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AESKUBLOTS®

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INSTRUCTION MANUAL

AESKUBLOTS® Borrelia-G/-M

Ref 4006 / 4007

DIN EN ISO 13485

Instruction Manual

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AESKU.DIAGNOSTICS GmbH & Co. KG
Mikroforum Ring 2
55234 Wendelsheim, Germany
Tel: +49-6734-9622-0
Fax: +49-6734-9622-2222
Info@aesku.com
www.aesku.com

1 Intended Use

AESKUBLOTS® Borrelia-G/M is a sensitive membrane based enzyme immunoassay for qualitative detection of specific IgG/M against *Borrelia burgdorferi* in human serum. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay is a tool to confirm positive ELISA results.

2 Clinical Application and Principle of the Test

Lyme disease is a multisystem disease caused by infection with spirochete *Borrelia burgdorferi*. This pathogen was discovered in 1981 by Willy Burgdorfer (Burgdorfer et al., 1982). So far the disease has been classified into three stages (Stage I: Erythema migrans symptom, Stage II: early clinical manifestations after dissemination of the pathogen, Stage III: with late manifestations). However, this classification is obsolete because the symptoms of the various stages overlap. Today, an early and a late manifestation of the disease can be distinguished. The early stage corresponds to stage I / II of the previous classification and the late stage of the stage III. The late onset may have severe clinical symptoms and treatment with antibiotics is difficult. For these reasons, early diagnosis is of great importance.

Main vector of *Borrelia* in Europe is the common wood tick *Ixodes ricinus*, Genus: *Ixodidae* (ticks). According to their occurrence in densely wooded areas the disease is piling up here, especially in summer and autumn. Once the tick has taken blood, the pathogens multiply in their midgut. Subsequently, the bacteria migrate to the salivary gland and are injected with the saliva into the wound.

The diagnosis of the early form is based on the clinical symptom of Migrans Erythema. However, this symptom is missing in 20-40% of patients. Furthermore, the symptoms in later stages are very diverse. Serological tests are therefore an important factor in diagnosis. Although the surest evidence is the cultivation of pathogens from blood, cerebrospinal fluid or skin biopsy, this test is not qualified for routine diagnostics due to the long duration and its low sensitivity. Due to the lack of a gold standard test for diagnosis of borreliosis the German Society for Hygiene and Microbiology (DGHM) currently recommends performance of a two-stage testing procedure (MIQ Lyme Borreliosis), requiring an initial diagnostic assessment (typically ELISA) followed by confirmation of a positive or doubtful ELISA result by an additional test (1) such as Western blotting.

Used antigens:

Nomenclature of borrelia antigens	**Properties of proteins**	**type**	**origin**
p100	Protein of membran-vesicles	rec	B. afzelii
VlsE	**v**ariable major protein-**l**ike **s**equence **E**xpressed	rec	B. afzelii
p58	not characterized	rec	B. garinii
p41 (Flagellin)	structural protein of endoflagellin	rec	B. burgdorferi sensu stricto
p39 (BmpA)	Flagella complex **B**orrelia **m**embrane **p**rotein **A**	rec	B. burgdorferi sensu stricto, B. garinii, B. afzelii
p31 (OspA)	**O**uter **s**urface, **protein A**	rec	B. afzelii

Nomenclature of borrelia antigens	Properties of proteins	type	origin
p23 (OspC)	**O**uter **s**urface **p**rotein **C**,Mix from different borrelia subtypes	rec	B. burgdorferi sensu stricto, B. garinii, B. bavariensis, B. afzelii, B. spielmanii
p18 (DbpA)	**D**ecorin **b**inding **p**rotein **A**	rec	B. burgdorferi sensu stricto, B. garinii, B. afzelii, B. spielmanii, B. bavariensis

Principle of the test

The antigens are applied as lines on a nitrocellulose membrane. The membrane is blocked to prevent unspecific reactions. Membrane-strips with specific antigens at exactly defined positions are incubated in serum samples diluted 1:101. 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. Unbound conjugate is washed off in the following step. After the addition of the TMB-substrate it is converted by an enzymatic reaction to a blue precipitate. The reaction is stopped by distilled water.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Wash and sample buffer (5x)	2x 100 ml	white	colorless	5x concentrated for preparation of 1 L
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Conjugate, IgG (Ref 4006)	1 x 10 ml	blue	purple	Containing: Anti-human immunoglobulin G (IgG) conjugated to horseradish peroxidase
Conjugate, IgM (Ref 4007)	1 x 10 ml	green	green	Containing: Anti-human immunoglobulin M (IgM) conjugated to horseradish peroxidase
TMB Substrate	1 x 10 ml	black	colorless	Stabilized TMB/H_2O_2
Membrane strips	24 strips	color coding: green	N/A	Coated antigens see Clinical Application and Principle of the Test
incubation tray	3 pcs.	N/A	N/A	N/A
tweezers, reference template, scoring sheet	1 pcs. each	N/A	N/A	N/A
MATERIALS REQUIRED, BUT NOT PROVIDED				
rocking platform, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (10, 1000 µl), absorbent or filter 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 membrane-strips at 2-8°C/35-46°F in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for at least four weeks. Reagents and strips shall be used within the expiry date indicated on each respective component. Don't use components after the expiry dates. Avoid intense exposure of TMB solution to the light.

5 Precautions of Use and General Introductions

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 under the conditions of intended 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 avoiding contact with eyes and skin and wearing disposable gloves.

Washbuffer and conjugate contain kathon (1% v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membrane.

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

Handle patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

To differentiate between the various **AESKUBLOTS®**-tests available, a color coding is applied above the reference line:

Color coding	**AESKUBLOTS®**
red	ANA-17 Comp
orange	ANA-17 Pro
blue	Myositis Pro
brown	Liver Pro
purple	Vasculitis Pro
black	Gastro Pro
green	Borrelia-G and Borrelia-M

In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Wash- and samplebuffer may be interchanged between lots and test kits. All other components are specific for each test kit and are not to be interchanged. Do not exchange reagent components between autoimmunity and borrelia diagnostic tests!

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.

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 priorly used with other reagents.

The intensity of the band color does not necessarily correlate with antibody titers obtained by other reference methodologies.

Samples from apparent normal blood donors may contain autoantibodies.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

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 during the first 8 h. Alternatively, the samples should be stored in tightly closed vials at 2-8°C/35-46°F for up to 48 h, or frozen at -20°C/-4°F for longer periods. Avoid repeated thawing and freezing. Do not use heat inactivated samples (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4 Vol. 30 No. 10).

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated wash buffer 1 + 4 with distilled water (e.g. 20 ml plus 80 ml).

7.2 Test Steps

Important notes:

Follow exactly this protocol. Make sure that the two components mentioned in the protocol are added to the tray in step 6, 9.

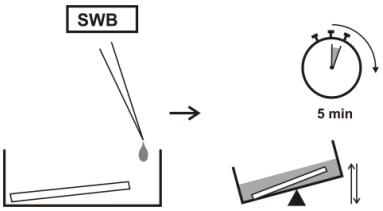
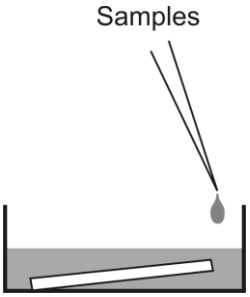
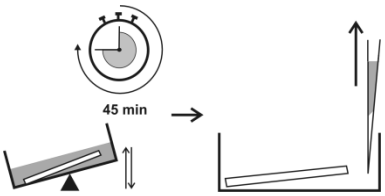
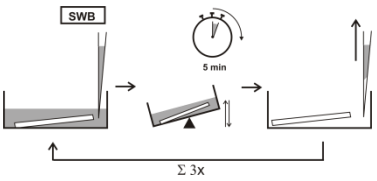
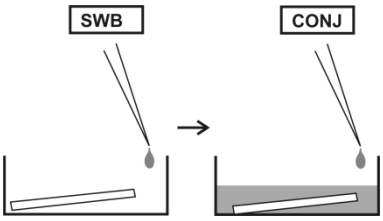
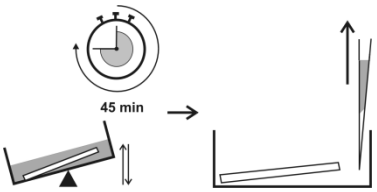
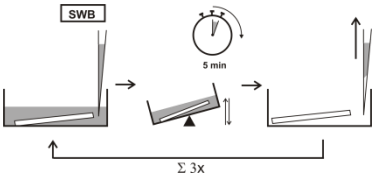
Do not let strip dry out during incubation steps.

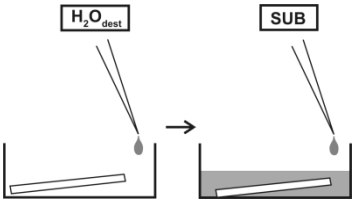
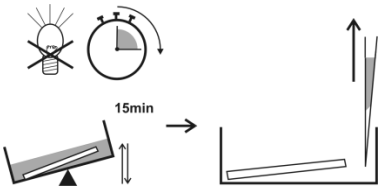
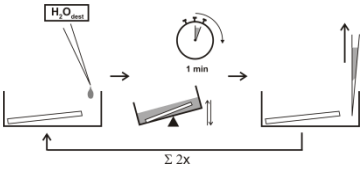
Do not touch strip with fingers, use tweezers.

Remove diluted samples completely after incubation of strip to avoid carry over.

Continuously shake strip during incubation steps.

Give sample buffer, conjugate and substrate together with the wash buffer to one side of the incubation tray. Do not allow to flow over the strip.

Step	*Description*
1.	Ensure the preparations, from step 7.1 above, have been carried out prior to test begin.
2.	 Put strip in correct orientation into incubation tray (reference line and color coding upwards). Moisten strip with 1 ml wash and sample buffer and incubate for 5 minutes with agitation .
CONTROLS & SAMPLES	
3.	 Pipette 10 µl serum sample into the designated incubation trays with sample buffer.
4.	 Incubate for 45 minutes at 20-32°C/68-89.6°F with agitation. After that remove sample completely.
5.	 Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.
CONJUGATE	
6.	 Pipette 700 µl wash buffer and 300 µl conjugate into each incubation tray with strip.
7.	 Incubate for 45 minutes at 20-32°C/68-89.6°F with agitation. Remove conjugate.
8.	 Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.

SUBSTRATE		
9.		Pipette 700 µl dH_2O and 300 µl substrate into each incubation tray with strip.
10.		Incubate for 15 minutes at 20-32°C/68-89.6°F with agitation, protected from intense light. Remove substrate.
STOP		
11.		Pipette 2 ml dH_2O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dH_2O. Repeat this step one time.
12.	Remove strip of the incubation tray. Dry strip between filter paper	
13.	Interpret the dried strip between 6 hours.	

AESKUBLOTS® Borrelia are also intended to be automatically processed and evaluated on the HELIA® line immunoassay analyzer.
Reagent preparation for HELIA®: Dilute 1 part wash buffer concentrate (WASH) with 4 parts ultrapure water (e.g. 100 mL wash buffer concentrate and 400 mL ultrapure water) to obtain a ready-to-use wash buffer. All other reagents are ready to use when processed in HELIA. For detailed handling of the test on HELIA® refer to the instruction manual of the HELIA®.

8 Qualitative Interpretation

Manual Analysis

On each **AESKUBLOTS® Borrelia-G/M** teststrip the following control bands are applied.

1.) Two function controls one at the top, one at the bottom of the strip (strongly positive reaction with every serum sample)
2.) Conjugate controls IgG and IgM (strongly positive reaction with the corresponding conjugate. Depending from the used serum the other conjugate control may develop a weak non specific color).
3.) Cut-Off control which intensity is used for result evaluation of the diagnostic bands.

Test results can be considered valid, if:

- Functional controls are visible
- Cut-off control is visible
- And the corresponding conjugate control become visible

Fix dried strip onto scoring sheet aligned with reference line (adhesive membrane). Align reference template with the strip reference line. Interpret results only in reference to cut-off control of each strip.

Interpret results of **AESKUBLOTS® Borrelia-G** like this:

Interpretation	**IgG**
negative	1 band except VlsE > Cut-Off
borderlined	VlsE or 1 band + p41 ≥ Cut-Off
positive	2 bands except p41 ≥ Cut-Off

Interpret results of **AESKUBLOTS® Borrelia-M** like this:

Interpretation	**IgM**
negative	No band except p41 > Cut-Off
borderlined	1 band except OspC or p41 or p18 ≥ Cut-Off
positive	OspC or p18 or 2 other bands ≥ Cut-Off

The results can be recorded on the scoring sheet.

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated.

The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample
Total incubation time:	142 minutes at 20-32°C/68-89.6°F
Storage:	at 2-8°C/35-46°F; use original vials only.
Number of determinations:	24 tests

10 Performance Data

10.1 Relative Sensitivity and Specificity

80 sera from patients with suspected Lyme Borreliosis were investigated in a comparative study using **AESKUBLOTS® Borrelia G/M** and another commercial line immunoassay.

n = 80		other commercial - line immuno assay	
		positive	negative
AESKUBLOTS® Borrelia-G/M	positive	52	7
	negative	2	19

A positive agreement (relative sensitivity) of 96.2 % was determined between the assays.

In addition, discrepant sera were examined in another blot-test. The results were conforming to the **AESKUBLOTS® Borrelia-G/M.**

Furthermore, in a comparative study 32 sera from patients with a suspected recent Borrelia infection were examined in the **AESKUBLOTS® Borrelia-M** and a further commercial line immunoassay.

n = 32		commercial - line immuno assay	
		positive	negative
AESKUBLOTS® Borrelia- M	positive	9	3
	negative	1	19

A positive agreement (relative sensitivity) of 90 % was determined between the assays.

In addition, discrepant sera were examined in another blot-test. Two of the three sera were found positive. The negative serum was also confirmed.

In order to determine the negative agreement (relative specificity), 400 healthy blood donors were investigated using **AESKUBLOTS® Borrelia-G/M**.

35 were determined positive for IgG with **AESKUBLOTS® Borrelia-G**. This means **91.3 %.**

11 were determined positive for IgM with **AESKUBLOTS® Borrelia-M**. This means **97.3 %.**

11 Literature

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP (1982). Lyme disease – A tick-borne spirochetosis?. Science. 216:1317–1319.

For further reading:

Engstrom SM, Shoop E, Johnson RC (1995). Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol. 33:419–27.

Wilske B (2005). Epidemiology and diagnosis of Lyme borreliosis. Annals of Medicine. 37,8: 568–579.

Stanek G, Strle F (2003). Lyme borreliosis. Lancet. 362: 1639–1647.

Lothar Thomas: Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books

CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

IVD	¨ Diagnosi in vitro	¨ For in vitro diagnostic use
	¨ Pour diagnostic in vitro	¨ Para uso diagnóstico in vitro
	¨ In Vitro Diagnostikum	¨ In Vitro Διαγνωστικό μέσο
	¨ Para uso Diagnóstico in vitro	
REF	¨ Numero d'ordine	¨ Cataloge number
	¨ Référence Catalogue	¨ Numéro de catálogo
	¨ Bestellnummer	¨ Αριθμός παραγγελίας
	¨ Número de catálogo	
LOT	¨ Descrizione lotto	¨ Lot
	¨ Lot	¨ Lote
	¨ Chargen Bezeichnung	¨ Χαρακτηρισμός παρτίδας
	¨ Lote	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Ευρωπαϊκή συμφωνία
	¨ Déclaracão CE de Conformidade	
Σ 24	¨ 24 determinazioni	¨ 24 tests
	¨ 24 tests	¨ 24 pruebas
	¨ 24 Bestimmungen	¨ 24 προσδιορισμοί
	¨ 24 Testes	
	¨ Rispettare le istruzioni per l'uso	¨ See instructions for use
	¨ Voir les instructions d'utilisation	¨ Ver las instrucciones de uso
	¨ Gebrauchsanweisung beachten	¨ Λάβετε υπόψη τις οδηγίες χρήσης
	¨ Ver as instrucões de uso	
	¨ Da utilizzarsi entro	¨ Use by
	¨ Utilise avant le	¨ Utilizar antes de
	¨ Verwendbar bis	¨ Χρήση μέχρι
	¨ Utilizar antes de	
+2°C +8°C	¨ Conservare a 2-8°C	¨ Store at 2-8°C (35-46°F)
	¨ Conserver à 2-8°C	¨ Conservar a 2-8°C
	¨ Lagerung bei 2-8°C	¨ Φυλάσσεται στους 2-8°C
	¨ Conservar entre 2-8°C	
	¨ Prodotto da	¨ Manufactured by
	¨ Fabriqué par	¨ Fabricado por
	¨ Hergestellt von	¨ Κατασκευάζεται από
	¨ Fabricado por	
STRIP	¨ Strip di nitrocelluslosa rivestita	¨ Coated nitrocellulose strip
	¨ Strip de nitrocellulose couché	¨ Tira de nitrocelulosa recubierta
	¨ Nitrozellulosemembran-Streifen mit aufgebrachten Antigenen	¨ Επίστρωση λωρίδα νιτροκυτταρίνης
	¨ Tira de nitrocelulose revestido	
WASH 20x	¨ Tampone di lavaggio	¨ Wash buffer
	¨ Tampon de Lavage	¨ Solución de lavado
	¨ Waschpuffer	¨ Ρυθμιστικό διάλυμα πλύσης
	¨ Solucão de lavagem	
Block-Reag	¨ Reagente bloccante	¨ Blocking Reagent
	¨ réactif de blocage	¨ Reactivo bloqueante
	¨ Blockier-Reagenz	¨ Αντιδραστήριο αποκλεισμού
	¨ Bloqueio de reagente	¨
RCNS 10ml	¨ Ricostituire con 10 mL	¨ Reconstitute with 10 mL
	¨ reconstituer avec 10 mL	¨ reconstituir con 10 mL
	¨ rekonstituiren mit 10 mL	¨ Ανασύσταση με 10 mL
	¨ reconstituir com 10 mL	¨
SB	¨ Tampone campione	¨ Sample buffer
	¨ Tampon Echantillons	¨ Tampón Muestras
	¨ Probenpuffer	¨ Ρυθμιστικό διάλυμα δειγμάτων
	¨ Diluente de amostra	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύζευγμα
	¨ Conjugado	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	