

*AESKULISA* Intrinsic factor

REF 3512

# Instruction manual

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## 1. Intended Use

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**AESKULISA Intrinsic factor** is a solid phase enzyme immunoassay with recombinant human intrinsic factor for the quantitative and qualitative detection of IgG antibodies against intrinsic factor. The assay is a tool in the diagnosis of pernicious anaemia (Biermer's anaemia)

## 2. Clinical Application and Principle of the Assay

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Pernicious anaemia is the end stage of an autoimmune gastritis (type A gastritis) resulting in destruction of the gastric mucosa. A small proportion (10-15%) of patients with autoimmune gastritis develop pernicious anaemia. Pernicious anaemia is the most common cause of vitamin B12 deficiency in Western populations. It is caused by a lack of intrinsic factor, a glycoprotein needed to absorb vitamin B12 from the gastrointestinal tract. Intrinsic factor is produced by gastric parietal cells. Vitamin B12, in turn, is necessary for the formation of red blood cells.

Pernicious anaemia usually does not appear before the age of 30. The average age at diagnosis is 60 years. In fact, one recent study revealed that nearly 2 percent of individuals over 60 years old suffer from pernicious anaemia. Furthermore, slightly more women than men are affected. The disease can affect all ethnic groups, but occurs more often among people of Scandinavian or Northern European descent.

Autoantibodies in pernicious anaemia target parietal cells and intrinsic factor. Antibodies against parietal cells are 80-90% sensitive but can be detected also in up to 5% of the healthy population. Antibodies against intrinsic factor show a sensitivity of 50-70% with a specificity of 100% in a population of healthy blood donors.

### ***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 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 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/39°F, 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 **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 IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.041	0.4
3 U/ml	0.164	2.5
10 U/ml	0.322	2.2
30 U/ml	0.615	1.3
100 U/ml	1.239	3.0
300 U/ml	2.141	1.3

### Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.275/ 1.259	1.267	101.0
P 02	0.668 /0.673	0.671	34.3

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.°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 Intrinsic factor (REF7512)* gave an analytical sensitivity of 1.0 U/ml.

### 10.2 Specificity and Sensitivity

The microplates are coated with **recombinant human gastric intrinsic factor**. No crossreactivities to other autoantigens have been found. Antibodies against intrinsic factor show a diagnostic sensitivity of 50-70% for pernicious anaemia and show a specificity of 100% in a population of healthy blood donors. Antibodies against parietal cells are 80-90% sensitive but can be detected also in up to 5% of the healthy population. The data has been acquired with the *AESKULISA Intrinsic factor (REF7512)*.

### Correlation:

The comparability of performance data was assessed with at least 30 sera tested on both, Aeskulisa 7512 and Aeskulisa 3512. A linear regression analysis of the two products showed that the two products are equivalent. Data can be received upon request.



### 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	160.0	163.0	98.2
	1 / 200	79.08	81.5	97.9
	1 / 400	37.4	40.8	91.7
	1 / 800	18.9	20.4	92.6
2	1 / 100	101.0	104.0	97.1
	1 / 200	53.0	52.0	101.9
	1 / 400	28.0	26.0	107.7
	1 / 800	12.0	13.0	92.3

### 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	285.0	6.1
2	149.0	4.8
3	63.0	5.2

Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)
1	279.0	4.8
2	159.0	3.2
3	58.0	5.6

### 10.5 Calibration

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

## 11. Literature

1. **Carmel, R. (1992).**

*Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race.*

Clin Exp Immunol, 89: 74-77 (11): 2617-20.

2. **Oh R, Brown DL (2003).**

*Vitamin B12 deficiency.*

Am Fam Physician, 67: 979-986.

3. **Toh Ban-Hock, Alderuccio, F. (2004).**

*Pernicious anaemia*

Autoimmunity 37: 357-361.

4. **Toh Ban-Hock, van Driel Ian R. (1997).**

*Pernicious anaemia.*

NEJM Vol. 337: 1441-1448.

## 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

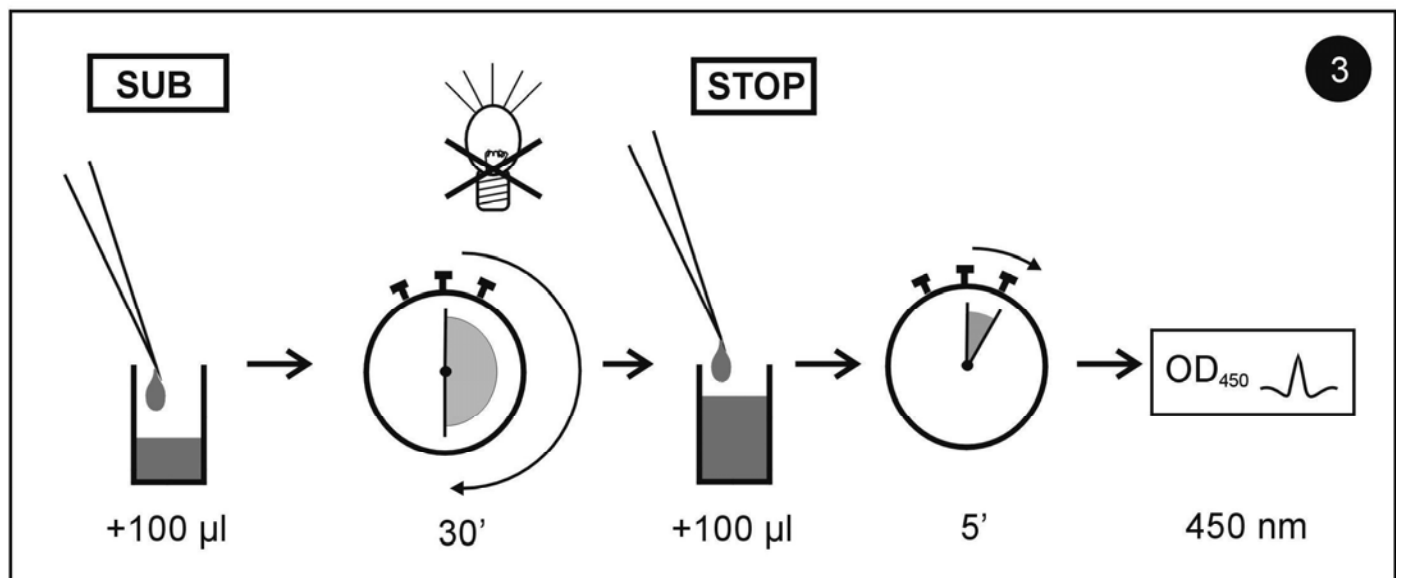
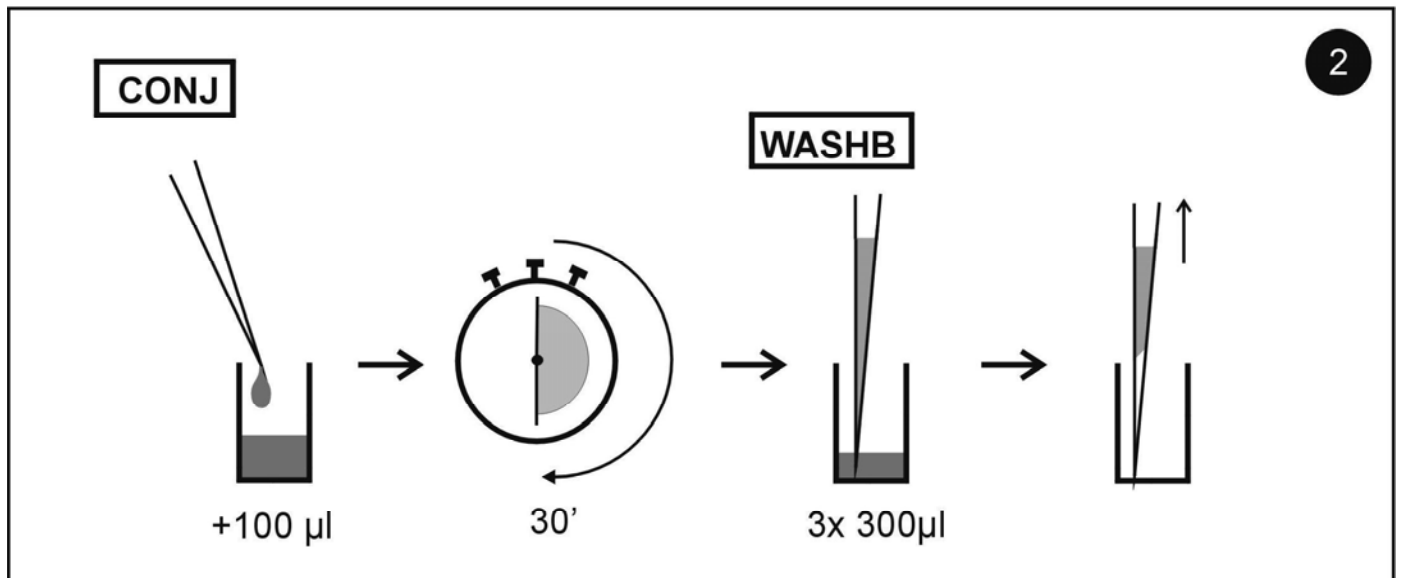
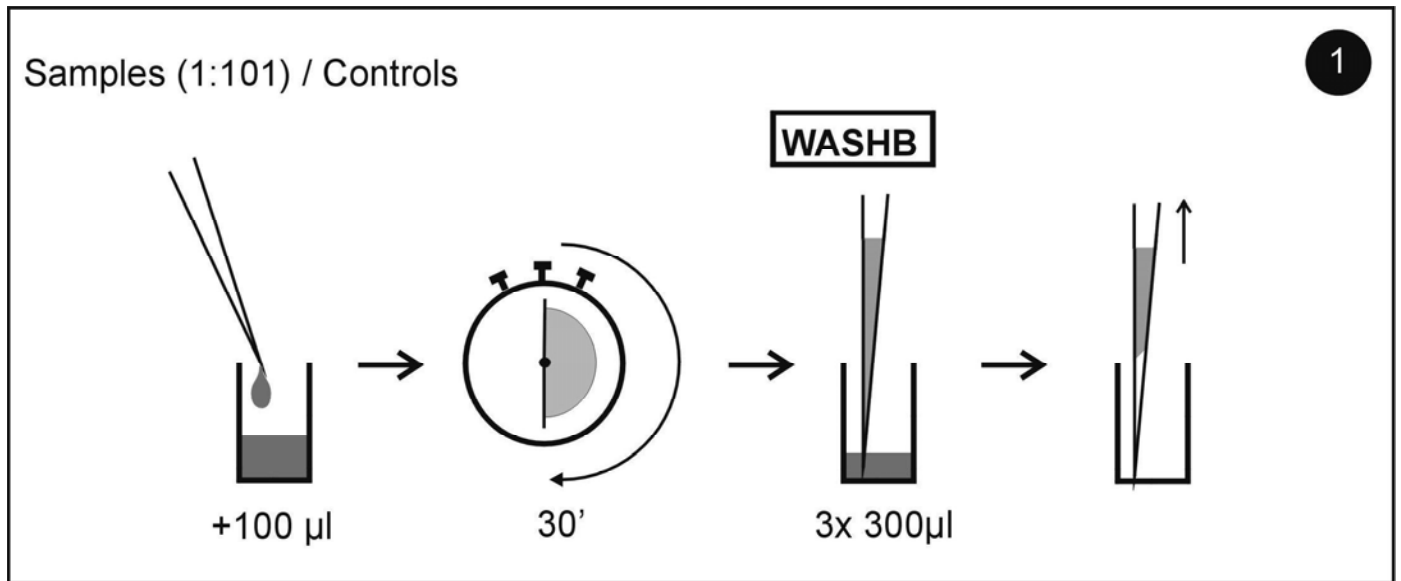
CC: Cut-off calibrator

P1: patient 1

P2: patient 2

P3: patient 3

## Annex B: Test Procedure














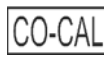








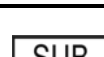
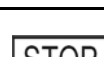
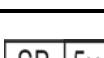
Assay/Test: \_\_\_\_\_ Incubation / Inkub. : 1. \_\_\_\_\_ min Date/ Datum: \_\_\_\_\_

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												
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>
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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>