

*AESKULISA* Cardiolipin-A

REF 3203

# Instruction manual

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## 1. Intended Use

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**AESKULISA Cardiolipin-A** is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native *human*  $\beta$ 2-glycoprotein I for the quantitative and qualitative detection of IgA antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed out of cardiolipin and  $\beta$ 2-glycoprotein I which are expressed only when  $\beta$ 2-glycoprotein I interacts with cardiolipin. The assay is an aid in the diagnosis and risk estimation of thrombosis in patients with systemic lupus erythematosus (SLE).

## 2. Clinical Application and Principle of the Assay

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Antibodies against cardiolipin belong to the group of anti-phospholipid antibodies specific for negatively charged phospholipids, components of biological membranes. Cardiolipin is an acidic phospholipid derived from glycerol and was named because of its isolation from bovine heart in 1941. Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The prevalence of anti-cardiolipin antibodies in SLE is 24-50 %.

The occurrence of anti-cardiolipin antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-cardiolipin antibodies in patients with no other autoimmune diseases characterize the primary anti-phospholipid syndrome (APS). Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanism by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

### ***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% and (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 APL/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)

Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase (preservative)

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 Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

### 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.

- 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 **APL/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 **APL/ml**.

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

### *Example of a standard curve*

We recommend pipetting calibrators in parallel for each run.

Calibrators IgA	OD 450/620 nm	CV % (Variation)
0 APL/ml	0.066	3.2
3 APL/ml	0.162	0.4
10 APL/ml	0.291	1.7
30 APL/ml	0.597	1.3
100 APL/ml	1.101	2.9
300 APL/ml	2.039	0.4

### *Example of calculation*

Patient	Replicate (OD)	Mean (OD)	Result (APL/ml)
P 01	0.772/0.752	0.762	48.8
P 02	1.058/1.038	1.048	82.9

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 APL/ml
<b>Analytical sensitivity:</b>	1.0 APL/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

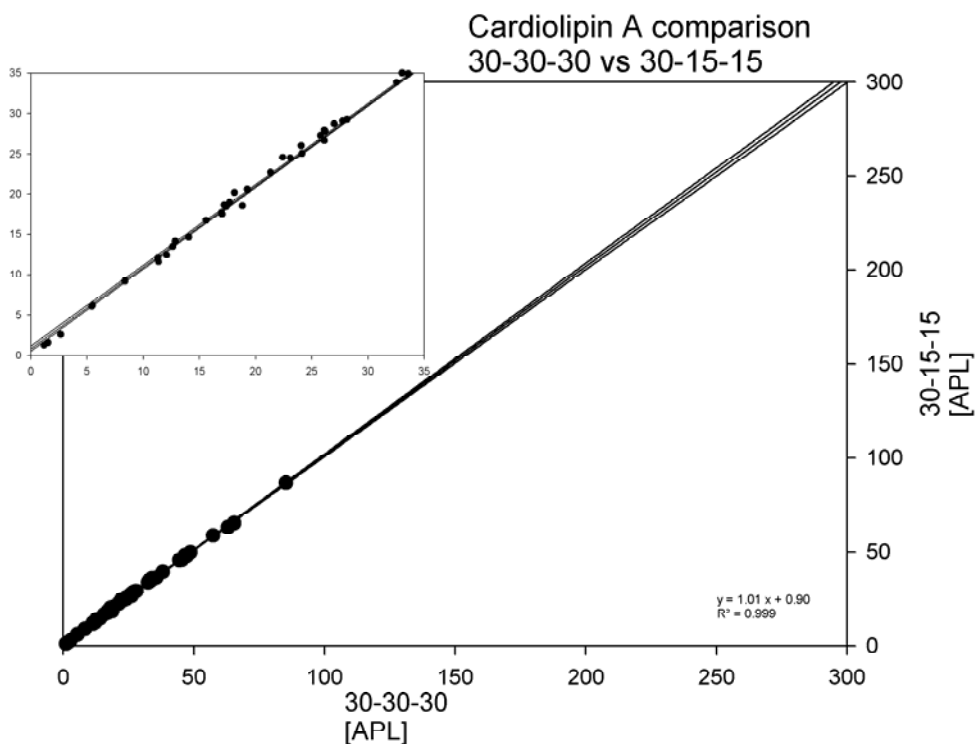
Testing sample buffer 30 times on *AESKULISA Cardiolipin-A (REF7203)* gave an analytical sensitivity of 1.0 APL/ml.

### 10.2 Specificity and Sensitivity

The microplate is coated with **highly purified cardiolipin and native human β2-glycoprotein I**. No crossreactivities to other autoantigens have been found. Antibodies against Cardiolipin can be found in 70% of SLE patients. The data has been acquired with the *AESKULISA Cardiolipin-A (REF7203)*.

### Correlation:

The comparability of performance data was assessed with 50 sera tested on both, *AESKULISA 7302* and *AESKULISA 3203*. A linear regression analysis of the two products showed that the two products are equivalent. Included in these sera are 33 sera close to cut-off.





### 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 (APL/ml)	expected concentration (APL/ml)	Recovery (%)
1	1 / 100	63.1	68.0	93.0
	1 / 200	33.7	34.0	99.1
	1 / 400	15.9	17.0	93.5
	1 / 800	9.0	8.5	105.9
2	1 / 100	138.6	141.8	97.7
	1 / 200	70.1	70.9	98.9
	1 / 400	33.2	35.5	93.5
	1 / 800	17.9	17.7	101.1

### 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 (APL/ml)	CV (%)
1	586.2	1.5
2	67.4	3.4
3	34.5	7.6

Inter-Assay		
Sample No.	Mean (APL/ml)	CV (%)
1	499.8	0.9
2	68.9	1.7
3	40.7	4.6

### 10.5 Calibration

Because of the lack of WHO reference material, Aeskulisa Cardiolipin-A is calibrated against reference sera from N.E. Harris, Louisville. The results are expressed in APL/ml for IgA.

## 11. Literature

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*Anticardiolipin antibodies - Clinical associations.*  
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*Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant.*  
Br. Med. J. 287, 1021-1023.
- Gastineau, D.A., Kazmier, F.J., Nichols, W.L., Bowie, E.J. (1985).**  
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- Harris, E.N., Gharavi, A.E., Boey, M.L., et al. (1983).**  
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*Clinical relevance of antibodies against different phospholipids.*  
Journal of Autoimmunity 15, A60.

## 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
A	CalA	CalE	P1				NC	P2				
B	CalA	CalE	P1				NC	P2				
C	CalB	CalF	P2				CC	P3				
D	CalB	CalF	P2				CC	P3				
E	CalC	PC	P3				PC	...				
F	CalC	PC	P3				PC	...				
G	CalD	NC	...				P1	...				
H	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

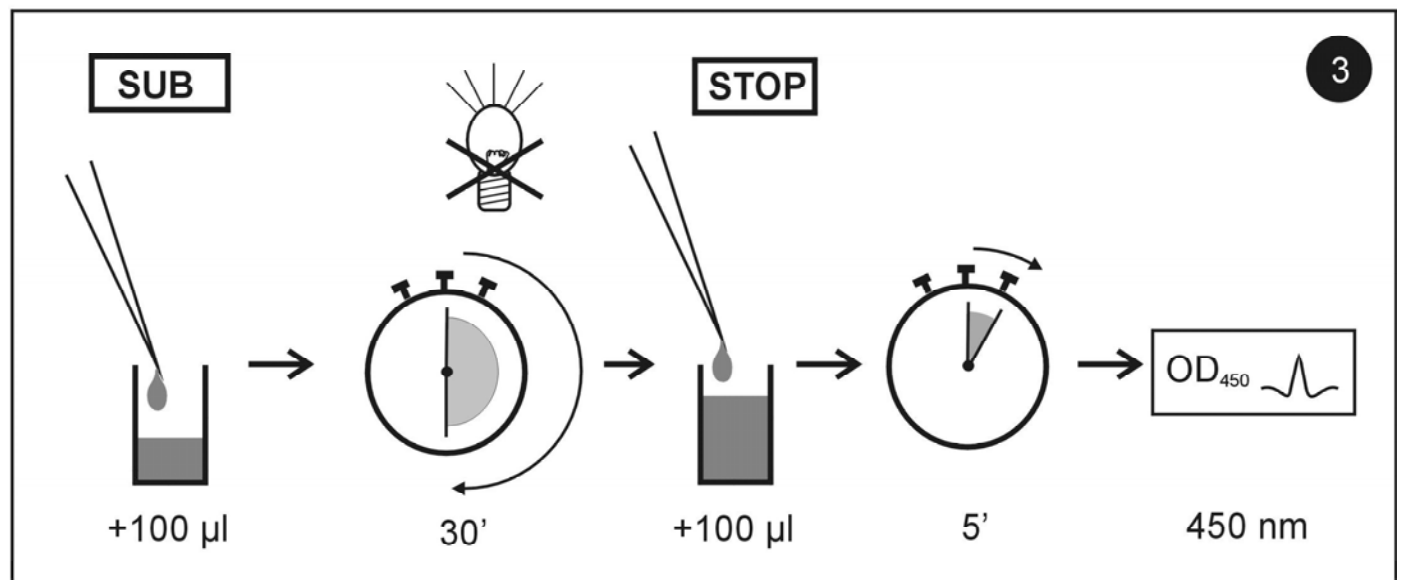
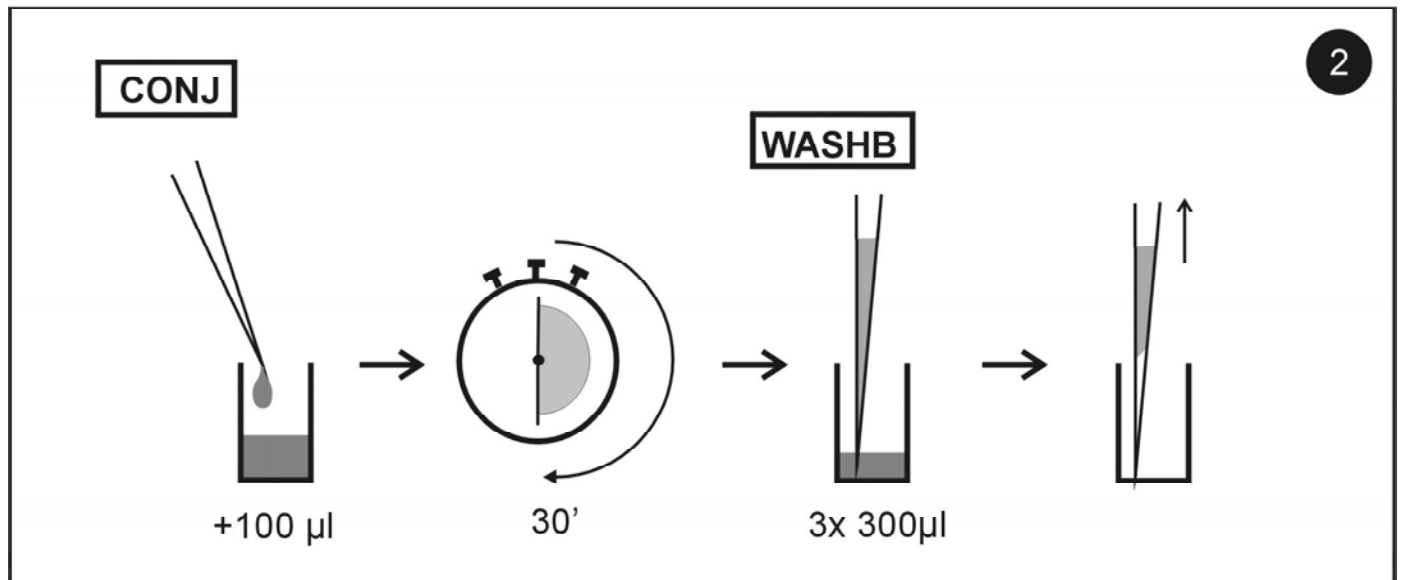
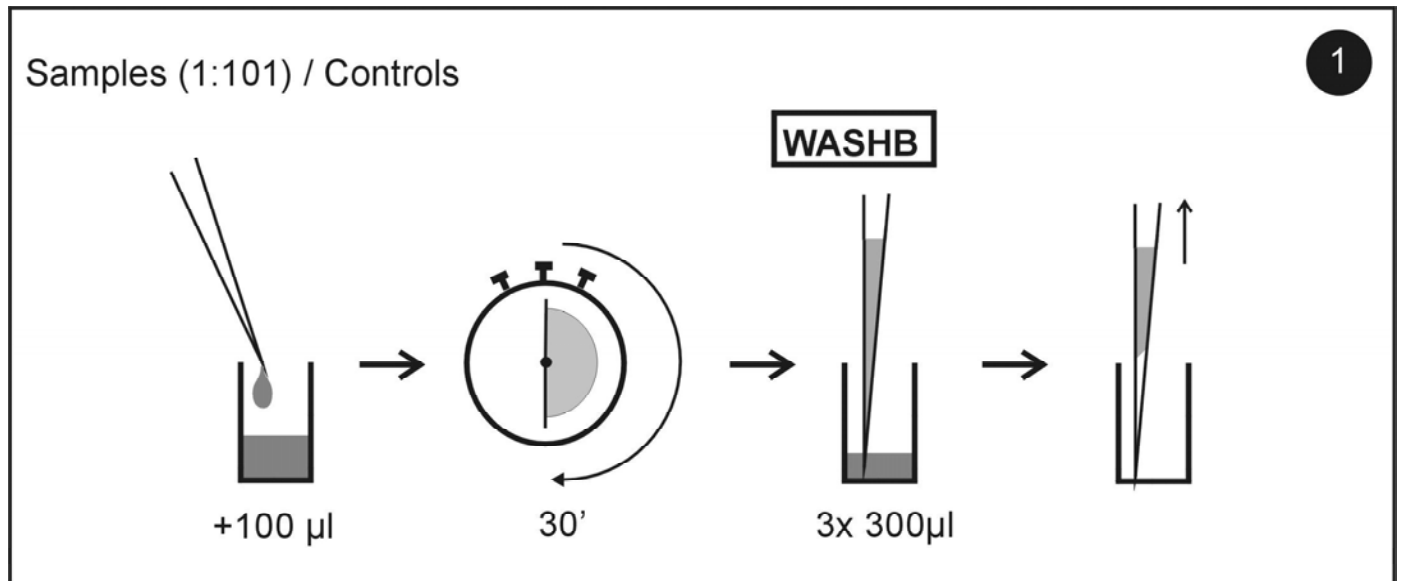
CC: Cut-off calibrator

P1: patient 1

P2: patient 2

P3: patient 3

## Annex B: Test Procedure












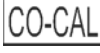










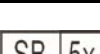


Assay/Test: \_\_\_\_\_ Incubation / Inkub. : 1. \_\_\_\_\_ min Date / Datum: \_\_\_\_\_

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												
C												
D												
E												
F												
G												
H												

	<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>
	<ul style="list-style-type: none"> <li>◆ Prodotto da</li> <li>◆ Fabriqué par</li> <li>◆ Hergestellt von</li> <li>◆ Fabricado por</li> </ul>	<ul style="list-style-type: none"> <li>◆ Manufactured by</li> <li>◆ Fabricado por</li> <li>◆ Κατασκευάζεται από</li> </ul>
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	<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>