CE **IVD** Σ 24

ANA GRANULAR 12 Ag IgG Dot for BDI
Order Code: AD ANAGR12DBD
BlueDiver Protocol: 02

1. INTENDED USE

ANA GRANULAR 12 Ag IgG Dot for BDI is an Immunodot kit intended for the detection, in human sera only, of IgG autoantibodies against the following antigens: SSA/Ro 60kD, SSB, Scl-70, Mi-2, Ku, TIF1-γ, SAE1/2, NXP-2, RNA Polymerase III, DFS-70, Sm/RNP and Sm.

This kit is intended to confirm results of speckled/granular patterns obtained by immunofluorescence, the screening and reference method in autoimmunity; the kit is intended as an aid in the diagnosis of several autoimmune diseases (for more details, see 11.5 *Auto-antibodies diagnostic values*).

The test is intended for a large, routine population. This kit is strictly reserved for professional use in clinical analysis laboratories. This kit is strictly foreseen as an automated test and can only be used in a BlueDiver Instrument Model I or II (hereinafter named BDI I or BDI II respectively).
For a semi-quantification of the test results, it is necessary to use the BlueDiver scanner system and the Dr Dot software. This system is not included in the BDI I, but is included in the BDI II (see point 4).

2. PRINCIPLE OF THE TEST

This kit and all its components are intended to be performed exclusively on the BDI I or II.
The test is based on the principle of an Enzyme Immunoassay. The strips are composed of a membrane fixed on a specific plastic support. During the automated test procedure, the BDI sequentially incubates the strips in the wells of ready-to-use reagent cartridges. Briefly: the strips are first incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing. Upon further incubation into AP-conjugated goat antibodies against human IgG, the enzyme conjugate binds to the antigen-antibody complexes. After removal of excess conjugate by washing, the strips are finally incubated into a substrate solution. Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.
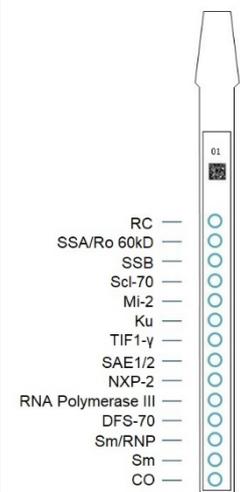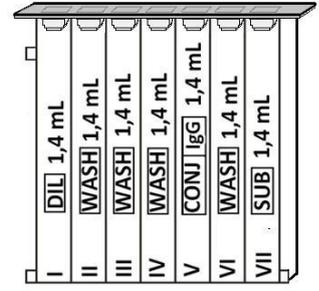The kit is composed of 24 single-use tests.

3. KIT CONTENTS

Prior to any use of the kit, please check that all the items listed are present. Please also check if characteristics of the product are corresponding to those described hereafter.
If one of the items is missing or damaged, please do not use the kit and contact your distributor.

3.1 COMPONENTS

		STRIP	CARTRIDGE
Strips	3 x 8 units on plastic supports, breakable individually; sealed in aluminium pouch. Each strip is for single-use. 14 dots on each: 1 positive control (RC) 12 antigens 1 negative control (CO)		
Cartridge	24 units having each 7 compartments; sealed.		
Diluent buffer	Ist position, 1 x 1,4 ml (yellow) *contains H$_2$O • TBS • NaCl • Tween • BSA • Preservatives • dye • antifoam emulsion*		
Wash buffer	IInd, IIIrd, IVth and VIth position, 4 x 1,4 ml (colourless) *contains H$_2$O • TBS • NaCl • Tween • Preservatives • antifoam emulsion*		
Conjugate	Vth position, 1 x 1,4 ml (red) *contains H$_2$O • TBS • NaCl • KCl • MgCL$_2$ • AP-conjugated goat anti-human IgG • Preservatives • dye • antifoam emulsion*		**Documents:** Instructions for Use (IFU), Certificate of Analysis (CoA)
Substrate	VIIth position, 1 x 1,4 ml (pale yellow solution) *contains H$_2$O • Preservatives • MgCL$_2$ • TBS • NBT stabilizer • NBT • BCIP*		

Abbreviations in alphabetic order:
AP = Alkaline Phosphatase; BCIP = Bromo-Chloro-Indolyl-Phosphate; BSA = Bovine Serum Albumin; KCl = Potassium Chloride; MgCl$_2$ = Magnesium Chloride; NaCl = Sodium Chloride; NBT = NitroBlue Tetrazolium; TBS = Tris Buffer Saline

For more information on the composition and concentration of the active ingredients used, please refer to the MSDS available on request or on www.alphadia.be.

Symbols used on kit labels

	Attention : consult instructions for use Attenzione : consulti le istruzioni per uso Achtung :Gebrauchsanwendung beachten Attention : consulter le mode d'emploi Atention : consultar las instruccions Atenção : consultar instruções para uso Προσοχή : Συμβουλευτειτε τις οδηλιες χρήσης	STRIP	Coated strip Strips rivestita Streifen Bandelette Tira Tira Στιγμάτων
IVD	In vitro diagnostic medical device Dispositivo medico diagnostico in vitro Zur medizinischen diagnostischen Anwendung in vitro Dispositif médical de diagnostic in vitro Dispositivo médico para uso diagnostico in vitro Dispositivo médico para uso diagnostico in vitro Ιατρικό υλικό για διάγνωση In Vitro	$C\epsilon$	CE Mark Marcatura CE CE-Kennzeichnung Marquage CE Marca CE Marcação CE μονογράφηση CE
8°C / 2°C	To be stored from 2°C to 8°C Conservazione da 2 – 8°C bei 2°C bis 8°C lagern A conserver de 2°C à 8°C Almacenar a 2 - 8°C Armazenar a 2 – 8ºC Αποθηκεύστε στους 2 έως 8°C	Σ	For … uses Per … dosaggi Für … Anwendungen Pour … utilisations Para … usos Para … utilização για … χρήσεις
LOT	Batch Number Lotto numero Chargennummer Désignation du lot Denominacion de lote Numéro do lote Κωδικός	REF	Code Codice Artikelnummer Référence Código Código Κωδικός
	Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Verwendbar bis (letzter Tag des Monats) Utiliser avant (dernier jour du mois indiqué) Estable hasta (usar antes de ultimo dia del mes) Data limite para utilização (ultimo dia do mês) Χρήση έως (τελευταια ημέρα του μήνα)		To be protected from direct sunlight Proteggere dalla luce Vor Licht schützen Protéger de la lumière Proteja de la luz Proteger da exposição à luz Προστατεύετε τον αντιδραστήριο
CART	Cartridge Cartuccia Patrone Cartouche cartucho cartucho κασέτα		Manufactured by Fabbricado da Hergestellt von Fabriqué par Fabricado por Fabricado por Κατασκευάζεται από την

3.2 Antigens used

SSA/Ro 60kD	Ro 60 kD protein (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
SSB	La 50 kD protein (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
Scl-70	DNA topoisomerase I (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
Mi-2	CHD4 protein (chromodomain helicase DNA binding protein), subunit Mi-2 beta (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
Ku	Regulatory subunit of DNA-dependent protein kinase (70/80 kD heterodimer) (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
TIF1-γ	**T**ranscriptional **I**ntermediary **F**actor 1-gamma (Trim 33) (recombinant, human, expressed in human HEK293 cells)
SAE-1/2	Small ubiquitin-like modifier Activating Enzyme subunit 1 (recombinant, human, expressed in E.coli) Small ubiquitin-like modifier Activating Enzyme subunit 2 (recombinant, human, expressed in E.coli)
NXP-2	Nuclear Matrix Protein 2 (also called anti-MJ) (recombinant, human, expressed in E.coli)
RNA Polymerase III	155 kD subunit (RPC 155) of the RNA Polymerase III complex (Recombinant, human, expressed in E.coli)
DFS-70	Lens Epithelium Derived Growth Factor (**D**ense **F**ine **S**peckles protein 70 kD) (recombinant, human, full length, expressed in E.coli)
Sm/RNP	snRNP particles. Contains essentially 68kD, A, BB', C and D proteins. A significant amount of snRNA is detectable (purified from bovine thymus)
Sm	Core proteins of snRNP particles. Contains mainly D protein. E, F, G subunits are detectable. BB' proteins are not detectable (purified from bovine thymus)

4. MATERIAL REQUIRED BUT NOT PROVIDED

BDI I:

The BDI I is an instrument that performs the various steps of incubation and washing of automated Alphadia strips, from the deposit of the sample to the final colour development. The maximum capacity is of 24 strips which are incubated simultaneously. Each strip is associated with a cartridge containing the various reagents making it possible to carry out the test. The BlueDiver Instrument has a barcode reader which controls the correct association between a strip and its cartridge.
Prior training is strongly recommended (see your distributor).
Please consult The User Manual before using the BDI I.

The BlueDiver scanner and Dr dot software:

The BlueDiver scanner and Dr Dot software are intended for reading test results of automated Alphadia strips. The Dr Dot software and BlueDiver scanner have to be used in combination.
The scanner has been specifically developed for the reading of the strips with "BlueDiver" design. Based on the image of the scanned strips, the Dr Dot software converts the intensity of each dot/line into a numerical value (the numerical scale is based on a greyscale). Results are expressed in arbitrary units (from 0-100). 1-24 strips can be read.
Prior training is strongly recommended (see your distributor).
Please contact your distributor do obtain the latest version of Dr Dot software.
Please consult The User Manual before using the BlueDiver scanner and Dr Dot Software.

BDI II:

The BlueDiver Instrument II is an instrument that performs the various steps of samples pipetting, incubation, washing, drying and reading of automated Alphadia strips, from the deposit of the sample tube to the final reading of the strips.
The maximum capacity of the BDI II is of 24 strips which are incubated simultaneously. Each strip is associated with a cartridge containing the various reagents making it possible to carry out the test. The BDI II has a barcode reader which controls the correct association between a strip and its cartridge.
It includes the BlueDiver scanner and Dr Dot reading system.
Prior training is mandatory (see your distributor).
Please consult The User Manual before using the BDI II

Other Material: Micropipettes, absorbent paper, protective equipment (see point 6).

5. STORAGE

The test kit must be stored at a temperature between +2°C to +8°C throughout its validity period (see expiration date on the kit). Do not freeze.
After initial opening of the kit, unused reagent cartridges must be stored at 2-8°C protected from (sun)light preferably inside the original kit box.
Unused strips have to be placed back into the provided pouches, sealed and stored at 2-8°C preferably inside the original kit box. When stored properly, all test kit components are stable until the indicated expiry date.

6. SAFETY PRECAUTIONS

1. All reagents are for in vitro diagnostic and professional use only. The test kit should be processed by trained technical staff only.
2. The reagents in the kit are considered as not dangerous, as the concentrations of potentially dangerous chemicals are below the thresholds specified by European regulations. More information is available on the MSDS of the kit (available upon request).
    Nevertheless, the product contains preservatives which may have (in their given concentration), slightly polluting properties or causing skin sensitization. Therefore, contact with the skin, eyes or mucous membranes should be avoided. As with any chemical containing specific hazards, the product/components of the product should only be handled by qualified personnel and with the necessary precautions.
3. Patient samples should be handled as if they were capable of transmitting infectious diseases; they therefore require suitable protection (gloves, laboratory coat, goggles). In any case, GLP should be applied with all the general or individual safety rules in force.
4. Waste disposal: Patient samples, incubated test strips and used cartridges should be handled as infectious waste. The boxes and other containers do not need to be collected separately, unless stated otherwise in official regulations.

7. RECOMMANDATIONS

1. Alphadia and its authorized distributors cannot be held responsible for damages caused indirectly or due to: a change or modification in the indicated procedure, an improper use of the kit and / or the use of an incomplete or damaged kit. The use of this kit is reserved for qualified technical personnel only.
2. Alphadia's responsibility is limited in all cases to the replacement of the kit.
3. In the event of a serious incident (injury, deterioration in health, or death) with this IVD device, please report it immediately to the manufacturer (see address below) and to the competent authority in your country.

8. SAMPLE COLLECTION, HANDLING AND STORAGE

The test should be used on recently collected sera samples only! Sera with particles should be centrifuged at low speed. Blood samples should be collected in dry tubes. Please avoid using a pool of different sera, as this can lead to inconsistent results (see point 10.4). After separation, the serum samples should be used immediately or aliquoted and stored at 2-8 ° C (for storage for a few days) or frozen at -20°C (for longer storage periods). Repeated freezing/ thawing cycles of the samples must be avoided.

9. ASSAY PROCEDURE

BASIC INFORMATION, HANDLING AND TIPS:
TEST PROCESS principle:
After the manual loading of the strips and reagent cartridges, the incubation and washing steps are automatically processed by the *BDI;* the continuous up and down agitation of the strips in the wells of the ready-to-use reagent cartridges ensures an efficient circulation of the fluids over the strips. The whole test procedure is run at room temperature.
Description of the STRIPS:

The reactive (front) side of the strips is coated with antigens which appear as faint blue dots. This coloration ensures that all antigens have been correctly spotted onto the membrane. The coloration disappears during the processing of the test. This front side also displays a strip number and a 2-dimensional square barcode for traceability of the strips after removal from the BDI at the end of the test.

The non-reactive (back) side of the strips displays both alphanumeric and bar-coded information for identification of the strip type and lot number by the BDI.

The strips must be manually inserted into the dedicated clamp before starting the automated process (see 9.1 and 9.2 *Test Preparation* hereafter). During this operation, avoid touching the membrane zone of the strips with fingers. Always wear laboratory gloves and use the plastic parts (strip support) for handling or manipulation.

Description of the CARTRIDGES: (see image page 1)
The reagent cartridges are composed of 7 different wells filled with ready-to-use reagents. The cartridges are sealed, and the reagent wells are hermetically separated. The sealing has to be removed before starting the test. Once opened, manipulate the cartridges with care in order to avoid reagent spilling and contamination from well to well.
The rear (back) side of the cartridges is labelled with both alphanumeric and bar-coded information for identification of the cartridge type and lot number by the BDI.
The cartridges must be manually loaded onto the dedicated cartridge holder before starting the automated process ((see 9.1 and 9.2 *Test Preparation* hereafter). The front and rear (back) sides of the cartridges have, respectively, a bottom triangular and two (bottom + top) square plastic edges for secure position and orientation into the holder.

Description of the CONTROLS:
The **Positive Control or RC (Reaction Control)** consists of a protein fixing all the immunoglobulins present in the test sample. If the test has been carried out correctly, this control will show a colouring at the end of the test (with an intensity depending on the effective concentration of immunoglobulins in the sample).
The absence of any colouring of this dot at the end of the test may indicate that the sample has not been pipetted on the strip (see 10.4 *Troubleshooting*).
The **Negative Control or CO (Cut-Off Control)** consists of a protein reacting with the enzymatic substrate and with certain constituent elements of the tested sample. If the test has been carried out correctly, this control is coloured at the end of the test, with a signal depending on the kinetics of the substrate and the characteristics of the sample. The intensity of this control serves as a threshold value for the final interpretation of the results (see 10 *INTERPRETATION OF RESULTS*).

STRIPS/CARTRIDGES association
The strips and cartridges of a same test kit share the same lot number and are dedicated to be associated in lot-specific pairs. Do not associate a strip and a cartridge with different lot numbers as this will be detected as an invalid setting by the BDI and will stop the process.
As long as each strip/cartridge pair is valid, the BDI can process strips/cartridges associations of different kits; However, only kits having the same protocol number (same incubation time and sequence) can be processed together in one same run (please refer to the protocol number indicated under the kit reference at the top of first page).

9.1 Test preparation on BDI I
Before any usage of the BDI, please refer to the manual of use supplied with the instrument.
- Allow all kit components to equilibrate at room temperature (+18°C to +25°C) before use.
- A working list (either edited from Dr Dot software, or external) should always be prepared for easy loading and correct association of strips, cartridges and patient samples.
- Make sure that the cartridge holder is fixed in its emplacement in the BDI.
- Make sure that the BDI is plugged in.

The following steps sequence summarizes the loading and preparation of the BDI, test strips, reagent cartridges and patient samples before starting the test. For detailed information or in case of any problem met at one of the following steps, please refer to the Manual of Use of the BDI.

1. Switch ON the BDI and wait a few seconds until the date and time are displayed on the touch screen.
2. Confirm the correct Date and Time by pressing **V** on the touch screen (in case of first use or for reset, refer to the manual of use of the BDI) → "***Initialize?***" is displayed on the screen.
3. Confirm Initialization by pressing **V** on the touch screen → the horizontal arm of the instrument automatically moves forward to a central (stand-by) position → "***Load strips (24)***" is displayed on the screen.
4. (Do not set nor confirm the number of strips at this step). Remove the clamp from its emplacement on the arm by gently pulling it upwards and load the strips to be tested: handle the clamp with numbered side facing up (open position) and insert the strips, also with numbered (reactive) side facing up, by slipping the upper plastic part (tongue) into the dedicated holes of the clamp. Apply a gentle pressure to ensure that the plastic tongue has reached the bottom end of the hole.
   Notes:
   Always start loading into position 1 of the clamp (left side) and do not leave empty spaces between the strips!
   After complete loading, check visually the vertical, horizontal and lateral alignment of the strips. Any obvious misalignment should be corrected by unloading the strip(s) from the clamp and loading them again.
   Be careful: any plastic bits remaining after breaking apart the individual strip holders may hinder the processing on the instrument and/or the reading with the BlueDiver scanner; please remove them with scissors.
5. Replace the clamp in its emplacement on the arm by gently pushing it downwards.
6. Set the number of loaded strips using the up and down arrows on the touch screen.
7. Confirm the number of loaded strips by pressing **V** on the touch screen → the horizontal arm automatically moves backward to stand over the alignment holes of the cartridge holder → "***Check alignment***" is displayed on the screen.
8. Use the "JOG" function on the screen to check the correct alignment of the strips: maintain a gentle pressure on the down arrow on the touch screen until the bottom of the strips enters into the alignment holes of the cartridge holder. If correctly aligned, the strips will not touch the outlines of the holes.
   Note:
   In case of misalignment (contact of the strips with the cartridge holder), please refer to the Manual of Use of the BDI.
9. Confirm the correct alignment of the strips by pressing **V** on the touch screen → the BDI lowers the strips completely into the alignment holes and reads the barcodes of the strips → after complete barcode reading, "***Load reagent***" is displayed on the touch screen.
   Note:
   In case of failure to read one or more strip barcode(s) (flashing LED at the unread position), please refer to the Manual of Use of the BDI.
10. Unseal the reagent cartridges and insert them under their respective strips in the dedicated notches of the cartridge holder.
11. Confirm complete loading by pressing **V** on the touch screen → the BDI reads the barcodes of the cartridges and checks the correct association with the strips → after complete barcode reading, the number of strips (validated strips/cartridges associations) is displayed on the screen.

Note:
In case of failure to read one or more cartridge barcode(s), or in case of detection of a wrong strip/cartridge association (flashing LED at the corresponding position), please refer to the Manual of Use of the BDI.

12. Confirm the number of strips by pressing **V** on the touch screen → the protocol number identified on the barcodes is displayed on the screen (***Protocol ID xx***).
13. Confirm the protocol number by pressing **V** on the touch screen → "***Please close cover.***" is displayed on the screen.
14. Close the cover of the BDI and confirm closing by pressing **V** on the touch screen → the BDI proceeds to a first washing (pre-treatment) step by incubating the strips into the 2nd well of the cartridges (processing time: 1 minute) → At the end of the wetting step, "***Please open cover.***" is displayed on the screen.
15. Open the cover of the BDI and confirm opening by pressing **V** on the touch screen → the horizontal arm automatically moves forward to the front of the instrument and swings the strips to an oblique position → "***Dry strips***" is displayed on the screen.
16. Dry the strips by gently applying absorbent paper onto the basis of the bottom small cavity (sample loading hole).
17. Confirm drying by pressing **V** on the touch screen → "***Apply samples***" is displayed on the screen.
18. Apply samples by pipetting 10 µl of patient serum into the bottom sample loading holes of the strips.
Note:
If preferred the 10µl of the serum can be directly pipetted into the Diluent Buffer ("Well I") of the cartridge. This operation can be done at any time from opening of the cartridges (see 9.1.10).
19. Confirm samples' loading by pressing **V** on the touch screen → "***Please close cover***" is displayed on the screen.
Close the cover of the BDI and confirm closing by pressing **V** on the touch screen → the BDI starts the test automatically by proceeding the steps sequence of the protocol (see 9.3). After completion of the process, the clamp moves to a central (stand-by) position in the BDI to allow easy manipulation of the clamp. The instrument beeps and "***Finished test***" is displayed on the screen.
20. Gently apply absorbent paper onto the basis of the strips to remove liquid from the bottom small cavity (sample loading hole) and allow the strips to dry for 30 minutes before interpretation of the results. The interpretation has to be done in the 24 hours following the test processing. In case of use of the BlueDiver scanner for help of results interpretation, <u>please leave the processed strips attached to the clamp</u>

TEST DATA REGISTRATION
The test protocol can be downloaded by pressing the USB stick symbol and following the indications on the screen (Insert USB → Writing USB → Remove USB). This step is not obligatory but is highly recommended for traceability and regulatory matters.

9.2 Test preparation on BDI II
Before any usage of the BDI II, please refer to the manual of use supplied with the instrument.
- Allow all kit components to equilibrate at room temperature (+18°C to +25°C) before use.
- All the preparatory steps requiring the operator's intervention are clearly indicated in the BDI's user interface. It is the instrument which indicates the number and type of tests to be run according to the indications made by the operator at the sample identification stage.
The operator is guided by the user interface from the insertion of the samples and kits to be tested onwards, to the final interpretation of the results.
- Remember to open the reagent cartridges before inserting them into the holder.

9.3 Test Processing (Protocol 02 for all Alphadia immunodot kits on BDI I and BDI II):

Step	Description	Processing time
01.	The strips are incubated into the 1st well of the cartridge (*Diluent Buffer*). Upon contact with the liquid in the wells and agitation, the pre-loaded patients' samples (see 9.1.18) are released from the small cavity at the bottom of the strips and are diluted in the buffer.	30 min
02.	The clamp moves forwards and the strips are incubated into the 2nd well of the cartridge (*Wash Buffer*)	2 min
03.	The clamp moves forwards and the strips are incubated into the 3rd well of the cartridge (*Wash Buffer*)	2 min
04.	The clamp moves forwards and the strips are incubated into the 6th well of the cartridge (*Wash Buffer*)	2 min
05.	The clamp moves backwards and the strips are incubated into the 5th well of the cartridge (*Conjugate*)	10 min
06.	The clamp moves backwards and the strips are incubated into the 4th well of the cartridge (*Wash Buffer*)	2 min
07.	The clamp moves backwards and the strips are incubated into the 3rd well of the cartridge (*Wash Buffer*)	2 min
08.	The clamp moves backwards and the strips are incubated into the 2nd well of the cartridge (*Wash Buffer*)	2 min
09.	The clamp moves forwards and the strips are incubated into the 7th well of the cartridge (*Substrate*)	10 min
10.	The clamp moves backwards and the strips are incubated into the 6th well of the cartridge (*Wash Buffer*)	2 min

10. INTERPRETATION OF RESULTS
For *BDI* I: a visual (qualitative) interpretation of the results is possible, however the use of the BlueDiver scanner and the Dr Dot software is generally recommended for more precision and for a semi-quantitative interpretation.
For *BDI* II: the semi-quantitative interpretation of the results is systematically performed at the end of the test.
IMPORTANT NOTICE: The positivity of all parameters of this kit is NOT possible and in such a case the test is not valid. An additional test has to be performed to establish the diagnosis.

10.1. Qualitative Interpretation
1. Remove the clamp from the BDI and unload the strips from the clamp.
2. Place the strips with reactive face up onto the marked fields of the Diver Interpretation Template provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
3. The first upper dot (Positive Control) must be positive for all patients.
Only a clearly coloured Positive Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded. If the first upper dot is not coloured, the test has failed and cannot be interpreted further.
4. Compare the **specific antigen dots** to the **Negative Control Dot** (which always is the last bottom dot). The colour intensity of the Antigen dots is directly proportional to the titer of the specific antibody in the patient sample.
The colour intensity of the Negative Control Dot may vary depending on the sample characteristics. If the sample is free of interfering substances the Negative Control Dot may be even close to uncoloured. In contrast, a highly coloured Negative Control Dot indicates a high rate of unspecific binding in the sample.

***POSITIVE RESULT*:**
A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen dot is higher than the intensity of the Negative Control Dot.
***<u>NEGATIVE RESULT</u>*:**

A sample is negative for a specific antibody if the colour intensity of corresponding Antigen dot is lower than or equal to the intensity of the Negative Control Dot.

NB: A weak coloration of an antigen dot, when close to the colour intensity of the Negative Control Dot may be difficult to differentiate by visual inspection only. In such cases, it is recommended to use Dr Dot software and scanning system (see 10.2) and refer to the corresponding instructions for more accurate interpretation.

10.2 Results semi-quantification: use of Dr Dot Software and Scanning system

The BlueDiver scanner is an especially designed system for the reading of Alphadia immunodot strips. It allows precise and easy insertion of test strips.

The Dr Dot software allows a semi-quantification of results. Based on the image obtained, each result will be quantified in grayscale value and compared to the reference scale integrated in the BlueDiver scanner cover.

These grayscale intensities will be transformed and displayed in arbitrary units (AU, from 0 to 100) based on the intensities of the controls (RC and CO, see point 9) present on the strip, according to the following conversion formula:

$$Result\ of\ antigen\ X\ (AU) = \frac{Grayscale\ intensity\ of\ antigen\ X - Grayscale\ intensity\ of\ CO}{Grayscale\ intensity\ of\ RC - Grayscale\ intensity\ of\ CO} * 100$$

1. Remove the clamp from the BDI. Leave the processed strips attached to the clamp. Be careful: the strips have to be completely dry before starting the scanning step!
2. Insert the clamp, the reactive side of the strips facing down, into the dedicated emplacement in the cover of the BlueDiver scanner.
3. Start scanning the strips using the Dr Dot software.
4. The software semi-quantifies the results, and the interpretation of the obtained values is as follows

Dr Dot arbitrary unit (AU)	Interpretation
< 5	Negative
5 – 10	Equivocal
>10	Positive

For detailed information about the BlueDiver scanner and Dr Dot software please refer to the Manual of Use of your Dr Dot software

10.3 Important recommendations for the interpretation of results

1. Alphadia's kits constitute a diagnostic aid. In consequence, no diagnosis can be established solely on the basis of our kits. The results should always be interpreted by taking into account the clinical examination, the patient's history and the results obtained by other methods.
   No single technique can rule out the possibility of false positive or false negative results. With this in mind, an indirect immunofluorescence test should, as far as possible, be carried out prior to the use of the present kit (immunofluorescence being recognized as a reference method in autoimmunity).
2. The intensity of a result is not necessarily related to the degree of intensity of the disease, but rather to the level of antibodies detected.
3. Low titers of auto-antibodies may occur in healthy patients. For this reason, low positive results (close to the CO, between 5 and 10 DrDot AU), although valid, should be considered equivocal. In such cases, the retesting of the patient, preferably by using a new sample, is recommended. If the result remains equivocal on retesting, other diagnostic tests and/or clinical information should be used to help determine the autoimmune status of the patient.
4. For various reasons, and under certain conditions, the kit may show a defect in performance (see 10.4 *Troubleshooting*). In such cases, the results are not valid and cannot be interpreted. It is recommended to repeat the test. If the error persists, please contact your distributor.
5. The intensity of the results may decrease when the device is used at the end of its life. However, the performance of the kit is not affected (detection of positives and negatives) under normal conditions of use and storage.
6. Sequential sampling (at different dates) of an autoimmune patient can sometimes lead to different results from one sample to another. This difference can have several reasons: the patient's treatment, the evolution of the disease, or a seroconversion. In the specific case of seroconversion, the result can be positive for an auto-antibody in an early sampling of the patient, and become positive for another auto-antibody in a later sampling of the same patient.

10.4 Troubleshooting

Problem	Possible causes + Action		
Discrepancy of results as compared to a reference method	- Use	- incorrect pipetting of serum - incorrect volume dispensed - Use of two different samples of the same patient (see point 10.3.6) or wrong sample handling/storage between tests - erroneous visual interpretation - erroneous DrDot reading ➔ repeat the test	
	- Material	- Interfering substance in the sample - Sample is a pool of different human sera ➔ repeat the test and confirm by other methods	
	- Method	- intrinsic performance of the kit (see 11.2 *Analytical sensitivity and specificity*) - expired kit - stability problem	
	Please contact your distributor for any further technical support requests.		
Different results in the same batch or between several batches	- Use	- incorrect pipetting of serum - incorrect volume dispensed - erroneous visual interpretation or - bad DrDot reading ➔ repeat the test	

	- Method	- intrinsic performance of the kit (see 11.1 *Repeatability and Reproducibility*)	
Contamination between neighbouring strips	- Use	- incorrect pipetting of serum ➔ repeat the test - incorrect verticality of the strips on the BDI ➔ correct the verticality **Please contact your distributor for any further technical support requests.**	
RC absent or weak	- Use	- Serum not pipetted at all ➔ repeat the test - Patient with immunoglobulin deficiency ➔ repeat the test to confirm patient status - Damaged reagents ➔ check the integrity of the reagents ➔ contact your supplier if you suspect a problem - Spot not on the strip ➔ count the number of dots on the strip; if not correct, contact your supplier	
CO absent		- damaged reagents ➔ check the integrity of the reagents, contact your dealer if you suspect a problem - Spot absent from the strip ➔ count the number of spots present on the strip, contact your distributor in case of incorrect number	
Non-specific bindings / high background / high CO value		Suspected presence of a contaminant or an interfering substance in the patient sample ➔ repeat the test and confirm through another method **Please contact your distributor for any further technical support requests.**	
Barcode of the strips or cartridges cannot be read		Manufacturing problem, please contact your distributor	
Kit content incorrect		Manufacturing problem, please contact your distributor	
Positive results for all the biomarkers of the kit		Problem with reagents, please contact your distributor	

NOTE:

The major residual risks of the kit, as given in the risk analysis of the kit at the end of design (after mitigation), are the following:

1) Risk of false results based on a pipetting error (bad serum)
2) Risk of false results based on an interfering substance contained in the sample

11. PERFORMANCES

11.1 Repeatability and Reproducibility

Reference samples were tested for each antibody in successive statistically representative series, both in the same test as in different tests and between different batches in order to calculate the intra-assay, inter-assay and inter-lot variations respectively. In all the cases, the variations in colour intensity, when semi-quantified on the Dr Dot software, were within the following expected limits:

CV ≤ 10% for intra-assay runs
CV ≤ 15% for inter-assay runs
CV ≤ 20% for inter-lot runs

11.2 Analytical sensitivity

Measurement range (semi-quantified results): From 0 AU (negative) to 100 AU (high positive).
Limit of detection: the lowest measured value of the test is 5 AU (considered as equivocal following the interpretation algorithm, see point 10.2)
As not any international standard is available for the auto-antibodies, trueness of measurement and linearity are not applicable on this product.

11.3 Analytical specificity

1. The main known interfering substances were tested on each biomarker of the present kit.
   For each concentration of interfering substance tested, the difference between the result of the sample without the interfering substance and the result obtained in the presence of the interfering substance did not exceed 15%.

Interfering substance	Maximum Concentration	Intermediate Concentration	Minimum Concentration	Difference <15%
Bilirubin	100 mg/dL	50 mg/dL	25 mg/dL	Yes
Haemoglobin	200 mg/dL	100 mg/dL	50 mg/dL	Yes
Cholesterol	224.3 mg/dL	112 mg/dL	56 mg/dL	Yes
Rheumatoid factor IgM	~500IU/ml	~300IU/ml	~100IU/ml	Yes

Note: It is impossible to test all the possible interfering substances described in the literature. Other interferences, amongst others drug-induced interferences, are possible.

2. The high analytical specificity of the test is guaranteed by the quality of the antigen used. This kit detects IgG antibodies against SSA/Ro 60kD, SSB, Scl-70, Mi-2, Ku, TIF1-γ, SAE1/2, NXP-2, RNA Polymerase III, DFS-70, Sm/RNP and Sm. No cross reactions with other autoantibodies have been found.

11.4

Clinical sensitivity and specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) were assayed following the test instructions. Sensitivity and Specificity were calculated from the results generated by the Dr Dot software. A detailed clinical report is available upon request.

SSA/Ro 60kD

+	-
True positive 95	False positive 1
False negative 2	True negative 181

Sensitivity $\frac{95}{97}$ = 98 %

Specificity $\frac{181}{182}$ = 99 %

SSB

+	-
True positive 63	False positive 1
False negative 1	True negative 200

Sensitivity $\frac{63}{64}$ = 98 %

Specificity $\frac{200}{201}$ = 99 %

Scl-70

+	-
True positive 13	False positive 0
False negative 0	True negative 254

Sensitivity $\frac{13}{13}$ = > 99 %

Specificity $\frac{254}{254}$ = > 99 %

Mi-2

+	-
True positive 2	False positive 0
False negative 0	True negative 68

Sensitivity $\frac{2}{2}$ = > 99 %

Specificity $\frac{68}{68}$ = > 99 %

Ku

+	-
True positive 2	False positive 0
False negative 0	True negative 38

Sensitivity $\frac{2}{2}$ = > 99 %

Specificity $\frac{38}{38}$ = > 99 %

TIF1-γ

+	-
True positive 1	False positive 0
False negative 0	True negative 39

Sensitivity $\frac{1}{1}$ = > 99 %

Specificity $\frac{39}{39}$ = > 99 %

SAE 1/2

+	-
True positive 1	False positive 0
False negative 0	True negative 39

Sensitivity $\frac{1}{1}$ = > 99 %

Specificity $\frac{39}{39}$ = > 99 %

NXP-2

+	-
True positive 1	False positive 0
False negative 0	True negative 39

Sensitivity $\frac{1}{1}$ = > 99 %

Specificity $\frac{39}{39}$ = > 99 %

RNA Polymerase III

+	-
True positive 2	False positive 0
False negative 0	True negative 38

Sensitivity $\frac{2}{2}$ = > 99 %

Specificity $\frac{38}{38}$ = > 99 %

DFS-70

+	-
True positive 2	False positive 0
False negative 0	True negative 44

Sensitivity $\frac{2}{2}$ = > 99 %

Specificity $\frac{44}{44}$ = > 99 %

Sm/RNP

+	-
True positive 41	False positive 2
False negative 2	True negative 218

Sensitivity $\frac{41}{43}$ = 95 %

Specificity $\frac{218}{220}$ = 98 %

Sm

+	-
True positive 26	False positive 1
False negative 0	True negative 239

Sensitivity $\frac{26}{26}$ = > 99 %

Specificity $\frac{239}{240}$ = 99 %

Note: Sensitivity and specificity values of 100 % are strictly related to sample cohorts used in clinical evaluations. In theory, a diagnostic kit shouldn't be considered to be 100% sensitive or specific (at least > 99%).

11.5 Auto-antibodies diagnostic values

Anti-SSA/Ro 60kD	Diagnostic marker and classification criterion for Sjögren's Syndrome (SjS). By EIA: Found in 96% of patients with primary SjS, Found in 80% of patients with secondary SjS, Found in 25-60% of patients with systemic lupus erythematosus (SLE), Found in 90-100 % of patients with subacute cutaneous lupus erythematosus (SCLE) Found in 90% of patients with neonatal cutaneous lupus erythematosus (NLE) Found more rarely (5-15%) in patients with rheumatoid arthritis (RA) and Found in 9% of patients with systemic sclerosis (SSc).
Anti-SSB	Diagnostic marker for Sjögren's Syndrome (SjS) By EIA: Found in 70% of patients with primary SjS, Found in 50% of patients with secondary SjS Found in 25% of patients with systemic lupus erythematosus (SLE), Found in 80% of patients with subacute cutaneous lupus erythematosus (SCLE) Found in 70% of patients with neonatal cutaneous lupus erythematosus (NLE)
Anti-Scl-70	Diagnostic marker for Systemic Sclerosis (SSc) Diagnostic specificity of 99%, sensibility of 10 % for limited SSc and up to 65% for diffuse SSc.
Anti-Mi-2	Diagnostic marker for idiopathic myositis, with a diagnostic sensitivity of 4-18%. Detectable in 15-31% of patients with adult dermatomyositis, and in 10-15% of those with juvenile dermatomyositis. Prognostic marker for a relatively mild clinical course, but associated with an increased risk of cancer. Detectable in the early stages of myositis development.
Anti-Ku	Found in 23% of patients with "primary" pulmonary hypertension Found in 1.8 à 23% of patients with systemic lupus erythematosus (SLE). Found in 1.2 à 14 % of patients with systemic sclerosis (SSc). Found in 2 à 33% of patients with an overlap syndrome with myositis.
Anti-TIF1 γ	Highly specific for dermatomyositis (DM), found in 17-23% of DM patients. Early diagnostic marker for tumors in older patients with DM. Associated with juvenile dermatomyositis (JDM), detectable in 23-36% of cases). Can disappear during therapy.
Anti-SAE-1/SAE-2	Found in 8 % of cases of adult dermatomyositis.
Anti-NXP2	Highly specific for autoimmune myositis (found in up to 28% of cases) Strong correlation with juvenile dermatomyositis (detected in 33% of the cases).

	Associated with a good response to immuno-suppressive therapy.
Anti-RNA-Polymerase III	Diagnostic specificity of 98-100% for Systemic Sclerosis (SSc)
Anti-DFS70	The highest frequencies of DFS70 antibodies have been reported in patients with Vogt-Koynagi-Harada syndrome (77%), atopic dermatitis (30-71%) and asthma (16%).
	Detected in 5-11% of healthy blood donors.
	The presence of isolated anti-DFS70 antibodies could be used as a biomarker to exclude the diagnosis of systemic autoimmune rheumatic diseases (SARD)
Anti-Sm/RNP	Sm : Diagnostic marker (ACR and SLICC criterion) for systemic lupus erythematosus (SLE) Diagnostic specificity of 99% for systemic lupus erythematosus (SLE) Diagnostic sensitivity of 5-40 % for systemic lupus erythematosus (SLE) RNP 68kD/A/C : Diagnostic criterion of Mixed connective tissue disease (MCTD). Highly specific and extremely sensitive (100%) in the absence of Sm and dsDNA antibodies. Found in 13 à 32 % of patients with systemic lupus erythematosus (SLE), Found in 10 % of patients with systemic sclerosis (SSc)
Anti-Sm	Diagnostic marker (ACR and SLICC criterion) for systemic lupus erythematosus (SLE) Diagnostic specificity of 99% for systemic lupus erythematosus (SLE) Diagnostic sensitivity of 5-40 % for systemic lupus erythematosus (SLE)

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12. TEST LIMITATIONS

1. The results obtained with this confirmatory test are dependent on the intrinsic performance of the kit and must be considered as an aid to the final diagnosis, taking into account the results obtained by a reference technique and the clinical data of the patient.

2. In case of hyper-lipemic samples, it is recommended to centrifuge it before the pipetting of the 10µl of sample, which must be done into the supernatant.