFTR* gene that alter the structure, function or production of the encoded chloride channel protein. It is a very common autosomal recessive disorder affecting the pulmonary, pancreatic, gastro-intestinal or reproductive system. The milder CFTR-related disorders are monosymptomatic clinical entities associated with CFTR dysfunction, e.g. congenital bilateral absence of the vas deferens (CBAVD), disseminated bronchiectasis or chronic pancreatitis, that do not fulfill the diagnostic criteria for CF. CBAVD is a common cause of male infertility in cystic fibrosis.

III. METHODOLOGY

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

The CF StripAssay® EXT detects 38 common mutations plus the IVS8 polyT variants in the *CFTR* gene:

	legacy name	HGVS nomenclature	RefSNP
1	CFTRdele2,3(21kb)	c.54-5940_273+10250del	--
2	E60X	c.178G>T	rs77284892
3	G85E	c.254G>A	rs75961395
4	394delTT	c.262_263delTT	rs121908769
5	E92X	c.274G>T	rs121908751
6	E92K	c.274G>A	rs121908751
7	R117H	c.350G>A	rs78655421
8	621+1G>T	c.489+1G>T	rs78756941
9	1078delT	c.948delT	rs121908744
10	R334W	c.1000C>T	rs121909011
11	I336K	c.1007T>A	rs397508721
12	R347H	c.1040G>A	rs77932196
13	R347P	c.1040G>C	rs77932196
14	IVS8 5T / 7T / 9T	c.1210-12T[5]/T[7]/T9]	rs1805177
15	A455E	c.1364C>A	rs74551128
16	I507del	c.1519_1521delATC	rs121908745
17	F508del	c.1521_1523delCTT	rs113993960
18	1677delTA	c.1545_1546delTA	rs121908776
19	1717-1G>A	c.1585-1G>A	rs76713772
20	G542X	c.1624G>T	rs113993959
21	G551D	c.1652G>A	rs75527207
22	R553X	c.1657C>T	rs74597325
23	2043delG	c.1911delG	rs121908778
24	2143delT	c.2012delT	rs121908812
25	2183AA->G	c.2051_2052delAAinsG	rs121908799
26	2184delA	c.2052delA	rs121908746
27	2184insA	c.2052dupA	rs121908786
28	2789+5G>A	c.2657+5G>A	rs80224560
29	3120+1G>A	c.2988+1G>A	rs75096551
30	3272-26A>G	c.3140-26A>G	rs76151804
31	Y1092X (C>A)	c.3276C>A	rs121908761
32	M1101K	c.3302T>A	rs36210737
33	R1158X	c.3472C>T	rs79850223
34	R1162X	c.3484C>T	rs74767530
35	3659delC	c.3528delC	rs121908747
36	3849+10kb C>T	c.3718-2477C>T	rs75039782
37	3905 insT	c.3773dupT	rs121908789
38	W1282X	c.3846G>A	rs77010898
39	N1303K	c.3909C>G	rs80034486

Reference Sequence (RefSeq): NM_000492.3

IV. KIT COMPONENTS

REF

	4-440	4-440-A	4-440 -TRIAL
1. **Lysis Solution**	50 ml	---	50 ml
2. **GENXTRACT™ Resin**	5 ml	---	5 ml
3a. **Amplification Mix A** *(yellow cap)*	250 µl	2x 250 µl	250 µl
3b. **Amplification Mix B** *(green cap)*	250 µl	2x 250 µl	250 µl
4. **Taq Dilution Buffer** *(transparent cap)*	500 µl	500 µl	500 µl
5. **HS-Taq DNA Polymerase (5 U/µl)** *(red cap)*	125 U	175 U	125 U
6. **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			
7. **Typing Trays**	3	---	2
8a. **Teststrips A** *(black cap)*	10	24	5
8b. **Teststrips B** *(white cap)*	10	24	5
9. **Hybridization Buffer** *(white cap)*	25 ml	65 ml	25 ml
10. **Wash Solution A** *(white cap)*	80 ml	200 ml	80 ml
11. **Conjugate Solution** *(transparent cap)*	25 ml	65 ml	25 ml
12. **Wash Solution B** *(transparent cap)*	80 ml	200 ml	80 ml
13. **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			
14. **Instructions For Use**	1	1	1
15. **Collector™ Sheet**	1	3	1

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

name of component	composition
Lysis Solution	hypotonic solution containing $KHCO_3$, NH_4Cl, EDTA
GENXTRACT™ Resin	Chelex 100 Resin MB in a buffered solution
Amplification Mix A/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

name of component	composition
DNAT	basic solution containing 1.6 % sodium hydroxide and a blue dye indicating a change of pH
Typing Trays	plastic tray with eight wells
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:

- Thermoblock or thermomixer for 1.5 ml reaction tubes with temperature control up to 99°C
- Adjustable microcentrifuge capable of 3,000-12,000 rpm (1,000-12,000 x g)
- 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

1.1. DNA-Extraction from EDTA-anticoagulated Blood

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.

Bring blood samples to room temperature. Mix well by carefully inverting blood collection tubes several times. Allow Lysis Solution and GENXTRACT™ Resin to reach room temperature.

- Pipette **100 µl blood sample** into a 1.5 ml microtube with screw cap.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Let stand for **15 min.** at room temperature.
- Centrifuge for **5 min.** at **3,000 rpm** (approx. 1,000 x g) in a microcentrifuge.
- Remove and discard the upper (top) 1 ml of supernatant.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Centrifuge for **5 min.** at **12,000 rpm** (approx. 12,000 x g) in a microcentrifuge.
- Remove and discard the supernatant except for approx. 50 µl of a visible, soft pellet.
- Resuspend GENXTRACT™ Resin by swirling the bottle thoroughly.
- Add **200 µl GENXTRACT™ Resin** to the pellet. Close tube and vortex for 10 sec.

Note: GENXTRACT™ Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot.

- Incubate for **20 min.** at **56°C**. Vortex for 10 sec.
- Incubate for **10 min.** at **98°C**. Vortex for 10 sec.
- Centrifuge for **5 min.** at **12,000 rpm** in a microcentrifuge. Cool on ice.

The resulting supernatant contains DNA template suitable for immediate use in PCR. For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2°C to 8°C; up to one week) or frozen at -30°C to -15°C (for long term).

Use of other DNA isolation methods with the CF StripAssay® EXT 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.

1.2. DNA Extraction from Dried Blood Spot (DBS)

By using a lancet collect blood drops from a finger or heel prick onto an absorbent filter paper suitable for neonatal screening (Whatman 903 Protein Saver or PerkinElmer 226 filter papers). Allow the blood spot to air-dry for maximum 24 hours at room temperature. For long term archiving up to one year, store DBS at 2°C to 8°C in a resealable bag with a desiccant pouch.

Allow Lysis Solution and GENXTRACT™ Resin to reach room temperature.

- Place **two 3 mm punches of a DBS** into a 1.5 ml microtube with screw cap.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.

- Let stand for **10 min.** at room temperature.
- Centrifuge for **1 min.** at **12,000 rpm** (approx. 12,000 x g) in a microcentrifuge.
- Remove and discard the supernatant completely.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Let stand for **10 min.** at room temperature and mix by inverting several times.
- Centrifuge for **1 min.** at **12,000 rpm** in a microcentrifuge.
- Remove and discard the supernatant completely.
- Resuspend GENXTRACT™ Resin by swirling the bottle thoroughly.
- Add **200 µl GENXTRACT™ Resin** to the punches. Close tube and mix gently by tapping the bottom of the tube. Punches should be completely submerged by the resin.

Note: GENXTRACT™ Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot.

- Incubate for **20 min.** at **56°C**.
- Incubate for **10 min.** at **98°C**.
- Centrifuge for **5 min.** at **12,000 rpm** in a microcentrifuge. Cool on ice.

The resulting supernatant contains DNA template suitable for immediate use in PCR. For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2°C to 8°C; up to one week) or frozen at -30°C to -15°C (for long term).

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

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

- Freshly prepare each time an appropriate amount of working solution (1:25, final conc. 0.2 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)	0.2 µl	2 µl
Taq Dilution Buffer	4.8 µl	48 µl
working solution	5 µl	50 µl

- Prepare two reaction tubes for each sample to be amplified. Place tubes on ice.
- For each sample prepare 2 final PCR reaction mixes (A and B) on ice:

  A: **15 µl Amplification Mix A** (yellow cap)
  5 µl diluted HS-Taq DNA Polymerase (1 U)
  5 µl DNA template

  B: **15 µl Amplification Mix B** (green cap)
  5 µl diluted HS-Taq DNA Polymerase (1 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 94°C.
- Insert reaction tubes and run the following thermocycling program:

  pre-PCR: 94°C/3 min.
  thermocycling: 94°C/15 sec. - 60°C/45 sec. - 72°C/1min. (35 cycles)
  final extension: 72°C/3 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: 472, 345, 315, 278, 257, 236/213, 194, 165 bp (A)
386, 350, 322, 297, 248, 225, 200, 172, 155 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 **10 µl DNAT** (blue cap) into the lower corner of each lane to be used in the Typing Trays.
- Add **10 µl amplification product A** into the corresponding drop of DNAT.
- 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.

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

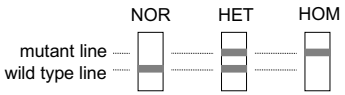

Fig. 2: Genotypes – staining patterns on the Teststrip

	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 19 (Fig. 3).

Some of the point mutations covered by the CF StripAssay® EXT are located within a few nucleotides on the *CFTR* gene. On the Teststrips these are represented by a common wild type probe:

line	wild type probe	mutation
22	codon 507 to 508	I507del (-ATC), F508del (-CTT)
26	codon 551 to 553	G551D, R553X
29	2183 to 2184	2183AA>G, 2184delA, 2184insA
31	codon 1158 to 1162	R1158X, R1162X
55	codon 85 and 394	G85E, 394delTT
56	codon 92	E92X, E92K
60	codon 334 to 336	R334W, I336K
61	codon 347	R347H, R347P

Samples that are compound heterozygous for two of these mutations (e.g. I507del + F508del or G551D + R553X) will be lacking the common wild type signal (see example G, page 19).

In addition to their wild type signal, homozygous CFTRdel2,3 samples will be lacking the wild type signal for codon 60 and for codon 85 and 394 (see example F, page 19).

The benign variants I506V, I507V and F508C do not cause any assay interference and will appear as CFTR wild type in the CF StripAssay® EXT.

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

VIII. PERFORMANCE EVALUATION

Accuracy of the CF StripAssay® EXT was determined by analyzing pretyped human genomic DNA samples and synthetic control DNA samples. Results of all samples were completely concordant with the reference methods used ((CF-EU2 kit (Elucigene), LINEAR ARRAY CF Gold or Amplicor® Cystic Fibrosis kit (both Roche), Sanger sequencing, HRM analysis, DGGE or RFLP)). The CF StripAssay® EXT correctly identified 326 mutant alleles (= 100% Positive Percent Agreement) and 254 wild type alleles (= 100% Negative Percent Agreement) in a total of 580 tested *CFTR* alleles.

Precision of the highly related CF StripAssays® were assessed as variability between specimen types, operators, days, different lots of Ampification Mixes, thermocyclers and hybridization devices. These parameters were investigated using the CF StripAssay® (REF 4-410) in a total of 58 runs, out of which all 58 showed the expected genotyping results. Only negligible differences in staining intensity of Teststrips were visible, and no background staining was observed. The CF StripAssays® were validated on the AB GeneAmp® PCR System 2700, AB GeneAmp® PCR System 9700, MJ Research PTC-200, AB Veriti™ and Eppendorf Mastercycler® X50s, which together represent a heating and cooling rate in the range of 1.7°C to 6.3°C/sec and 1.4°C 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: Assessment of the clinical performance of the highly related CF StripAssays® comprised data from an unpublished multi-center comparison study as well as published data on using CF StripAssays® in a clinical setting or as reference method.
In the comparison study 155 patient samples from the catchment area of four CF centers across Europe were tested in parallel with the CF StripAssay® (REF 4-410) and the reference methods routinely used in the participating laboratories. The CF StripAssay® accurately called 209 out of 236 mutant alleles (88,6% diagnostic sensitivity) and 74 out of 74 wild type alleles (100% diagnostic specificity). The literature search revealed 9 publications pertaining to the safety and performance of the CF StripAssays®. No adverse events or deviations were identified within these studies when compared to other devices or clinical symptoms, which demonstrate the clinical performance, the benefits and the safety of the CF StripAssays®.

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 CF StripAssay® EXT is exclusively designed for the diagnosis of 38 known mutations and the IVS8 5T/7T/9T variants as listed in section III, which are represented by allele-specific capture probes on the Teststrips. Other point mutations or large deletions in the *CFTR* gene 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 present in the homozygous state.

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

In cases where a large CFTR deletion is present on the second allele, a hemizygous mutation is misleadingly reported as homozygous. However, the clinical phenotype of CF is associated with both the hemi- and homozygous mutation status, and hence such situations will ultimately lead to the same diagnosis.

The CF StripAssay® EXT 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 CF StripAssay® EXT 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)
- Cystic Fibrosis Foundation (www.cff.org)
- Cystic Fibrosis Mutation Database (www.genet.sickkids.on.ca)
- CF Network (http://cf.eqascheme.org)

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
Σ	Sufficient for <n> tests
	Storage temperature limits
	Use by
	Caution
	Manufacturer
	Date of manufacture
	Consult Instructions For Use

XVII. EXAMPLES OF TEST RESULTS

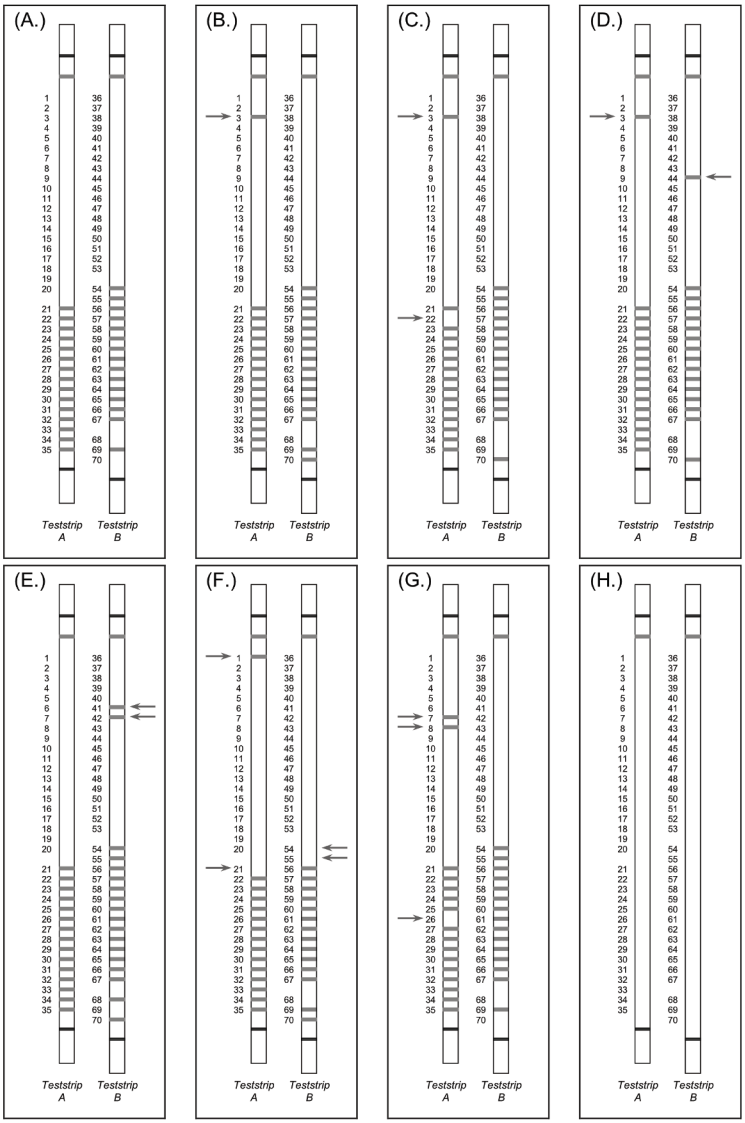

Fig. 3: Examples of results obtained with the CF StripAssay® EXT

(A.) normal [7T/7T]
(B.) F508del heterozygous [7T/9T]
(C.) F508del homozygous [9T/9T]
(D.) F508del / R334W heterozygous [9T/9T]
(E.) R117H / 621+1G>T heterozygous [5T/9T]
(F.) CFTRdel2,3 (21kb) homozygous [7T/9T]
(G.) G551D / R553X heterozygous [7T/7T]
(H.) negative control or PCR failure

XVIII. RELATED PRODUCTS

REF		Σ
4-410	CF StripAssay®	10 tests
4-420	CF StripAssay® TUR	10 tests
4-430	CF StripAssay® GER	10 tests
4-440	CF StripAssay® EXT	10 tests
CS-012	StripAssay® Detection Reagents	20 tests
CS-017	StripAssay® Detection Reagents 48	48 tests
2-014	GENXTRACT™ Blood DNA Extraction System	100 extractions
2-020	Spin Micro DNA Extraction Kit	20 extractions
6-080	Typing Trays	5

Distributor:

Manufacturer:

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