



# Evaluation of a new ELISA test for the determination of anti-actin antibodies in autoimmune hepatitis

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## BACKGROUND

Autoimmune hepatitis type I (AIH-I) is an inflammatory disease of the liver characterized by a hypergammaglobulinemia and the presence of anti-nuclear (ANA) and anti-smooth muscle antibodies (ASMA). ASMA could be directed against different cytoskeleton proteins such as actin, microtubules or intermediate filaments detected by indirect immunofluorescence assay (IFA) on mouse stomach kidney (MSK) slides. ASMA are also detected in other liver diseases such as primary biliary cirrhosis and viral hepatitis leading to a low predictive value for AIH-I. Although actin is the antigen recognized by ASMA in AIH-I, no methods have been until now standardized to be used in the routine medical laboratory. Therefore, in order to improve the diagnosis of AIH-I, a new ELISA test using actin as solid phase has been developed.

## MATERIALS

A retrospective study was conducted using frozen sera.

Eight groups of sera (n = 235) were studied.

Group 1: 65 ASMA positive sera. Among them, 25 were IFI titer < 1:160 and 40 were IFI titer > 1:320

Group 2: 37 sera from patients with hepatopathies (autoimmune hepatitis, cirrhosis, cholangitis).

Group 3: 33 sera from patients with viral hepatitis (20 HCV and 13 HBV).

Group 4: 20 rheumatoid factor (RF) positive sera between 50 and 100 IU/ml.

Group 5: 10 sera with hypergammaglobulinemia.

Group 6: 20 ANA positive sera.

Group 7: 20 ANCA positive sera.

Group 8: negative control consist of 30 sera from healthy donors.

All sera were stocked at -80 °C and thawed just before analyses were performed.

## METHODS

\* **Indirect immunofluorescence test:** was performed using a commercial Bio-rad (Switzerland) and Inova Diagnostics, (Inc., San Diego, CA; commercialized in Switzerland by Ruwag AG) mouse stomach kidney sections and Hep-2 cells with FITC anti-human Ig conjugate. Sera and conjugate have been incubated for 20 min. at room temperature (RT) under humidity. Sera with a titer < 1:80 were considered as negative, titer = 1:80 equivocal and titer > 1:80 positive.

\* **ELISA technique.** Anti-actin antibodies were measured using the QUANTA Lite™ Actin ELISA (Inova diagnostics, Inc. San Diego, CA; commercialized in Switzerland by Ruwag AG). Pre-diluted high and low controls and diluted (1:101) patient sera were added in duplicate to separate well and incubated for 30 min. at RT, allowing anti-actin antibodies present to bind to the immobilized purified F-actin. Unbound sample was washed away and an enzyme labelled anti-human IgG antibody was added to each well and incubated for 30 min. at RT. After washing, the remaining enzyme activity was measured by adding the TMB chromogenic substrate and the optical density (OD) at 450 nm was measured using a microplate reader. The reactivity for each sample was then calculated by dividing the average OD of the sample by the average OD of the actin ELISA low positive control found on the label. The sera were classified as negative (< 20 Units), weak positive (20 – 30 Units) and moderate to strong positive (> 30 Units).

## RESULTS :

- On the 235 sera tested, 96 (40.8%) were ASMA positive by IFA method and 57 (24.2%) were anti-actin positive using the ELISA method (Fig. 1 and 2, Tab.1).

Fig. 1 anti-actin ( ELISA)

	+	-	
ASMA +	48	48	96 (40,8 %)
(IFA) -	9	130	139
	57	178	235
			(24,2 %)

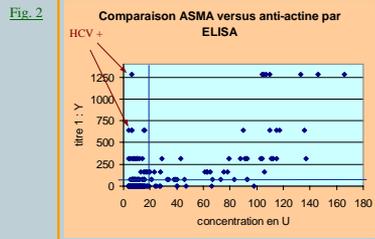


Fig. 3 Autoimmune hepatitis serum samples

	+	-	
ASMA +	20	5	25 (71,4 %)
(IFA) -	4	6	10
	24	11	35
			(68,5 %)

Tab.1: Anti-actin positive / ASMA negative samples (n = 9)

No	Anti-actine (U)	Pathologie
1	40	Hépatite autoimmune
2	28	Hépatite autoimmune
3	66	LED
4	47	CBP - Sjögren - HAI
5	24	Hépatite B
6	40	Waldenström
7	27	LED + syndr. Inflammatoire
8	25	Susp. Churg-Strauss
9	98	?

Tab.2: Anti-actin negative / ASMA positive with AIH (n = 5)

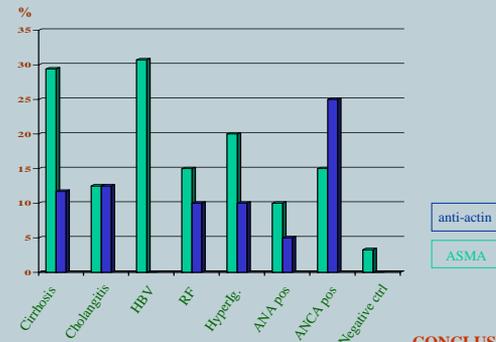
No	Titre ASMA	Titre ANA	Anti-actine (U)	Remarques
1	1:320	1:320 Hom/Mou	4	Anti-vimentine +
2	1:320	négatif	12	HAI I et II
3	1:160	1:160 Hom/Mou	18	HAI - Médic. ?
4	1:80	négatif	9	?
5	1:80	1:320 Mou	9	?

Tab.3: Anti-actin positive / ASMA negative with AIH (n = 4)

No	Titre ANA	Anti-actine (U)	diagnostic
1	1:1280 Hom.	40	HAI + cirrhose
2	?	28	HAI I et II ...
3	1:5120 Hom.	66	Susp. HAI + LED
4	1:1289 Hom.	47	Sjögren, CBP, HAI

Tab.4: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ASMA and anti-actin

	ASMA	Anti-Actin
Sensitivity	71.4 %	68.5 %
Specificity	64.5 %	83.5 %
PPV	26 %	42.1 %
NPV	92.8 %	93.8 %



## CONCLUSIONS :

We propose the use of the anti-actin ELISA test as a confirmatory test to discriminate AIH-I associated positive ASMA from non-specific non AIH-I associated positive ASMA.