

Revised 21 July 2009 (Vers. 2.0)

Please use only the valid version of the package insert provided with the kit

1 INTENDED USE

HepAK plus Dot is used for the qualitative determination of IgG autoantibodies to M2, LKM1, LC1, SLA and F-actin in human serum or plasma for the differential determination of autoimmune liver diseases.

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 (1). 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 neutrophile. PBC is characterized by occurrence of antibodies to mitochondrial antigens. Antibodies to M2 seem to be the most specific out of 9 described so far for PBC. M2 antibodies react with epitopes of the E2 subunit of the pyruvate-dehydrogenase complex and other ketoacid-dehydrogenase complexes.

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" (2).

1. Czaja AL. Natural history, clinical features and treatment of autoimmune hepatitis. *Semin Liver Dis* (1987) 4: 1 - 12
2. Johnson PJ, McFarlane IA. Meeting Report: International Autoimmune Hepatitis Group. *Hepatology* (1993) 18: 998 - 1005

2 PRINCIPLE OF THE TEST

HepAK plus Dot is a sensitive immunodot assay for the determination of IgG antibodies to M2, LKM1, LC1, SLA and F-actin in human serum or plasma, respectively.

HepAK plus Dot includes 24 numbered test strips with 7 dots fixed on a plastic support: Five test dots are coated with antigens M2, LKM1, LC1, SLA and F-actin. Two test dots serve as positive and negative controls.

Patient sera and strips are incubated in the test tray. During the first incubation antibodies of the patient sample bind to the target antigens immobilized on the solid-phase of the strips. Following an incubation period of 30 min unbound serum components are removed by a washing step.

Bound antibodies react specifically with anti-human-IgG conjugated to alkaline phosphatase. Following an incubation period of 30 min excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

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Alkaline phosphatase converts the colourless substrate solution into a dark purple precipitating dot. After 10 - 12 minutes while shaking the reaction is stopped by a washing step.

Strips are dried for at least 30 min by pressing the reactive side onto absorbent paper. Results are regarded to be positive if the colouration of the test dot is more intense than the colouration of the negative control.

3 PATIENT SAMPLES

3.1 Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can also be used.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

3.2 Preparation before use

Allow all components to reach room temperature prior to use in the assay. Take care to agitate serum samples gently in order to ensure homogeneity.

4 TEST COMPONENTS FOR 24 X 5 DETERMINATIONS

A	Dot strips 24 numbered strips coated with 5 specific antigens: M2 (mitochondrial membrane enzymes: PDC-E2, OGDC-E2 and BCOADC-E2; human recombinant) LKM1 (cytochrome P450 2D6: fusion tri-peptides AA 193-218, AA 261-287, AA 321-351; human recombinant) LC1 (liver cytosolic antigen: formiminotransferase cyclodeaminase; human recombinant) SLA (soluble liver antigen, murine) F-actin (rabbit) - Positive control - Negative control	24 dot strips for the determination of five antibody specificities each
B	Wash buffer 10 X sufficient for 400 ml solution	40 ml concentrate capped blue
C	Sample diluent	40 ml

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	(coloured yellow)	capped yellow
D	Conjugate anti-human-IgG (goat) coupled with alkaline phosphatase (coloured red)	40 ml ready for use capped red
E	Substrate nitroblue tetrazolium with bromo-chloro-indolyl-phosphate (black bottle)	40 ml ready for use capped black
F	Incubation tray for 8 strips	3 x

4.1 Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- graduated cylinders
- distilled or deionised water
- plate shaker
- plastic pincers
- paper towel

4.2 Size and storage

HepAK plus Dot has been designed for 24 x 5 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt all components of the HepAK plus Dot have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

4.3 Preparation before use

Allow all components to reach room temperature prior to use in the assay.

Prepare a sufficient amount of wash solution by diluting the concentrated washing buffer 10 times (1 + 9) with deionised or distilled water. *For example*, dilute 10 ml of the concentrate with 90 ml of deionised or distilled water.

For each test strip 15 ml of washing buffer are requested.

The wash solution prepared is stable at 2 - 8 °C up to 30 days.

All other components are ready for use and so stable until the expiry date.

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Avoid exposure of the substrate solution to light.

After each filling of wells with solution, agitate the incubation tray manually to ensure strips are completely immersed and to remove air bubbles which may be trapped under the strip.

5 ASSAY PROCEDURE

1. Bring all reagents to room temperature (RT) (18-25°C) before use. Mix gently without causing foam.
2. Place the strips with the reactive side up (labels on top) into the respective well. Dispense 2 ml of wash solution (made of B) into the respective wells.
3. Cover tray, incubate **10 min** at RT (18-25°C) while shaking.
4. Discard wash solution. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
5. Add **1.5 ml** sample diluent (C) and **10 µl** patient serum or plasma to the respective wells.
6. Cover tray and incubate **30 min** at RT (18-25°C) while shaking.
7. Decant or aspirate, wash each well **three times three minutes** with **1.5 ml** wash solution (made of B) while shaking. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
8. Add 1.5 ml conjugate (D) to each well
9. Cover tray and incubate **30 min** at RT (18-25°C) while shaking.
10. Decant or aspirate, wash each well **three times three minutes** with **1.5 ml** wash solution (made of B) while shaking. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
11. Add **1.5 ml** of substrate (E) to each well.
12. Cover plate, incubate **10-12 min** while shaking.
13. Decant or aspirate, wash each well **once three minutes** with wash solution (made of B) while shaking to stop the reaction. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
14. Collect the strips from the wells and dry the membranes by pressing briefly the reactive side of the strip onto absorbent paper. After approximately 30 min the strips are to be interpreted.

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6 EVALUATION OF RESULTS

6.1 Evaluation

Results should be interpreted only after strips have been dried for at least 30 minutes.








The **positive control** must be positive in all cases. The colouration of the dot ensures that the test has been run correctly and the kit components are not degraded. If the positive control dot shows no colouration the results cannot be interpreted.

The **negative control** demonstrates the extent of non-specific antibody binding of the sample in the test. The colouration of the dot corresponds to the minimal intensity above which a sample is considered positive.

The test dots are coated with autoantigens and detect specific antibody binding of the sample in the test. The colour intensity of the test dot depends on the titer of specific antibody binding in the sample.

The patient sample is positive concerning a certain antibody if the test dot colouration is stronger (more intense) than the negative control.

6.2 Test example

1	HepAK plus Dot result:	
	- Positive control	positive
	- M2	
	- LKM1	
	- LC1	
	- SLA	positive
	- F-actin	
	- Negative control	

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Positive result:

A sample is considered to be positive for autoantibodies to M2, LKM1, LC1, SLA or F-actin if the colouration of the test dot is more intense than the colouration of the negative control dot.

The colour intensity of the negative dot depends on the test conditions (e.g. incubation times, temperature, washing efficiency) and on the composition of each individual sample. It might be uncoloured even if the test has been run in optimal conditions.

Negative result:

A sample is considered to be negative for autoantibodies to M2, LKM1, LC1, SLA or F-actin if the colouration of the test dot is less intense than the colouration of the negative control dot.

6.3 Limitations of Method

Healthy individuals should be tested negative by the HepAK plus Dot. However, M2, LKM1, LC1, SLA or F-actin autoantibody positive apparently healthy individuals do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

7 CHARACTERISTIC ASSAY DATA

7.1 Specificity and Sensitivity

Clinically defined populations (confirmed positive with disease specific reference methodologies) have been used for checking the sensitivity. Specificity was checked with control groups that embrace a normal healthy population as well as clinically defined control groups.

Sensitivity:

M2, LKM1, LC1, SLA, F-actin: > 99

Specificity:

M2, LKM1, LC1, SLA, F-actin: > 99

7.2 Reproducibility

The dot assay is a qualitative test and the precision is evaluated in terms of variation of the visual colour of the test. Three control sera (high, medium, low positive) were assayed for intraassay and interassay imprecision in a statistically relevant repetition.

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7.3 Comparison to reference methods

The evaluation of the kit was performed in collaboration with the Laboratoire d'Immunopathologie du Centre Hospitalier du Luxembourg (CHL, Prof. Dr. R.L. Humbel).

negative sera (n=20): sera of asymptomatic individuals, negative for respective autoantibodies in ELISA, IFA, Western Blot and immunodiffusion

M2 positive sera (n=15): positive in indirect immunofluorescence (IFA) on cryostat sections of rat liver / kidney / stomach and HEP-2 cells as well as in ELISA (CHL, Anti-PDH ELISA)

		ELISA		IFA	
		+	-	+	-
Dot	+	15	0	15	0
	-	0	20	0	20

LKM1 positive sera (n=23): sera of 15 patients with autoimmune hepatitis type 2 (AIH-2) and 8 patients with AIH-2 and associated chronic hepatitis C (HC), all sera positive in IFA on rat liver / kidney sections and in ELISA with recombinant P450-2D6

		ELISA		IFA	
		+	-	+	-
Dot	+	23	0	23	0
	-	0	20	0	20

LC1 positive Sera (n=10): positive in IFA on rat liver sections and in Ouchterlony immunodiffusion (ID) using rat liver extract as antigen

		IFA		ID	
		+	-	+	-
Dot	+	10	0	10	0
	-	0	20	0	20

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SLA positive Sera (n=5): sera from patients with autoimmune hepatitis, positive in ELISA (INOVA, USA) and Western Blot (WB) using rat liver extract

		ELISA		WB	
		+	-	+	-
Dot	+	5	0	5	0
	-	0	20	0	20

F-Actin positive Sera (n=29): smooth muscle antibodies (SMA) positive in IFA on rat liver / kidney / stomach sections

		IFA (SMA)	
		+	-
Dot	+	28	0
	-	1	20

8 INCUBATION SCHEME

1.	Bring all reagents and the requested number of strips to room temperature (18-25°C)	
2.	Place the strips with the reactive side upside in the tray and dispense 2 ml of wash solution (made of B) into the respective wells	
3.	Seal plate and incubate while shaking 10 minutes, room temperature (18-25°C)	
4.	Discard wash solution	
5.	Pipette 1.5 ml sample diluent (C) and 10 µl patient serum or plasma (1 + 150) into each well	
6.	Incubate while shaking 30 minutes, room temperature (18-25°C)	
7.	Decant, wash strips while shaking 3 x 3 minutes with 1.5 ml (made of B)	
8.	Pipette 1.5 ml conjugate (D) in the respective well	
9.	Incubate while shaking 30 minutes, room temperature (18-25°C)	
10.	Decant, wash strips while shaking 3 x 3 minutes with 1.5 ml (made of B)	
11.	Pipette 1.5 ml substrate (E)	
12.	Incubate while shaking 10 - 12 minutes, room temperature (18-25°C)	

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13.	Decant, wash strips to stop reaction while shaking	1 x 3 minutes with 1.5 ml (made of B)
14.	Dry membranes by pressing the strip onto absorbent paper. After approximately 30 min the strips are ready to be interpreted.	

9 SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. DRG and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only. In the United States, this kit is intended for Research Use Only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C prior use in the original shipping container.
- Some of the reagents contain small amounts of bromonitrodioxane (< 0.01 % w/w), methylisothiazolones (< 20 ppm) or sodium azide (< 0.05 %) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

DRG® HepAK plus Dot (DOT-4275)



RUO in the USA

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Distributed by					
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..	

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