

*AESKULISA* tTg-GA New Generation

**REF 3516**

# Instruction manual

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## 1. Intended Use

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The **AESKULISA tTg-GA** of the new generation is a solid phase enzyme immunoassay for the separate quantitative and qualitative detection of IgA and/or IgG antibodies against neo-epitopes of tissue transglutaminase (tTG) in human serum. The assay employing human recombinant transglutaminase crosslinked with gliadin-specific peptides displays neo-epitopes of tTg which ensures a significantly increased sensitivity and specificity of the test.

The assay is a tool for the diagnosis and monitoring of celiac disease (gluten-sensitive enteropathy).

## 2. Clinical Application and Principle of the Assay

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Gluten-sensitive enteropathy or celiac disease is characterized by atrophy of the small intestinal villi leading to a so-called flat mucosa. It is caused by a pathological intolerance to gliadin, the alcohol-soluble fraction of gluten in wheat, rye and barley. As celiac disease is caused by the uptake of gluten, consequently a gluten-free diet cures the disease completely and thus has to be maintained for life-time. Renewed consumption of gliadin leads to a return of the symptoms. The disease is HLA-associated (>95% of patients have DQ2 enREFd by DQA1\*0501 and DQB1\*0201) and manifests at any age with a peak onset in early childhood, even in neonatals. The incidence rates range from 1 in 4000 to 1 in 300 in european countries.

Diagnosis of celiac disease is made by small intestinal biopsy (demonstrating flat mucosa) supported by serological markers. Antibodies against gliadin and anti-endomysium antibodies (EMA) are of major significance. They are detected so far by indirect immunofluorescence, which is restricted to subclass IgA only. The identification of tissue transglutaminase (tTG) as the major target antigen of EMA provided the opportunity of a more easy and reliable diagnosis of celiac disease. tTG is an enzyme that upon wounding is released from cells where it is thought to aid in tissue repair.

Anti-tTG antibodies show higher sensitivity and specificity than anti-Gliadin antibodies. Furthermore they correlate tightly with the activity of the disease and thus are especially useful for diet monitoring. The cross-link of tTg with gliadin-specific peptides results in neo-epitopes of tTg. As these neo-epitopes are structurally closer to the physiological antigens, the new generation AESKULISA tTg tests show a markedly increased sensitivity and specificity. These epitopes show no cross-reactivities with gliadin. The determination of IgG antibodies to tTG is the only available specific serology for those 2% to 5% of patients with IgA deficiency. A high number of subclinical cases have been detected by screening for anti-tTg, fostering the theory that the majority of celiac disease cases is undetected and untreated (Iceberg model).

### ***Principle of the test***

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

### 3. Kit Contents

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#### **To be reconstituted:**

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)  
Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)  
Containing: Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)

#### **Ready to use:**

Negative Control 1 vial, 1.5 ml (capped green: colorless solution)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Cut-off Calibrator 1 vial, 1.5 ml (capped blue: yellow solution)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml  
(color increasing with concentration: yellow solutions)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Conjugate 1 vial, 15 ml IgA (capped red: red solution)  
1 vial, 15 ml IgG (capped blue: blue solution)  
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)  
Containing: Stabilized TMB/H<sub>2</sub>O<sub>2</sub>

Stop Solution 1 vial, 15 ml (capped white: colorless solution)  
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells  
Coating see paragraph 1

#### **Material required but not provided:**

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-channel pipette or automated system), adsorbent paper.

Our tests are designed to be used with purified water according to the definition of the United States

### 4. Storage and Shelf Life

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Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. **Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.**

## 5. Precautions of Use

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### 5.1 Health hazard data

**THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY.** Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety :

#### ***Recommendations and precautions***

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

**WARNING !** Calibrators, Controls and Buffers contain sodium azide ( $\text{NaN}_3$ ) as a preservative.  $\text{NaN}_3$  may be toxic if ingested or adsorbed by skin or eyes.  $\text{NaN}_3$  may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

### 5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32 °C/68-89.6 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30 °C/86 °F for automated systems.**

Never expose components to higher temperature than 37 °C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

**A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.**

## 6. Sample Collection, Handling and Storage

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Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8 °C/35-46 °F up to three days, or frozen at -20 °C/-4 °F for longer periods.

## 7. Assay Procedure

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### 7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

#### **Samples:**

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

#### **Washing:**

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

#### **Automated washing:**

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

#### **Manual washing:**

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

#### **Microplates:**

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8 °C/35-46 °F).

### 7.2 Work flow

**For pipetting scheme see Annex A, for the test procedure see Annex B**

**We recommend pipetting samples and calibrators in duplicate.**

**Cut-off calibrator should be used for qualitative testing only.**

**NOTE: If antibody classes (IgG, IgA and/or IgM) are determined in parallel, calibrators, controls and samples have to be done for each antibody class separately.**

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32 °C/68-89.6 °F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32 °C/68-89.6 °F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32 °C/68-89.6 °F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

## 8. Quantitative and Qualitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **U/ml**.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

### Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgGA	OD 450/620 nm	CV % (Variation)
0 U/ml	0.073	3.1
3 U/ml	0.179	2.3
10 U/ml	0.342	1.2
30 U/ml	0.662	0.1
100 U/ml	1.310	0.9
300 U/ml	2.263	0.3

### Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.808/0.831	0.820	39.6
P 02	1.081/1.071	1.076	66.1

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

### **Do not use this example for interpreting patients results!**

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient's OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

**Negative:**  $OD_{\text{patient}} < 0.8 \times OD_{\text{cut-off}}$

**Equivocal:**  $0.8 \times OD_{\text{cut-off}} \leq OD_{\text{patient}} \leq 1.2 \times OD_{\text{cut-off}}$

**Positive**  $OD_{\text{patient}} > 1.2 \times OD_{\text{cut-off}}$

## 9. Technical Data

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<b>Sample material:</b>	serum
<b>Sample volume:</b>	10 µl of sample diluted 1:101 with 1x sample buffer
<b>Total incubation time:</b>	90 minutes at 20-32 °C/68-89.6 °F
<b>Calibration range:</b>	0-300 U/ml
<b>Analytical sensitivity:</b>	1.0 U/ml
<b>Storage:</b>	at 2-8 °C/35-46 °F use original vials, only
<b>Number of determinations:</b>	96 tests

## 10. Performance Data

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### 10.1 Analytical sensitivity

Testing sample buffer 30 times on *AESKULISA tTg-GA (New Generation)* gave an analytical sensitivity of 1.0 U/ml.

### 10.2 Specificity and Sensitivity

The microplates are coated with **recombinant human tissue-transglutaminase and gliadin-specific peptides**. No crossreactivities to other autoantigens have been found, especially the gliadin-specific peptides do not crossreact with gliadin.

The diagnostic specificity of tTg antibodies for celiac disease is 95%. The diagnostic sensitivity of tTg antibodies for celiac is 95.2-96.9%.



### 10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	76.5	71.0	107.7
	1 / 200	36.6	35.5	103.1
	1 / 400	17.2	17.8	96.8
	1 / 800	8.5	8.9	95.8
2	1 / 100	62.2	59.0	105.4
	1 / 200	29.7	29.5	100.7
	1 / 400	13.3	14.8	90.2
	1 / 800	7.0	7.4	94.9

### 10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-Assay		
Sample No.	Mean (U/ml)	CV (%)
1	13.8	7.0
2	56.7	5.2
3	166.5	5.5

Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)
1	10.1	2.3
2	38.9	0.8
3	169.4	4.2

### 10.5 Calibration

Due the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

## 11. Literature

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- Dietrich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. (1997).**  
*Identification of tissue transglutaminase as the autoantigen of celiac disease.*  
Nat Med 3: 797-801.
- Dietrich W, Laag E, Schöpfer H, Volta U, Ferguson A, Gillett H, Riecken EO, Schuppan D. (1998).**  
*Autoantibodies to tissue transglutaminase as predictors of celiac disease.*  
Gastroenterology 115: 1317-1321.
- Mäki M, Collin P (1997).**  
*Coeliac disease.*  
Lancet 349: 1755-1759.
- Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C (2002).**  
*Structural basis for gluten intolerance in Celiac Sprue.*  
Science 297: 2275-2279.

## ANNEX A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:

For **quantitative interpretation** use calibrators to establish a standard curve.

For **qualitative interpretation** use cut-off calibrator.

	for <b>quantitative interpretation</b> use calibrators to establish a standard curve						for <b>qualitative interpretation</b> use cut-off calibrator					
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	CalA	CalE	P1				NC	P2				
<b>B</b>	CalA	CalE	P1				NC	P2				
<b>C</b>	CalB	CalF	P2				CC	P3				
<b>D</b>	CalB	CalF	P2				CC	P3				
<b>E</b>	CalC	PC	P3				PC	...				
<b>F</b>	CalC	PC	P3				PC	...				
<b>G</b>	CalD	NC	...				P1	...				
<b>H</b>	CalD	NC	...				P1	...				

CalA: calibrator A, CalB: calibrator B, CalC: calibrator C, CalD: calibrator D, CalE: calibrator E, CalF: calibrator F

PC: positive control

NC: negative control

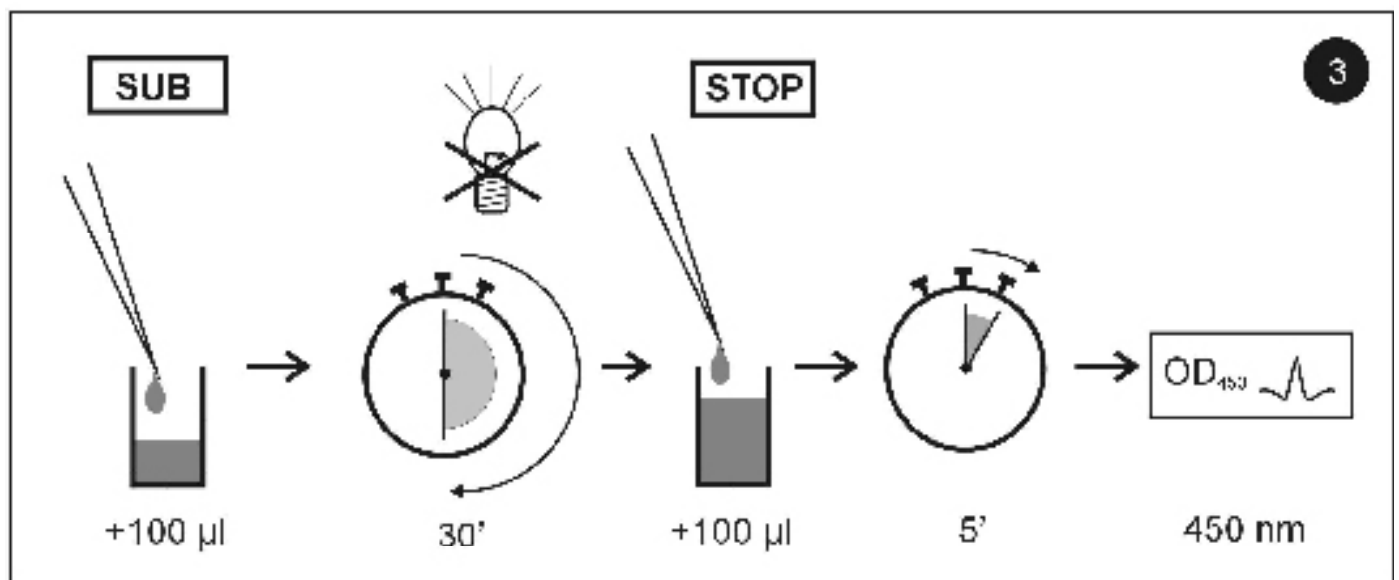
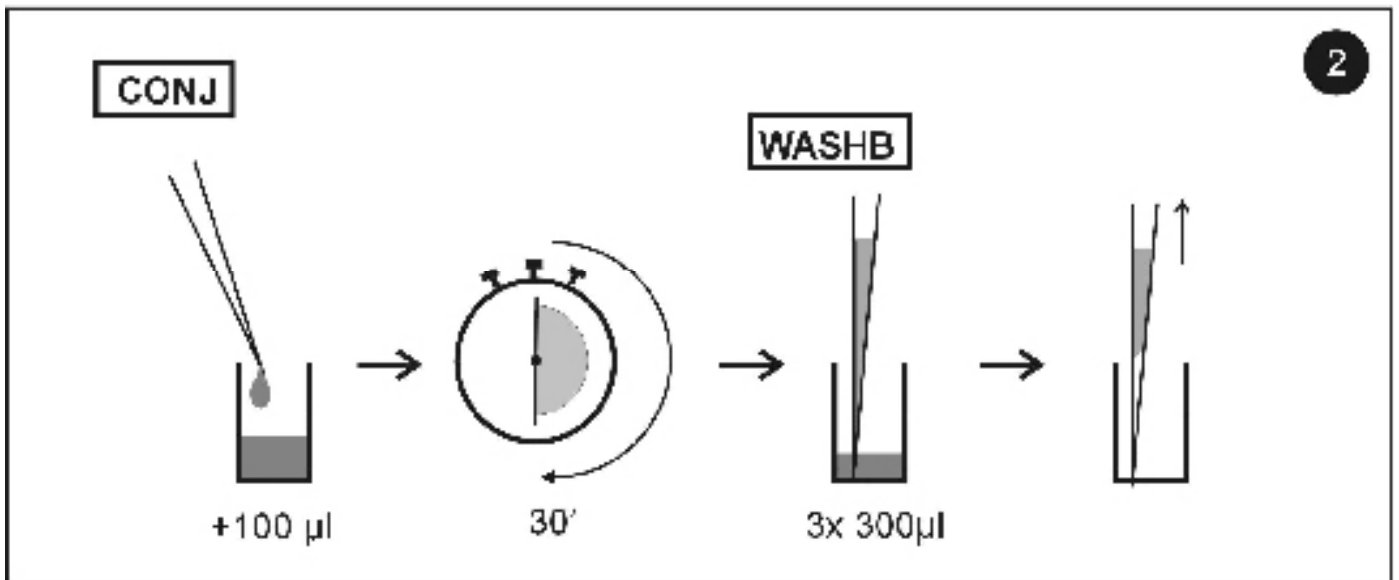
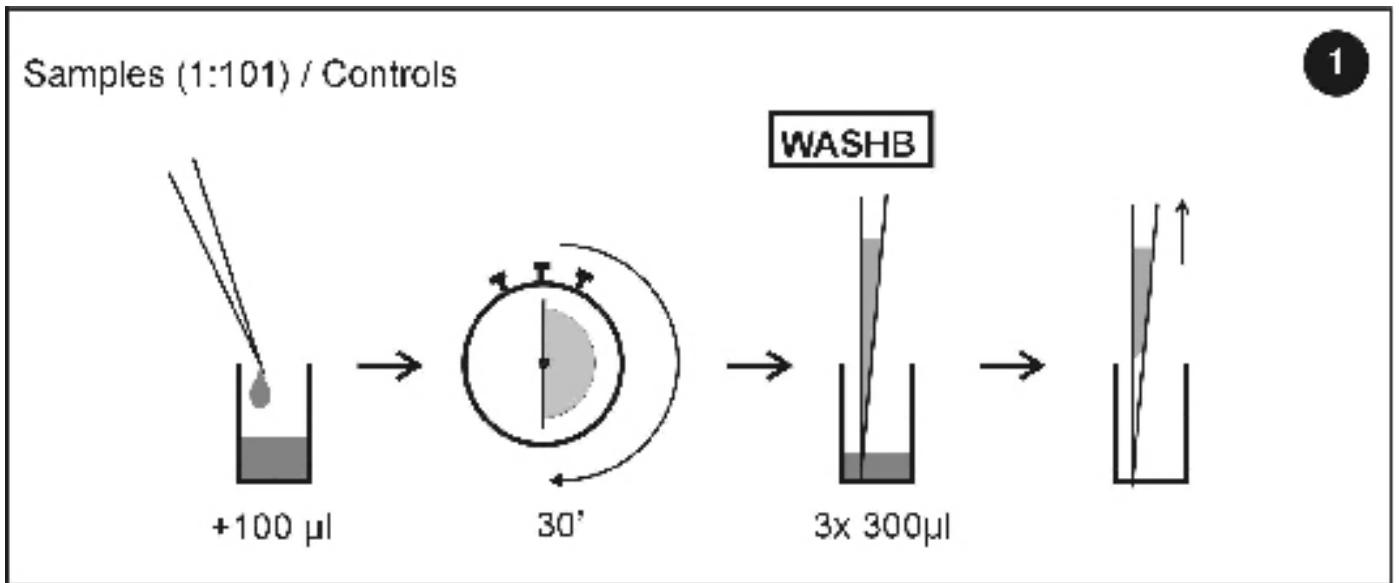
CC: Cut-off calibrator

P1: patient 1

P2: patient 2

P3: patient 3

## Annex B: Test Procedure










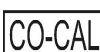




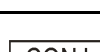
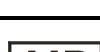
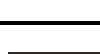
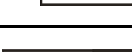
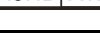
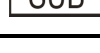
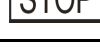




Assay/Test: \_\_\_\_\_ Incubation / Inkub. : 1. \_\_\_\_\_ min Date/ Datum: \_\_\_\_\_

Temperature/Temperatur: \_\_\_\_\_ °F \_\_\_\_\_ °C 2. \_\_\_\_\_ min  
Signature/Unterschrift: \_\_\_\_\_  
Name: \_\_\_\_\_ 3. \_\_\_\_\_ min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
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	<ul style="list-style-type: none"> <li>◆ Diagnosi in vitro</li> <li>◆ Pour diagnostic in vitro</li> <li>◆ In Vitro Diagnostikum</li> <li>◆ Para uso Diagnóstico in vitro</li> </ul>	<ul style="list-style-type: none"> <li>◆ For in vitro diagnostic use</li> <li>◆ Para uso diagnóstico in vitro</li> <li>◆ In Vitro Διαγνωστικό μέσο</li> </ul>
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	<ul style="list-style-type: none"> <li>◆ Rispettare le istruzioni per l'uso</li> <li>◆ Voir les instructions d'utilisation</li> <li>◆ Gebrauchsanweisung beachten</li> <li>◆ Ver as instruções de uso</li> </ul>	<ul style="list-style-type: none"> <li>◆ See instructions for use</li> <li>◆ Ver las instrucciones de uso</li> <li>◆ Λάβετε υπόψη τις οδηγίες χρήσης</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Da utilizzarsi entro</li> <li>◆ Utilise avant le</li> <li>◆ Verwendbar bis</li> <li>◆ Utilizar antes de</li> </ul>	<ul style="list-style-type: none"> <li>◆ Use by</li> <li>◆ Utilizar antes de</li> <li>◆ Χρήση μέχρι</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Conservare a 2-8°C</li> <li>◆ Conserver à 2-8°C</li> <li>◆ Lagerung bei 2-8°C</li> <li>◆ Conservar entre 2-8°C</li> </ul>	<ul style="list-style-type: none"> <li>◆ Store at 2-8°C (35-46°F)</li> <li>◆ Conservar a 2-8°C</li> <li>◆ Φυλάσσεται στους 2-8°C</li> </ul>
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	<ul style="list-style-type: none"> <li>◆ Calibratore</li> <li>◆ Etalon</li> <li>◆ Kalibrator</li> <li>◆ Calibrador</li> </ul>	<ul style="list-style-type: none"> <li>◆ Calibrator</li> <li>◆ Calibrador</li> <li>◆ Αντιδραστήριο βαθμονόμησης</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Recupero</li> <li>◆ Corrélation</li> <li>◆ Wiederfindung</li> <li>◆ Recuperação</li> </ul>	<ul style="list-style-type: none"> <li>◆ Recovery</li> <li>◆ Recuperado</li> <li>◆ Ανάκτηση</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Coniugato</li> <li>◆ Conjugé</li> <li>◆ Konjugat</li> <li>◆ Conjugado</li> </ul>	<ul style="list-style-type: none"> <li>◆ Conjugate</li> <li>◆ Conjugado</li> <li>◆ Σύζευγμα</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Micropiastro rivestita</li> <li>◆ Microplaque sensibilisée</li> <li>◆ Beschichtete Mikrotiterplatte</li> <li>◆ Microplaca revestida</li> </ul>	<ul style="list-style-type: none"> <li>◆ Coated microtiter plate</li> <li>◆ Microplaca sensibilizada</li> <li>◆ Επικαλυμμένη μικροπλάκα</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Piastra ad aghi rivestita</li> <li>◆ Pinplate sensibilisée</li> <li>◆ Beschichtete Pinplatte</li> <li>◆ Pinplate revestida</li> </ul>	<ul style="list-style-type: none"> <li>◆ Coated pinplate</li> <li>◆ Pinplate sensibilizada</li> <li>◆ Επικαλυμμένη πλάκα Pin</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Tampone di lavaggio</li> <li>◆ Tampon de Lavage</li> <li>◆ Waschpuffer</li> <li>◆ Solução de lavagem</li> </ul>	<ul style="list-style-type: none"> <li>◆ Wash buffer</li> <li>◆ Solución de lavado</li> <li>◆ Ρυθμιστικό διάλυμα πλύσης</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Tampone substrato</li> <li>◆ Substrat</li> <li>◆ Substratpuffer</li> <li>◆ Substrato</li> </ul>	<ul style="list-style-type: none"> <li>◆ Substrate buffer</li> <li>◆ Tampón sustrato</li> <li>◆ Ρυθμιστικό διάλυμα υποστρώματος</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Reagente bloccante</li> <li>◆ Solution d'Arrêt</li> <li>◆ Stopreagenz</li> <li>◆ Solução de paragem</li> </ul>	<ul style="list-style-type: none"> <li>◆ Stop solution</li> <li>◆ Solución de parada</li> <li>◆ Αντιδραστήριο διακοπής αντίδρασης</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Tampone campione</li> <li>◆ Tampon Echantillons</li> <li>◆ Probenpuffer</li> <li>◆ Diluente de amostra</li> </ul>	<ul style="list-style-type: none"> <li>◆ Sample buffer</li> <li>◆ Tampón Muestras</li> <li>◆ Ρυθμιστικό διάλυμα δειγμάτων</li> </ul>