Instructions For Use

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DotDiver HepAK 10

REF 5070

Dot immunoassay for the determination of IgG antibodies against liver specific antigens in human serum

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IVD CE Σ 24 x 10 2°C–8°C

1 Intended Purpose

The DotDiver HepAK 10 is a qualitative dot immunoassay for the determination of IgG antibodies against liver specific antigens (M2/nPDC, M2/OGDC-E2, M2/BCOADC-E2, M2/PDC-E2, gp210, sp100, LKM1, LC1, SLA and F-actin) in human serum.

The DotDiver HepAK 10 is intended as an aid in the diagnosis of autoimmune liver diseases in conjunction with other clinical and laboratory findings.

The immunoassay is designed for semi-automated use with the DotDiver instrument.

The immunoassay is designed for professional *in vitro* diagnostic use.

2 Diagnostic Relevance

The group of primary autoimmune liver disease (PAL) comprises autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). The clinical picture of PAL is in most cases not different from other chronic liver diseases. About 15% of all cases with chronic liver diseases show an autoimmune pathogenesis. Therefore, after exclusion of infectious etiology especially by viruses, the determination of different autoantibodies is recommended.

Patients with PSC show intestine related symptoms and with regard to serological diagnosis atypical ANCA patterns in indirect immune-fluorescence on neutrophiles.

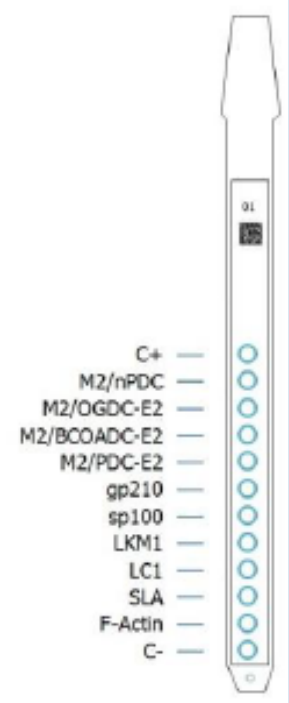
PBC is characterized by occurrence of antibodies to mitochondrial antigens. M2 antibodies react with epitopes of the pyruvate-dehydrogenase complex. PBC is also specified by disease-specific ANA mainly reacting with the nuclear pore protein gp210 and the nuclear body protein sp100. Recent data indicates that these PBC-specific ANA correlate with different types of progression in PBC. One that is represented by positive anti-gp210 antibodies leads to hepatic failure. ANA against gp210 or sp100 proteins were also associated with death or liver transplantation in a group of patients with variable response to ursodeoxycholic acid.

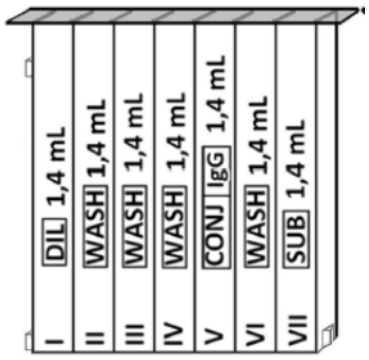
Patients suffering from AIH show a variety of autoantibodies. Due to the appearance of different antibody specificities classification of AIH into different subgroups is discussed. Type I is characterized by the occurrence of antinuclear antibodies (ANA) and antibodies to smooth muscles (ASMA). ASMA recognize antigenic structure formed by polymeric f-Actin. For type II a high prevalence of antibodies to liver and kidney microsomal antigens (LKM) has been described. LKM1 antibodies recognize fusion tri-peptide epitopes of cytochrome P450 2D6. LC1 antibodies are specific for type II hepatitis, too. The respective antigen is formiminotransferase / cyclodeaminase located in the cytosol of liver cells. Patients with type III autoimmune hepatitis exhibit antibodies to the soluble liver antigen (SLA). This type III is not yet fully accepted as independent subgroup for autoimmune hepatitis by the “International Hepatitis Group”.

3 Test Principle

Dot immunoassays are frequently used for the determination of specific antibodies directed against multiple antigens. The test strips are coated with various antigens in consistent intervals. If antibodies are present in the patient´s sample, they bind to the respective antigens. A secondary antibody conjugated with the enzyme alkaline phosphatase detects the generated immune complexes. A colorless substrate is converted into a colored, insoluble product. The signal intensity of the precipitated reaction product is proportional to the antibody activity in the sample.

4 Test Components

Component	Description
Test strips STRIP, 24 (3 x 8) pieces 	24 (3 x 8) breakable test strips on plastic supports (ready-to-use), each strip coated with dots of highly purified - M2/nPDC - M2/OGDC-E2 - M2/BCOADC-E2 - M2/PDC-E2 - gp210 - sp100 - LKM1 - LC1 - SLA - F-actin as well as positive and negative control
Cartridges CART, 24 pieces with 7 compartments	24 cartridges (ready-to-use) sealed with aluminum foil, each compartment contains reagents

	
Sample diluent **DIL**, 1 x 1.4 mL	Colored solution (contains ProClin 300)
Wash buffer **WASH**, 4 x 1.4 mL	Colorless solution (contains ProClin 300)
Conjugate IgG **CONJ**, 1 x 1.4 mL	Colored solution of polyclonal anti-human IgG antibody conjugated to alkaline phosphatase (contains ProClin 300)
Substrate BCIP / NBT **SUB**, 1 x 1.4 mL	Substrate solution of bromo-chloro-indolyl-phosphate and nitroblue tetrazolium (ready-to-use; contains sodium azide)
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipette (10 µL) and disposable pipette tips
- DotDiver2.0 instrument or, alternatively, DotDiver instrument with additional scanner and Blot GAlaxy evaluation software or equivalents
- Adsorbent paper or paper towel

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use of the immunoassay as well as of the DotDiver device must be read carefully. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers. Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

8 Preparation

8.1 Preparation of Reagents

All components including the test strips must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Test Strips

The breakable test strips are provided on plastic supports within sealed aluminium pouches. Unused test strips should always be stored refrigerated and protected from moisture.

8.1.2 Cartridges

The cartridges are ready-to-use. They contain ready-to-use reagents in sufficient amounts for the analysis of one sample. Unused cartridges should always be stored refrigerated.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Procedure

Start the DotDiver device. Place the test strips into the clamp and the cartridges into the corresponding positions of the cartridge holder and insert both into the instrument. Close the cover and follow the indications on the DotDiver screen.

Processing, analysis and evaluation of the immunoassay performed by the DotDiver2.0 are described below (Protocol 02):

Step	Description
1. Piercing	Piercing of well 1 of the cartridges by the instrument
2. Open cover	Open the cover of the instrument
3. Addition of samples	Add 10 µL of undiluted samples into well 1 of the cartridges and application of sample information
4. Close cover	Close the cover of the instrument
5. Piercing	Piercing of all other wells of the cartridges by the instrument
6. Pre-wash	Incubation of strips for 1 min. in well 2 (wash buffer) of the cartridge.
7. Sample Incubation	Incubation of strips for 30 min. in well 1 (dilution buffer) of the cartridge.
8. Wash	Incubation of strips for 2 min. in well 2 (wash buffer) of the cartridge.
9. Wash	Incubation of strips for 2 min. in well 3 (wash buffer) of the cartridge.
10. Wash	Incubation of strips for 2 min. in well 6 (wash buffer) of the cartridge.
11. Conjugate Incubation	Incubation of strips for 10 min. in well 5 (conjugate) of the cartridge.
12. Wash	Incubation of strips for 2 min. in well 4 (wash buffer) of the cartridge.
13. Wash	Incubation of strips for 2 min. in well 3 (wash buffer) of the cartridge.
14. Wash	Incubation of strips for 2 min. in well 2 (wash buffer) of the cartridge.
15. Substrate Incubation	Incubation of strips for 10 min. in well 7 (substrate) of the cartridge.
16. Wash	Incubation of strips for 2 min. in well 6 (wash buffer) of the cartridge.

17. Drying	Automated drying of test strips for about 25 min. by integrated fan.
18. Analysis	Automated imaging of the test strips by integrated camera
19. Evaluation	Automated evaluation of the test strips by integrated software

9.2 Automation

Processing, analysis and evaluation of the immunoassay is performed completely by the DotDiver2.0 device. For semi-automated use of the immunoassay with the DotDiver device, prepare test strips and cartridges according to the instructions for use of the instrument and follow the indications on the DotDiver screen. Processing of the immunoassay must be performed analogous to the described procedure. Processed strips must be dried for approximately 30 min. prior scanning and evaluation of images by use of the Blot GAlaxy evaluation software or equivalent.

10 Test Evaluation

10.1 Metrological Traceability

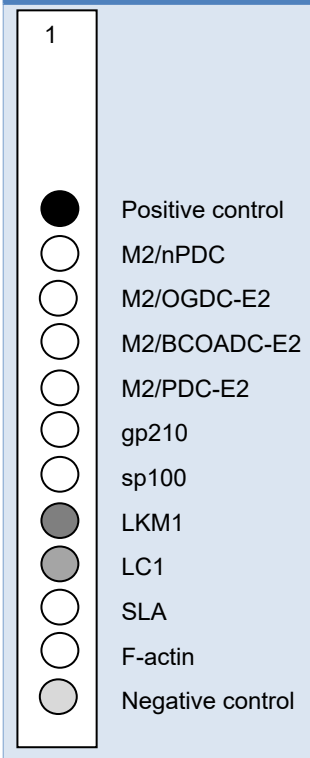
The immunoassay is calibrated using internal reference samples.

10.2 Evaluation

The qualitative evaluation of is performed by comparison of the antigen specific signal intensities of the patient´s sample with the negative control dot on the test strip. The negative control dot serves as a cut off control for qualitative evaluation.

The test strips are coated with antigens. The color intensity is proportional to the specific antibody activity in the sample. A sample is considered to be positive for a specific antigen, if the coloration of the antigen dot shows a more intense coloration than the negative control dot on the test strip. A sample is considered to be negative for a specific antigen, if the coloration of the antigen dot shows a less intense or equal coloration compared to the negative control dot on the test strip.

Example of a processed DotDiver HepAK 10 test strip



10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- Intensity negative control < intensity positive control
- The positive control must be evaluated positive.

The coloration of these controls ensures that the test has been performed correctly. If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities. No differences in the qualitative evaluation have been observed.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

The sensitivity and specificity were assessed by the analysis of characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies).

	Sensitivity	Specificity
M2/nPDC	94 %	94 %
n-PDC and/or OGDC-E2 and/or /BCOADC-E2	> 99 %	98 %
gp210	> 99 %	> 99 %
sp100	> 99 %	> 99 %
LKM1	> 99 %	> 99 %
LC1	> 99 %	> 99 %
SLA	> 99 %	> 99 %
F-actin	97 %	98 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially

hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 0.0015 %) as a preservative, and must not be swallowed or allowed to come into contact with skin or mucosa.

Some of the reagents contain sodium azide (< 0.1 %) as a preservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- Czaja AL. Natural history, clinical features and treatment of autoimmune hepatitis. Semin. Liver Dis. 1987, 4, 1 – 12.
- Bogdanos DP, Invernizzi P, Mackay IR, Vergani D.World J. Autoimmune liver serology: current diagnostic and clinical challenges. Gastroenterol. 2008, 14, 3374 – 87.
- Nakamura M et al. Specificity and Sensitivity of gp210 Autoantibodies detected using an Enzym-Linked Immunosorbent Assay and a Synthetic Polypeptide in the Diagnosis of Primary Biliary Cirrhosis. Hepatology 1996, 23, 1020 – 4.
- Corpechot C et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology. 2008, 48, 871 – 7.
- Johnson PJ, McFarlane IA. Meeting Report: International Autoimmune Hepatitis Group. Hepatology 1993, 18, 998 – 1005.

15 Symbols

	Manufacturer
CE	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Biological risk
	Keep away from sunlight
STRIP	Test strip
CART	Cartridge
DIL	Sample diluent
CONJ	Conjugate
WASH	Wash buffer
SUB	Substrate

16 Changes

Changes in current Instructions for Use	
Current Version	005/09.2022
Summary of Changes	Editorial changes in all sections.