



INSTRUCTION MANUAL

REF 4077

February 23, 2007

ENA Dot

- 24x6 determinations -



IVD *In vitro* diagnostic device

Immunodot for the determination of IgG antibodies to nuclear and cytoplasmic antigens in human serum or plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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INTENDED USE

ENA Dot is used for the separate qualitative determination of autoantibodies to nuclear and cytoplasmic antigens [Sm, Sm/RNP, SS-A, SS-B, Jo-1 (histidyl-t-RNA synthetase), and Scl-70 (topoisomerase I)] in human serum or plasma.

Systemic autoimmune diseases (such as systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Sjögren syndrome, dermatomyositis, mixed connective tissue disease) are characterized by the production of a variety of autoantibodies directed against components of the cell nucleus or plasma.

Although significance and pathological relevance of some autoantibodies have not completely understood yet, the detection of autoantibodies plays an important role in the diagnosis of systemic autoimmune diseases.

ENA Dot allows the detection of autoantibodies to extractable nuclear and cytoplasmic antigens.

ENA Dot offers a rapid and handsome opportunity for the determination of the whole autoantibody pattern in systemic autoimmune diseases in one single test. Allowing the analysis of large and small series ENA Dot represents an excellent alternative to other techniques. Employing a specific negative control non specific colouration depending on the test conditions and sample features are eliminated allowing an easy qualitative interpretation of the results.

Tan EM.: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989 44:93-151

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PRINCIPLE of the TEST

ENA Dot is a sensitive immunodot assay for the determination of antibodies to Sm, Sm/RNP, SS-A, SS-B, Jo-1, and Scl-70 in human serum or plasma.

ENA Dot includes 24 numbered test strips with dots each fixed on a plastic support: 6 dots are coated with Sm, Sm/RNP, SS-A, SS-B, Jo-1, and Scl-70 antigens, respectively. 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 autoantigens 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.

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.

PATIENT SAMPLES

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.

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.

Note: *Neat patient samples have to be used for the assay.*

TEST COMPONENTS for 24x6 determinations

A	Dot strips	24
Ag	24 numbered strips coated with specific antigens Sm (16 kDa D protein, bovine) Sm/RNP (68 kDa, A, C, BB', D; bovine) SS-A (60 kDa, human recombinant) SS-B (50 kDa; bovine) Jo-1 (bovine) Scl-70 (bovine)] - Positive control - Negative control	dot strips for the determination of six antibody specificities each
B	Wash buffer	40 ml
BUF WASH	sufficient for 400 ml solution	concentrate capped blue
	10x	
C	Sample diluent	40 ml
DIL	(coloured yellow)	capped yellow
D	Conjugate	40 ml
CONJ	anti-human-IgG (goat) coupled with alkaline phosphatase (coloured red)	ready for use capped red
E	Substrate	40 ml
SOLN NBT/BCIP	nitroblue tetrazolium with bromo-chloro-indolyl-phosphate (black bottle)	ready for use capped black
F	Incubation tray for 8 strips	3 x

Materials required

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

Size and storage

ENA Dot has been designed for 24 x 6 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 ENA 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.

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.

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.

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.

EVALUATION OF RESULTS

Evaluation:

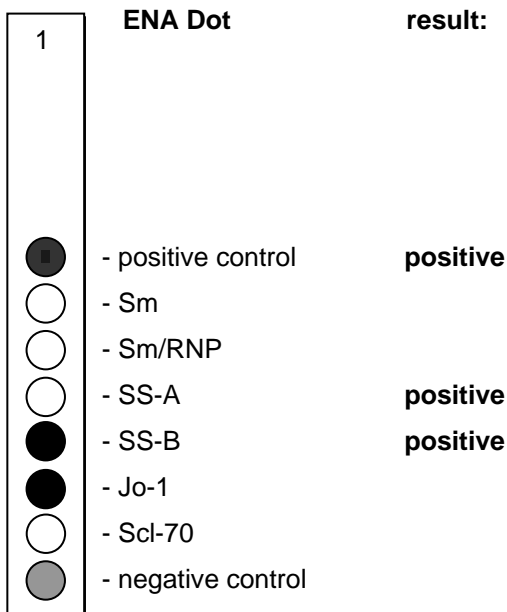
Results should be interpreted only after strips have been dried for at least 30 minutes.

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.

Test example



Positive result:

A sample is considered to be positive for autoantibodies to Sm, Sm/RNP, SS-A, SS-B, Jo-1, and Scl-70 if the colouration of the test dot is more intense than the colouration of the negative control.

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 serum.

Negative result:

A sample is considered to be negative for autoantibodies to Sm, Sm/RNP, SS-A, SS-B, Jo-1, and Scl-70 if the colouration of the test dot is less intense than the colouration of the negative control.

Limitations of Method

Healthy individuals should be tested negative by the ANA Dot. However, Sm, Sm/RNP, SS-A, SS-B, Jo-1, and Scl-70 autoantibody positive apparently healthy persons 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.

CHARACTERISTIC ASSAY DATA

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:

Sm, RNP, SS-A, SS-B, Jo-1, Scl-70 > 99%

Specificity:

Sm, RNP, SS-A, SS-B, Jo-1, Scl-70 > 99%

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.

Comparison to reference methods:

Evaluation of ANA Dot was performed in collaboration with the Laboratory for Immunology of the Central Hospital Luxembourg (Prof. Dr. R.L. Humbel). 306 sera positive for ANA in IFA on HEp-2 cells were tested on ANA Dot in daily routine.

155 positive and 151 negative ANA Dot results were confirmed when testing these sera in immunodiffusion (ID) regarding to Ouchterlony.

Results of anti-ANA reference panel (Laboratory for Immunology of the Central Hospital Luxembourg) in the ANA Dot: 10 anti-Sm +, 10 anti-RNP +, 10 anti-SS-A +, 10 anti-SS-B +, 7 anti-Jo-1 +, 10 anti-Scl-70 +, 50 ANA-IFA negative samples):

	Sm+ (n = 10)	RNP+ (n = 10)	SS-A+ (n = 10)	SS-B+ (n = 10)
Dot +	10 Sm+, Sm/RNP+	10 Sm/RNP+	10	10 SSA+, SSB+
Dot -	0	0	0	0

	Jo-1+ (n = 7)	Scl-70+ (n = 10)	ANA - (n = 50)
Dot +	7	10	0
Dot -	0	0	50

Results of ANA control panel from Center of Disease control (CDC), Atlanta, USA: 10 certified probes were investigated by ANA Dot and by reference ELISAs:

sample	CDC results	ELISA	ANA Dot
CDC 1	homogen	Sm +, Sm/RNP +	Sm +, Sm/RNP+
CDC 2	speckled, La (SS-B)	SS-A +, SS-B +	SS-A +, SS-B +
CDC 3	speckled	Sm +, Sm/RNP +, SS-A +, SS-B +	Sm +, Sm/RNP +, SS-A +, SS-B +
CDC 4	RNP	Sm/RNP +	Sm/RNP +
CDC 5	Sm	Sm +, Sm/RNP +	Sm +, Sm/RNP +
CDC 6	nucleolar	-	-
CDC 7	Ro (SS-A)	SSA +	SSA +
CDC 8	centromer	-	-
CDC 9	Scl-70	Scl-70 +	Scl-70 +
CDC 10	Jo-1	Jo-1 +	Jo-1 +

INCUBATION SCHEME

ENA Dot (4077)

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)
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.

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH 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.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for re-constituted 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.