Liver[10] IgG

Order Code: LI10D-24

1. INTENDED USE

BlueDot Liver[10] IgG is an immunodot kit intended for the detection, in human sera only, of IgG autoantibodies against M2/nPDC, M2/OGDC-E2, M2/BCOADC-E2, M2/PDC-E2, gp210, sp100, LKM1, LC1, SLA and F-actin antigens.

This kit is intended to confirm results of 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. Prior training is strongly recommended (please contact your distributor).
It can only be used manually on a platform shaker or in an open automated immunodot processing system, programmed according to the pipetting scheme described in point 9.2.

2. PRINCIPLE OF THE TEST

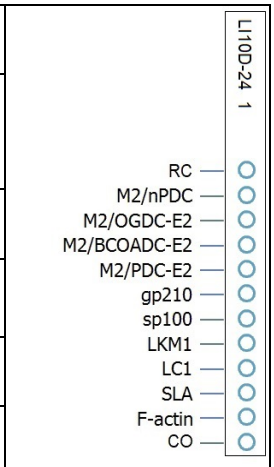
This kit and all its components are intended to be performed exclusively manually.
The test is based on the principle of an Enzyme Immunoassay. The strips are composed of a membrane fixed on a specific plastic support. During the test procedure, the strips are incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing. AP-conjugated goat antibodies against human IgG are added to the strips. This enzyme conjugate binds to the antigen-antibody complexes. After removal of excess conjugate by washing, a substrate solution is added. 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 the 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

TO BE DILUTED:	**(10 x) Wash Solution**	**1 x 40 ml - 10x concentrated** (colourless) *Contains: H_2O • TBS • NaCl • Tween • Preservatives*
READY TO USE:	**Dot strips**	**24 units** *12 dots each:* *1 negative control* **(CO)** *10 antigens* *1 positive control* **(RC)**
	Sample Diluent	**1 x 40 ml** (yellow) *Contains: H_2O • TBS • NaCl • Tween • BSA • Preservatives • Dye*
	Conjugate	**1 x 40 ml** (red) *Contains: H_2O • TBS • NaCl • KCl • $MgCL_2$ • AP-conjugated goat anti-human IgG • Preservatives • Dye*
	Substrate	**1 x 40 ml** (brown bottle, pale yellow solution) *Contains: H_2O • Preservatives • $MgCL_2$ • TBS • NBT • BCIP • NBT Stabilizer*
	Incubation trays	**3 units** *with 8 wells for incubation*



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.d-tek.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 Atención : consultar las instrucciones Atenção : consultar instruções para uso Προσοχή : Συμβουλευτειτε τις οδηλιες χρήσης		For ... uses Per ... dosaggi Für ... Anwendungen Pour ... utilisations Para ... usos Para ... utilização για ... χρήσεις
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	REF	Code Codice Artikelnummer Référence Código Código Κωδικός
2°C 8°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 – 8°C Αποθηκεύστε στους 2 έως 8°C		Manufactured by Fabbricado da Hergestellt von Fabriqué par Fabricado por Fabricado por Κατασκευάζεται από την
LOT	Batch Number Lotto numero Chargennummer Désignation du lot Denominacion de lote Numéro do lote 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)
CE	CE Mark Marcatura CE CE-Kennzeichnung Marquage CE Marca CE Marcação CE μονογράφηση CE		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
TRAY	Incubation tray Vaschetta d'incubazione Inkubationsschale Plaque d'incubation Bandejas de incubación Bandejas de incubação Δίσκοι επώασης	STRIP	Coated strip Strips rivestita Streifen Bandelette Tira Tira Στιγμάτων
DIL	Diluent Diluente campione Verdünnungspuffer Diluant Tampón diluyente Tampão de diluição Ρυθμιστικό διάλυμα αραίωσης	WASH ...x	(... x concentrated) wash buffer Tampone di lavaggio (concentrato... x) (... x konzentrierte) Spülpufferlösung tampon de lavage (... x concentré) (... x concentrado) tampones de lavado (... x concentrado) tampão de lavagem (... x συγκέντρωση) Ρυθμιστικό διάλυμα πλύσης
CONJ ...	Conjugate ... Coniugato ... Konjugat ... Conjugué ... Conjugado ... Conjugado ... Συζυγές ...	SUB	Substrate Substrato Substrat Substrat Sustrato Substrato Υπόστρωμα

3.2 Antigens used

M2/nPDC	E1, E2, E3 subunits of Pyruvate Dehydrogenase Complex (purified from bovine heart)
M2/OGDC-E2	Subunit E2 of OxoGlutarate Dehydrogenase Complex (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
M2/BCOADC-E2	Subunit E2 of Branched-Chain OxoAcid Dehydrogenase Complex (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
M2/PDC-E2	Subunit E2 of Pyruvate Dehydrogenase Complex (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
gp210	Glycoprotein of the nuclear pore complex (36-amino acid sequence corresponding to the C-terminal cytoplasmic tail of gp210, human, recombinant, expressed in E.coli)
sp100	100 kD protein of the nuclear body (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
LKM1	Cytochrome oxydase P450 2D6 (liver-kidney microsome type I antigen), Full length (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
LC1	Formiminotransferase cyclodeaminase (liver cytosol type I antigen) (recombinant, human, expressed in Baculovirus-infected Sf9 cells)
SLA	Soluble Liver Antigen (recombinant, human, expressed in E.coli bacterial cells)
F-actin	In-vitro polymerized actin filaments (prepared from purified G-actin (rabbit skeletal muscle))

4. MATERIAL REQUIRED BUT NOT PROVIDED

Platform shaker / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper.

5. STORAGE

The reconstituted wash solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube.
Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.
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 (see MSDS).
   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 reagent vials 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. D-tek 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. D-tek'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:
The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This blue coloration disappears during the first step of the incubation. During incubation with the wash solution, a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure.
During the procedure, agitation of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. A Rocking platform is the shaker of choice. Be sure to adjust the movement of the shaker in such a way that no spilling of solutions or cross-contamination between the wells can occur.
After each filling of the wells with solution, agitate manually the incubation tray until the strips are completely immersed in order to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip) on the upper part of the strip (plastic label zone).
Avoid touching the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run **at room temperature (18-25°C)**.

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*).

9.1 Reagents preparation

1. Allow all components to equilibrate at room temperature **(18-25°C)** before use.
2. **Dilute** the concentrated **wash solution 10x** with **distilled water.**
   Prepare 15 ml diluted wash solution per strip tested
   Example: 1,5 ml concentrated wash solution + 13,5 ml distilled water for one strip.
   Do not substitute reagents or mix strips with different batch numbers this may lead to variations in the results.

9.2 Pipetting flow chart

1. **Place** one **strip** per patient into the wells, blue dots **facing up**.
2. Add **2 ml diluted wash solution** per well. **Incubate** (shake) **for 10 min.**
   Upon correct incubation, the blue coloration of the dots completely disappears.
   If not prolong the procedure until the colour of the dots fades completely.
3. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
4. Add **1,5 ml sample diluent** per well.
5. Add **10 µl patient sample** per well. **Incubate** (shake) **for 30 min.**
   Avoid touching the membrane with the pipette tip. Preferentially dispense the sample into the solution over the upper part of the strip (plastic label zone).
   Note: Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1,5 ml sample diluent + 10 µl patient sample). Mix (Add to the well)
6. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
7. **Wash 3 x 3 minutes** with **1,5 ml diluted wash solution** per well (shake)**.**
   Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper
8. Add **1,5 ml Conjugate** per well. **Incubate** (shake) **for 30 min**.
9. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper
10. **Wash 3 x 3 min.** with **1,5 ml diluted wash solution** (shake)
    Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.
11. Add **1,5 ml Substrate** per well. **Incubate** (shake) **for 10 min.**
12. **Discard** solution from the wells.
    Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
13. **Wash 1 x 3 min.** with **1,5 ml diluted wash solution** per well to stop the reaction.
14. **Collect** the strips from the wells and allow them to dry for 30 minutes on absorbent paper. The interpretation has to be done in the 24 hours following the test processing.

10. INTERPRETATION OF RESULTATS

A visual (qualitative) interpretation of the results of BlueDOT kits is possible, however the use of the BlueScan scanner and the Dr Dot Software is generally recommended for more precision and a semi-quantitative interpretation.

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. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
2. The first upper dot (**Positive Control Dot**) 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.
3. 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.
   ***NEGATIVE RESULT*:**
   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 (material needed: BlueDiver Clamp, empty stripholders)

The BlueScan scanner is an especially designed system for the reading of D-tek 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 BlueScan 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. Prepare a BlueDiver Clamp and load it with as many empty stripholders as there are strips to analyse. Carefully insert a strip into each stripholder, RC showing upwards.
2. Insert the clamp, the reactive side of the strips facing down, into the dedicated emplacement in the cover of the BlueScan 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 BlueScan 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. D-tek'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 a BlueDot 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 Dr Dot 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 Dr Dot 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 Dr Dot 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
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 distributor 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.**
Strips not correctly labelled	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 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%.

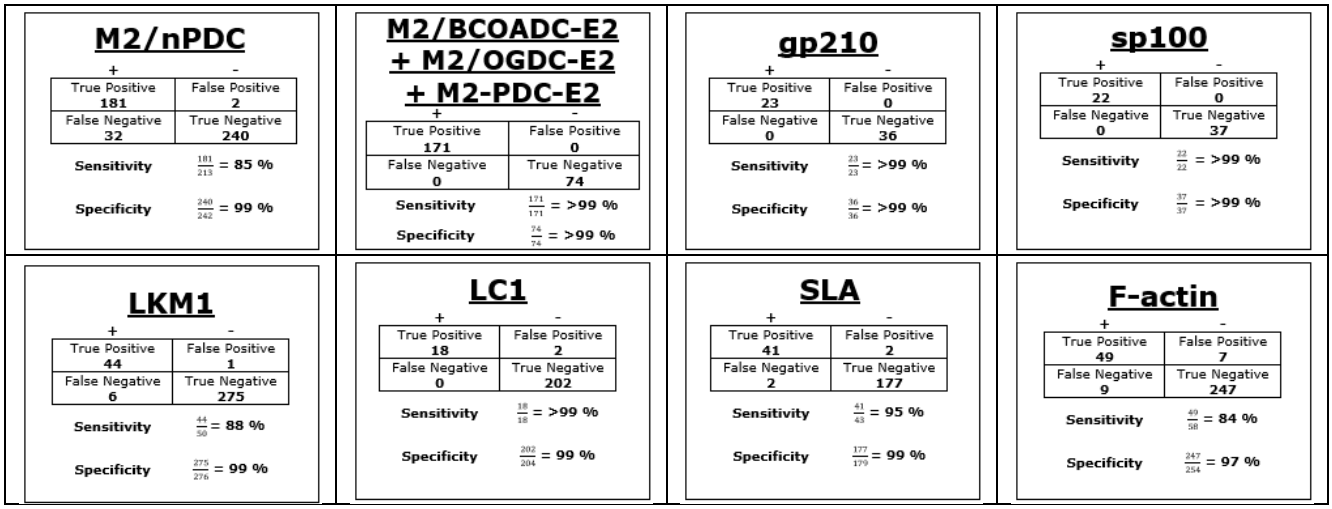
Interfering substance	Maximum Concentration	Intermediate Concentration	Minimum Concentration	Difference <15%
Bilirubin	100 mg/dL	50 mg/dL	25 mg/dL	Yes
Haemoglobin	200 mg/dL	100 mg/dL	50 mg/dL	Yes
Cholesterol	224.3 mg/dL	112 mg/dL	56 mg/dL	Yes
Rheumatoid factor IgM	~500IU/ml	~300IU/ml	~100IU/ml	Yes

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 M2/nPDC, M2/OGDC-E2, M2/BCOADC-E2, M2/PDC-E2, gp210, sp100, LKM1, LC1, SLA and F-actin. 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 obtained by external performance evaluations and EQAs control programs. A detailed clinical report is available upon request.



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-M2/nPDC	Anti-M2/nPDC are marker antibodies of primary biliary cholangitis (PBC) and are detectable in nearly 95% of cases. They count towards the three diagnostic criteria for PBC. Although they are highly specific for PBC, Anti-M2/nPDC can also be detected in patients with chronic inflammatory rheumatic diseases. It is believed that these patients are at an increased risk of developing PBC in addition to the underlying disease. Particularly in Anti-M2/nPDC positive CREST variant of systemic sclerosis there is an increased risk of PBC development (Fregeau et al., 1988; Zurgil et al., 1992). In patients with SLE, the presence of Anti-M2/nPDC is significantly associated with increased aminotransferase (Li et al., 2 006). Anti-M2/nPDC are detectable in 3–6% of autoimmune hepatitis (AIH) type 1 patients. These are most often cases of an AIH/PBC overlap syndrome. AIH/PBC overlap should be considered when the ALP to aminotransferase ratio is less than 1.5, IgG is elevated and the SMA are present with a titer greater than 1:80 (Bowlus & Gershwin, 2014). Anti-M2/nPDC can be predictive. They can appear years before manifestations of PBC. Individuals with persistently high Anti-M2/nPDC antibody levels have a higher risk of developing PBC. Prospective studies have shown that 76% of asymptomatic Anti-M2/nPDC positive patients over a period of observation from 11–24 years are diagnosed with PBC (Metcalf et al., 1996). The prevalence of Anti-M2/nPDC in the first-degree relatives of PBC patients is high (13.1%) (Nakamura et al., 2014). Anti-M2/nPDC titers do not change over time and are not associated with disease severity or progression (Benson et al., 2004). On the other hand some groups have been shown that the Anti-M2/nPDC titer decrease with the treatment with UDCA (Nakamura et al., 2014). Anti-M2/nPDC persist following liver transplantation
Anti-M2/OGDC-E2 Anti-M2/BCOADC-E2 Anti-M2/PDC-E2	AMA-M2 are directed against proteins of the E2 components of the 2-oxoacid dehydrogenase family of enzyme complexes (2-OACD). The central target antigens of these complexes are: • Pyruvate dehydrogenase complex (PDC-E2, PDH-E2) • Branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), sometimes known as branched chain keto acid dehydrogenase (BCKD) • 2-oxoglutarate dehydrogenase complex (OGDC-E2, OADC-E2), also known as α-ketoglutarate dehydrogenase (KGD) • Dihydrolipoamide dehydrogenase (E3)-binding protein (E3BP) E1α subunit of pyruvate dehydrogenase complex (PDC-E1α) Each of these antigens is composed of three subunits (E1, E2, E3), with the immunodominant epitope of each being E2. See Anti-M2/nPDC for diagnostic values.
Anti-gp210	Anti-gp210 antibodies are highly specific for primary biliary cholangitis (PBC) and are detectable by enzyme immunoassay in 10-45% of PBC patients with a specificity of 99.5%. They are rarely or very rarely observed in autoimmune hepatitis, chronic hepatitis B (12.6%), rheumatoid arthritis, polymyositis or Sjögren's syndrome. Their possible predictive value is currently unknown. The titer of anti-gp210 antibodies depends on disease activity or stage progression. Anti-gp210 antibodies are associated with extrahepatic manifestations, as arthritis. They are also considered as prognostic markers of a poor outcome and correlated to a higher risk of hepatic failure. Anti-gp210 antibodies persist after liver transplantation, and are therefore an unsuitable marker of possible disease recurrence.
Anti-sp100	Anti-sp100 antibodies are specific (97%) for primary biliary cholangitis (PBC) with a diagnostic sensitivity of 20-40%. These autoantibodies are found relatively often (48%) in the group of AMA negative patients with a clinically and histologically proven PBC. Anti-sp100 antibodies seem to be associated with urinary tract infections. 74% of PBC patients with urinary tract infections are positive for anti-sp100 antibodies (Bogdanos et al., 2003). In low frequencies, anti-sp100 antibodies have been found in rheumatoid disease (3% in rheumatoid arthritis, up to 10% in systemic lupus erythematosus, in ~5% in systemic sclerosis, in 2% in Sjögren's syndrome).

	Anti-sp100 antibodies persist following liver transplantation, and are therefore an inappropriate marker for possible disease recurrence.
Anti-LKM1	LKM1 antibodies are marker antibodies of autoimmune hepatitis (AIH) type 2 and are included in the diagnostic AIH criteria of the International Autoimmune Hepatitis Group with a sensitivity of 90-95% in (mainly) young patients. They also are part of the simplified criteria of AIH. Patients with AIH type 2 are typically ANA and SMA negative. In primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), LKM1 antibodies are rarely detected. LKM1 antibodies occur in ~50–60% of cases together with LC1 antibodies, however they can also be detected in isolation.
Anti-LC1	LC1 antibodies are detectable in 30–59% of patients with autoimmune hepatitis (AIH) type 2 and are a diagnostic criterion of the International Autoimmune Hepatitis Group. They are predominantly found in children and younger patients and are often associated with LKM1 antibodies. In 50–60% of LKM1 antibody positive patients, LC1 antibodies are also detected as a second marker antibody of AIH type 2. In ~10% of AIH type 2 patients however, LC1 antibodies are the only marker antibodies found. In pediatric AIH type 2, LC1 antibodies are more frequent (59%) than in adults (28.6%).
Anti-SLA	SLA/LP antibodies are highly specific for autoimmune hepatitis (AIH) type 3. Although the definition of AIH type 3 is controversial, as it is clinically and therapeutically not different from AIH type 1, it is clearly a separate entity due to the SLA/LP antibodies. The diagnostic sensitivity has been reported as 19–33%. Their positive predictive value is nearly 100%.
Anti-F-actin	High titers of anti-F-actin are marker antibodies and are accordingly diagnostic criteria of the International Autoimmune Hepatitis Group (three points in the scoring system for a titer >1: 80, two points for 1:80 and one point for 1:40) for autoimmune hepatitis (AIH) type 1. They are also part of the simplified criteria of AIH. They are very often associated with anti-nuclear antibodies (ANA), however they can be isolated positive in ~35% of AIH type 1 patients. The diagnostic sensitivity and specificity for AIH type 1 are ~80% and 96%, respectively. Therefore, a negative anti-F-actin result cannot completely rule out AIH. The titer has a limited correlation with disease activity. Only high titers >1:80 are associated with the disease activity. Neither the antibody titer at diagnosis nor the antibody behavior in the course of the disease are prognostic markers. Note: In children a titer of 1:20 can be diagnostically relevant. Most low titers of anti-F-actin can be found in viral infections, such as infectious mononucleosis, chronic hepatitis C (8–10%), however also in rheumatic diseases, primary biliary cholangitis (PBC) (22%), patients with alcoholic liver disease (3-16%) and neoplastic disease. Their prevalence in healthy individuals is ~5%.

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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 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.

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