AESKULISA IA2-GAD65 Screen

REF 3604

Instruction manual

Contents

1. Intended Use

The ***AESKULISA IA2-GAD65 Screen*** is a solid phase enzyme immunoassay employing a fusion protein of recombinant human tyrosine phosphatase-related protein islet antigen 2 (IA2) and recombinant human glutamate decarboxylase 65kDa isoform (GAD65) for the quantitative and qualitative detection of antibodies against islet cells in human serum.
The assay is a tool in the diagnosis of type 1 diabetes mellitus.

2. Clinical Application and Principle of the Assay

The insulin dependent diabetes mellitus, also called type I diabetes (T1DM), is a chronic autoimmune disease resulting from the autoimmune destruction of insulin producing ß-cells of Langerhans` islets. It is characterized by largely or complete lack of insulin production and the presence ofß-cell autoantibodies. The antigens recognized by these antibodies include insulin, glutamate decarboxylase (GAD65 kDa isoform) and tyrosine phosphatase-related protein islet antigen 2 (IA2). Autoantibodies against islet cell antigens are important preclinical markers as they may be present for years before diagnosis of diabetes and at a time where other metabolic assays show normal diagnostic findings. Recent studies show that a combined testing for antibodies against GAD65 and IA2 detects more than 90% of new-onset type 1 diabetes patients. The AESKULISA IA2-GAD65 Screen allows the simultaneous detection of autoantibodies against GAD65 and IA-2. It's an ideal screening assay to identify individuals of high risk for type 1 diabetes mellitus.

Principle of the test
Serum samples are incubated in the microtiter plates coated with the specific antigen over night. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards the IA2-GAD65 Biotin is incubated. During this step autoantibodies against IA2 and/or GAD65 form a bridge between the antigen immobilized on the plate and the IA2-GAD65 Biotin in the liquid phase. Unbound IA2-GAD65 Biotin is washed off in the following step. The amount of bound IA2-GAD65 Biotin is then determined by the addition of Streptavidin-Peroxidase (conjugate) that binds to Biotin. Unbound Streptavidin-Peroxidase 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 proportional to the initial concentration of the respective antibodies in the sample.

3. Kit Contents

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)

IA2-GAD65 Biotin 3 vials, Lyophilized (capped white)
Containing: Biotin conjugated to recombinant human IA2-GAD65; bovine serum albumin (BSA)

Ready to use:

Reconstitution Buffer 1 vial, 20 ml (capped red: red solution)
Containing: PBS, bovine serum albumin (BSA)

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

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

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

Calibrators 6 vials, 1 ml each 0, 25, 75, 125, 250, 500 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: streptavidin conjugated to horseradish peroxidase; bovine serum albumin (BSA)

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H_2O_2

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, ELISA plate cover, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multichannel 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

Store all reagents and the microtiter plate at 2-8°C/35-46°F, in their original containers. Reconstituted IA2-GAD65 Biotin has to be used on day of reconstitution. Once prepared, all other reconstituted solutions are stable for at least 1 month at 2-8°C/35-46°F. **Reagents and the microtiter plate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microtiter plates in designated foil, including the desiccant, and seal tightly.**

5. Precautions of Use

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 (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. 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

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

7.1 Preparations prior to starting

Please bear in mind that only reagents should be prepared which will be used at the same day!

Dilute concentrated buffers:
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).
To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:
Dilute serum samples 1:4 with sample buffer (1x)
e.g. 225 µl sample buffer (1x) + 75 µl serum. Mix well!

Reconstitute and dilute IA2-GAD65 Biotin:
Reconstitute lyophilized IA2-GAD65 Biotin in two steps: First, add 1 ml Reconstitution buffer to one vial, let it stand for 5 minutes and mix it until IA2-GAD65 Biotin is completely dissolved. Make sure that no remaining lyophilized or reconstituted IA2-GAD65 Biotin is in the cap. In a second step, transfer 1 ml reconstituted IA2-GAD65 Biotin to 5 ml Reconstitution buffer and mix well. One vial of reconstituted IA2-GAD65 Biotin is sufficient for six stripes. If more stripes will be used in one test, the two vials of lyophilized IA2-GAD65 Biotin have to be reconstituted and diluted as described above and then combined. 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.
- Cover the plate. Incubate for 16-20 hours at 2-8°C/35-46°F
- Let microtiter plate and reagents stand for 30 minutes at room temperature.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl of IA2-GAD65 Biotin (reconstituted and diluted) 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 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	Positive Results
< 30 U/ml	**> 30 U/ml**

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators	*OD 450/620 nm*	*CV % (Variation)*
0 U/ml	0.148	5.6
25 U/ml	0,276	3.2
75 U/ml	0.499	2.9
125 U/ml	0.768	4.7
250 U/ml	1.496	0.9
500 U/ml	2.627	2.1

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.499/0.463	0.481	76.2
P 02	1.494/1.496	1.495	258.4

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. All samples which are higher than cut-off are considered positive.

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

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

9. Technical Data

Sample material:	serum
Sample volume:	75 µl of sample diluted 1:4 with 1x sample buffer
Total incubation time:	16-20 hours at 2-8°C/35-46°F and 90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-500 U/ml
Analytical sensitivity:	3 U/ml
Storage:	at 2-8°C/35-46°F use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

Limit of detection

Testing sample buffer 60 times on *AESKULISA IA2-GAD65 Screen* and 8 low negative samples for 8 times gave an limit of detection of 3 U/ml.

10.2 Specificity and Sensitivity

The microtiter plate is coated with fusion protein of recombinant human tyrosine phosphatase-related protein islet antigen 2 (IA2) and recombinant human glutamate decarboxylase 65kDa isoform (GAD65). No crossreactivities to other autoantigens have been found. The diagnostic specificity of an Islet Cell Screen is 100% and the diagnostic sensitivity is up to 98%.

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 / 4	295.89	300.00	98.63
	1 / 8	157.17	150.00	104.78
	1 / 16	77.47	75.00	103.29
	1 / 32	39.64	37.50	105.71
2	1 / 4	183.42	184.00	99.68
	1 / 8	96.9	92.00	105.33
	1 / 16	49.56	46.00	107.74
	1 / 32	26.05	23.00	113.26

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 (IU/ml)	CV (%)
1	17.8	17.7
2	30.47	8.4
3	72.54	5.4
4	135.77	4.6
5	217.47	2.1

Inter-Assay		
Sample No.	Mean (IU/ml)	CV (%)
1	17.8	19.3
2	30.47	9.5
3	72.54	6.1
4	135.77	5.0
5	217.47	3.1

10.5 Calibration

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

11. Literature

1. **Chen S, Willis J, Maclean C, Ananieva-Jordanova R, Amoroso MA, Brooking H, Powell M, Collins A, Bennett S, Mitchell S, Burne P, Furmaniak J, Smith BR (2005).** Sensitive non-isotopic assays for autoantibodies to IA-2 and to a combination of both IA-2 and GAD65. Clin Chim Acta.357 (1): 74-83.

2. **Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ; Participating Laboratories (2008).** *Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glut amic acid decarboxylase and islet antigen-2. Diabetologia 51 (5): 846-852.*

3. **Lan MS, Wasserfall C, Maclaren NK, Notkins AL (1996).** *IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoanti gen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A.; 93 (13): 6367-6370.*

4. **Brooking H, Ananieva-Jordanova R, Arnold C, Amoroso M, Powell M, Betterle C, Zanchetta R, Furmaniak J, Smith BR (2003).** A sensitive non-isotopic assay for GAD65 autoantibodies. Clin Chim Acta. 331(1-2):55-59.

5. **Van den Driessche A, Eenkhoorn V, Van Gaal L, De Block C (2009).** *Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review. Neth J Med. 67(11):376-387.*

6. **Winter WE, Harris N, Schatz D (2002).** Type 1 diabetes islet autoantibody markers. Diabetes Technol Ther 4(6):817-839.

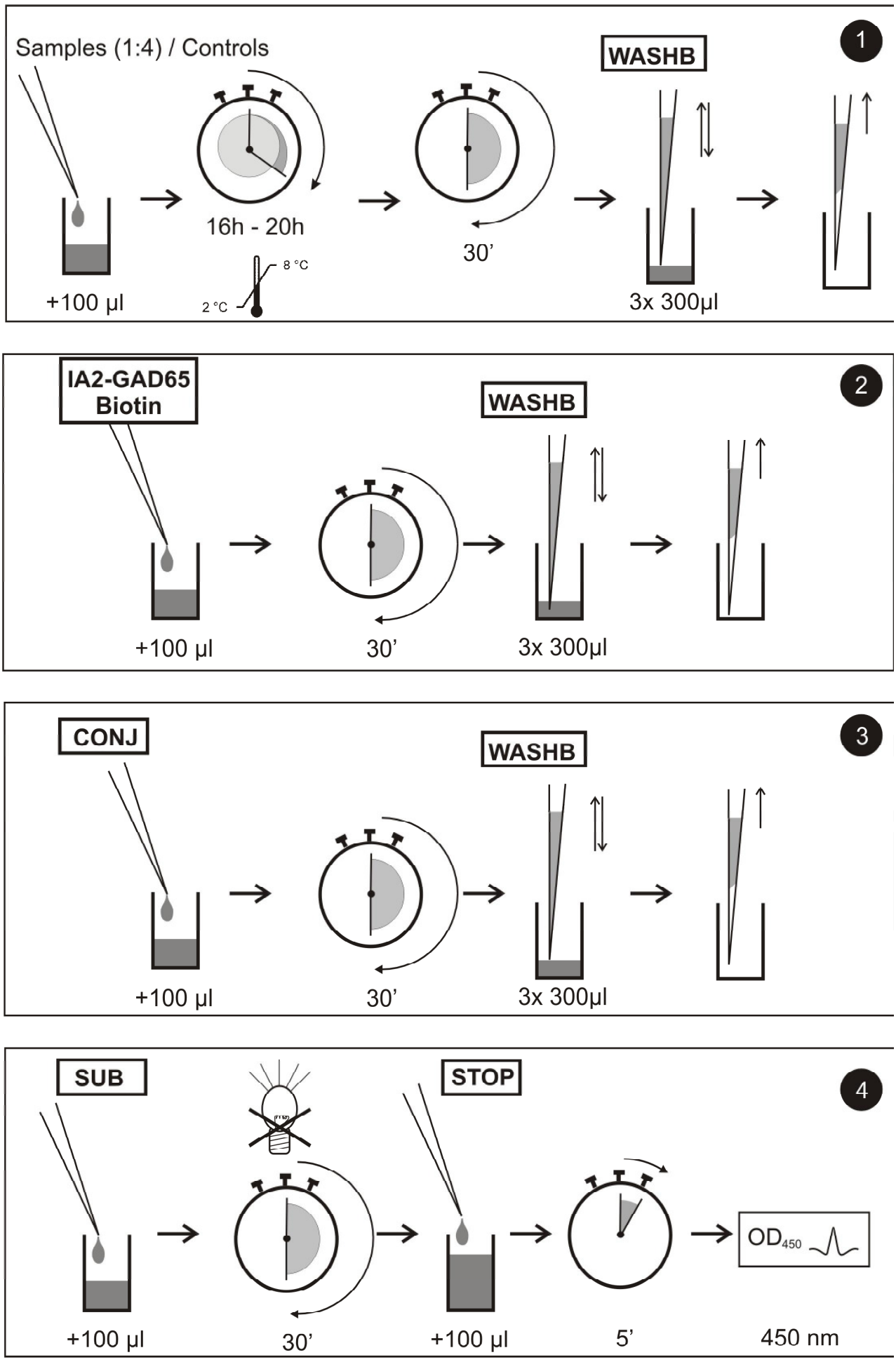
ANNEX A: Pipetting scheme

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 **quantitative interpretation** use calibrators to establish a standard curve						for **qualitative interpretation** 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												

AESKU.DIAGNOSTICS GmbH 55234 Wendelsheim - Mikroforum Ring 2, Germany Phone: + 49-6734-96270, Fax: + 49-6734-962727

IVD	♦ Diagnosi in vitro ♦ Pour diagnostic in vitro ♦ In Vitro Diagnostikum ♦ Para uso Diagnóstico in vitro	♦ For in vitro diagnostic use ♦ Para uso diagnóstico in vitro ♦ In Vitro Διαγνωστικό μέσο
REF	♦ Numero d'ordine ♦ Référence Catalogue ♦ Bestellnummer ♦ Número de catálogo	♦ Cataloge number ♦ Numéro de catálogo ♦ Αριθμός παραγγελίας
LOT	♦ Descrizione lotto ♦ Lot ♦ Chargen Bezeichnung ♦ Lote	♦ Lot ♦ Lote ♦ Χαρακτηρισμός παρτίδας
CE	♦ Conformità europea ♦ Déclaration CE de Conformité ♦ Europäische Konformität ♦ Déclaracão CE de Conformidade	♦ EC Declaration of Conformity ♦ Declaración CE de Conformidad ♦ Ευρωπαϊκή συμφωνία
96	♦ 96 determinazioni ♦ 96 tests ♦ 96 Bestimmungen ♦ 96 Testes	♦ 96 tests ♦ 96 pruebas ♦ 96 προσδιορισμοί
i	♦ Rispettare le istruzioni per l'uso ♦ Voir les instructions d'utilisation ♦ Gebrauchsanweisung beachten ♦ Ver as instrucões de uso	♦ See instructions for use ♦ Ver las instrucciones de uso ♦ Λάβετε υπόψη τις οδηγίες χρήσης
	♦ Da utilizzarsi entro ♦ Utilise avant le ♦ Verwendbar bis ♦ Utilizar antes de	♦ Use by ♦ Utilizar antes de ♦ Χρήση μέχρι
+2°C +8°C	♦ Conservare a 2-8°C ♦ Conserver à 2-8°C ♦ Lagerung bei 2-8°C ♦ Conservar entre 2-8°C	♦ Store at 2-8°C (35-46°F) ♦ Conservar a 2-8°C ♦ Φυλάσσεται στους 2-8°C
	♦ Prodotto da ♦ Fabriqué par ♦ Hergestellt von ♦ Fabricado por	♦ Manufactured by ♦ Fabricado por ♦ Κατασκευάζεται από
CO-CAL	♦ Calibratore cut-off ♦ Etalon Seuil ♦ Grenzwert Kalibrator ♦ Calibrador de cut-off	♦ Cut off Calibrator ♦ Calibrador de cut-off ♦ Οριακός ορός Αντιδραστήριο βαθμονόμησης
CON +	♦ Controllo positivo ♦ Contrôle Positif ♦ Positiv Kontrolle ♦ Controlo positivo	♦ Positive Control ♦ Control Positivo ♦ Θετικός ορός ελέγχου
CON −	♦ Controllo negativo ♦ Contrôle Négatif ♦ Negativ Kontrolle ♦ Controlo negativo	♦ Negative Control ♦ Control Negativo ♦ Αρνητικός ορός ελέγχου
CAL	♦ Calibratore ♦ Etalon ♦ Kalibrator ♦ Calibrador	♦ Calibrator ♦ Calibrador ♦ Αντιδραστήριο βαθμονόμησης
RC	♦ Recupero ♦ Corrélation ♦ Wiederfindung ♦ Recuperacão	♦ Recovery ♦ Recuperado ♦ Ανάκτηση
CONJ	♦ Coniugato ♦ Conjugé ♦ Konjugat ♦ Conjugado	♦ Conjugate ♦ Conjugado ♦ Σύζευγμα
MP	♦ Micropiastra rivestita ♦ Microplaque sensibilisée ♦ Beschichtete Mikrotiterplatte ♦ Microplaca revestida	♦ Coated microtiter plate ♦ Microplaca sensibilizada ♦ Επικαλυμμένη μικροπλάκα
PINP	♦ Piastra ad aghi rivestita ♦ Pinplate sensibilisée ♦ Beschichtete Pinplatte ♦ Pinplate revestida	♦ Coated pinplate ♦ Pinplate sensibilizada ♦ Επικαλυμμένη πλάκα Pin
WASHB 50x	♦ Tampone di lavaggio ♦ Tampon de Lavage ♦ Waschpuffer ♦ Solucão de lavagem	♦ Wash buffer ♦ Solución de lavado ♦ Ρυθμιστικό διάλυμα πλύσης
SUB	♦ Tampone substrato ♦ Substrat ♦ Substratpuffer ♦ Substrato	♦ Substrate buffer ♦ Tampón sustrato ♦ Ρυθμιστικό διάλυμα υποστρώματος
STOP	♦ Reagente bloccante ♦ Solution d'Arrêt ♦ Stopreagenz ♦ Solucão de paragem	♦ Stop solution ♦ Solución de parada ♦ Αντιδραστήριο διακοπής αντίδρασης
SB 5x	♦ Tampone campione ♦ Tampon Echantillons ♦ Probenpuffer ♦ Diluente de amostra	♦ Sample buffer ♦ Tampón Muestras ♦ Ρυθμιστικό διάλυμα δειγμάτων