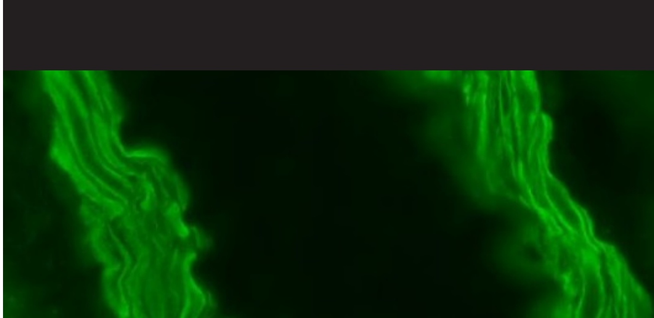

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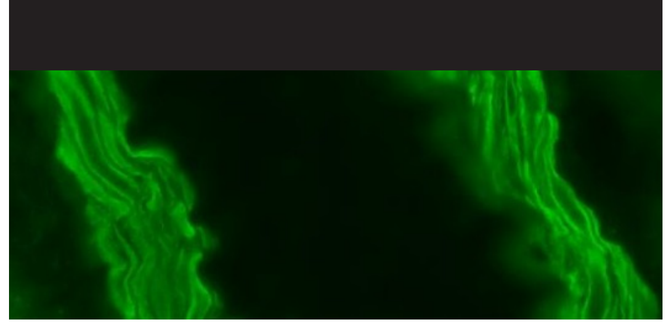

DETECTION OF SMOOTH MUSCLE ANTIBODIES USING THE QUANTA Lite™ ACTIN ELISA

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INTRODUCTION

Anti-actin autoantibodies are the main component of what have been called smooth muscle antibodies (ASMA). These antibodies display specificity towards the actin component of the cytoskeleton[1,2] and are traditionally detected by indirect immunofluorescence utilizing thin sections of rodent liver, kidney or stomach as substrate.[2,3]

Anti-actin antibodies are found in 52-85% of patients with autoimmune hepatitis (AIH) or chronic active hepatitis (CAH) and in 22% of patients with primary biliary cirrhosis (PBC).[3,4,5] Anti-actin antibodies have been reported, usually in low titers, in 3-18% of sera from the general healthy population.[6]

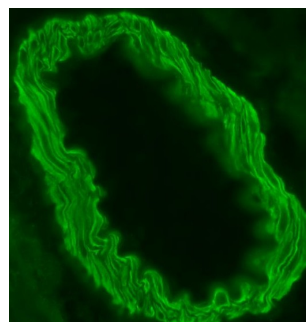
Immunofluorescent procedures for detection of actin or smooth muscle antibodies are subjective, with assay performance dependent on the type of tissue used, conjugate specificity, strength of the microscope system used to read the result as well as the experience of the observer. Newer ELISA methods first reported in the mid 1980's to early 1990's[4,5,7,8] have the potential for superior as well as more standardized and automatable performance. It has been reported that anti-smooth muscle specificity for actin is found primarily in patients with AIH; whereas, in viral infections such as infectious mononucleosis, viral hepatitis, measles and mumps, that anti-smooth muscle reactivity is due to reactivity to non-actin cytoplasmic antigens.[1,2,3,9,10]

Smooth muscle antibodies and anti-nuclear antibodies are the immunoserological hallmarks of type 1 autoimmune hepatitis.[11] Smooth muscle antibodies are directed against several components, the most important of which is actin but immunofluorescence can also detect antibodies to tubulin and intermediate filaments.[12]

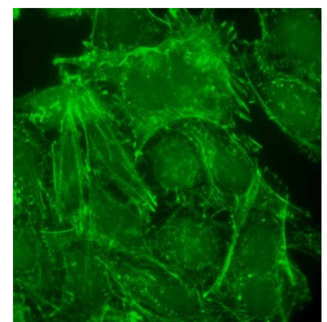
Recent studies have suggested that it is the anti-actin antibodies that have specificity for autoimmune liver disease and they have been advocated as better markers for autoimmune hepatitis compared with anti-smooth muscle.[12,13,14] In fact, the designation "anti-actin hepatitis" has been used to describe this condition.[15] Czaja et al.[14] have shown that anti-actin antibodies were present in 73 of 99 (74%) patients with type 1 autoimmune hepatitis and in 0 of 83 healthy blood donors and in 3-15% of patients with other types of chronic hepatitis. From this same study it was determined that nearly all (99%) of actin positive patients were also smooth muscle antibody positive while 46% of the actin negative group had smooth muscle reactivity. Anti-actin positive patients were more prone to be unresponsive to corticosteroid therapy (16% vs. 4%) and were more prone to suffer liver failure (20% vs. 4%).[14]

PRINCIPLES OF THE PROCEDURE

Purified F-actin is bound to the wells of a polystyrene microwell plate. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any anti-actin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG antibody is added to each well. A second incubation allows the enzyme label to bind to any patient IgG antibodies which have become attached to the microwells. After washing away any unbound enzyme conjugate, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated by comparing the color that develops in the patient wells with the color in the control wells.



Smooth muscle positive on kidney blood vessel



F-Actin positive on HEp-2 cell

NORMAL RANGE

One hundred and fifty random normal serum samples were selected and tested by the QUANTA Lite™ Actin ELISA. This population consisted of 89 males ranging in age from 18 to 70 years (mean 31 years) and 61 females ranging in age from 17 to 74 years (mean 36 years). Only 3 of these 150 samples tested positive (2%). Two samples had a moderate to strong result of 75 and 31 units and the other sample was only weakly positive at 21 units. The positive cutoff for this assay is 20 units. The average value for these 150 normal samples was 7.3 units.

The 3 positive normal samples were tested on the immunofluorescent-based NOVA Lite™ ANA Plus kit. The strongly positive samples (75 and 31 units)reacted with a classic actin positive smooth muscle pattern. These samples produced brightly fluorescing reactions with the smooth muscle of the blood vessels of the kidney, the muscularis of the stomach as well as with the contractile protein cords running between the gastric parietal cells. The more weakly reactive sample (21 units) exhibited an atypical, more reticulin-like staining of the smooth muscle tissue.

CROSS-REACTIVITY

To assess potential cross-reactivity of other autoantibodies with the QUANTA Lite™ Actin ELISA a total of 68 different sera were tested, each containing high levels of various commonly tested autoantibodies. Included in this group of 68 samples were 6 samples from patients with SLE, containing high levels of ANA and double stranded DNA, 2 rheumatoid factor sera, 6 sera positive for endomysial/tissue transglutaminase, 7 sera positive for thyroid peroxidase (TPO). There were 8 ANCA positives, 4 P-ANCA and 4 C-ANCA. One glomerular basement membrane (GBM), 1 lupus anti coagulant positive and 4 each of Sm, RNP, SS-A, SS-B, Scl-70 and Jo-1 were tested. The remaining sera were 4 anticardiolipin, 3 ß2 GPI, 2 prothrombin positives, 1 histone and 3 samples from type I diabetes patients that are ICA positive. Only 4 samples produced a positive result. Two samples were quite strongly positive at 83 and 66 units. When tested by IFA on the NOVA Lite™ ANA Plus (mouse kidney/stomach) kit both samples were found to be smooth muscle antibody positive to a titer of 1:320 with a characteristic Actin pattern. Two samples were weakly positive at 26 units each. Neither of these showed any reactivity with smooth muscle tissue by immunofluorescence. Both samples were ANA/DNA positives.

PRECISION AND REPRODUCIBILITY

The precision and reproducibility of the QUANTA Lite™ Actin ELISA was measured by running six replicates each of a negative (14 units), low positive (25 units) and strongly positive sample (110 units) in six separate assays. Within and between run precision were calculated as follows: The standard deviation and coefficient of variation for each sample are summarized in tables 1a, 1b and 1c.

Table 1a
Within-run

	Negative	Low Positive	High Positive
Mean	14 units	25 units	110 units
SD	1.1	1.4	3.8
%CV	8.0%	5.8%	3.5%

Table 1b
Between-run

	Negative	Low Positive	High Positive
Mean	14 units	25 units	110 units
SD	1.2	1.3	5.3
%CV	8.6%	5.3%	4.2%

Table 1c
Overall

	Negative	Low Positive	High Positive
Mean	14 units	25 units	110 units
SD	1.3	1.4	4.5
%CV	9.7%	5.5%	4.1%

ACTIN POSITIVITY OF 83 ANTI-SMOOTH MUSCLE POSITIVE SAMPLES

Overall 75% of smooth muscle positive samples titered >1:40 were positive by Actin ELISA. At higher titers the agreement was even better. 96.6% of samples >1:160 were actin positive as were 91.3% of samples >1:80.

Table 2

IFA Titer	Number	Number Actin Positive	% Positive
2560	1	1	100%
1280	1	1	100%
640	8	8	100%
320	7	6	86%
160	12	12	100%
80	17	14	82%
40	37	20	54%
Total	**83**	**62**	**75%**

RELATIVE SENSITIVITY AND SPECIFICITY

To determine the relative sensitivity and specificity of the assay, 83 samples submitted for smooth muscle antibody testing from the table above plus another 150 normal samples were tested by both the QUANTA Lite™ Actin ELISA and commercial IFA method. Of the 233 samples, 65 were positive and 136 negative by both methods. Thirty two samples were positive by IFA but not by the ELISA method. Of these 32 samples found IFA positive yet ELISA negative, 11 were from the normal group and 17 of the remaining 21 samples were IFA positive but only at a 1:40 dilution.

Table 3

		QUANTA Lite™ Actin +	QUANTA Lite™ Actin -	
IFA method	+	65	32	Relative Sensitivity = 67%
	_	0	136	Relative Specificity = 100%
				Relative Efficiency = 100%

SUMMARY OF CLINICAL DATA

Sensitivity for AIH may actually be higher than indicated since many of these patients were undergoing immunosuppressive therapy prior to sample being drawn. In addition, many patients had multiple draws. Seropositivity for table 3 was defined as positivity of the earliest bleed. An additional 7 AIH and 1 AIH/PBC and 1 AIH/PSC patient became positive on subsequent samples yielding sensitivities of 75.7% for the AIH group, 88.8% for the AIH/PBC group and 100% for the AIH/PSC group.

Table 4

Patient Group	Number	Number Positive	% Positive
Autoimmune Hepatitis (AIH)	214	155	72.4%
Cryptogenic Hepatitis	9	3	33%
Autoimmune cholangitis	8	5	62.5%
Primary biliary cirrhosis (PBC)	10	3	30%
AIH/PBC overlap	9	7	77.8%
AIH/PSC overlap	3	2	66%
Drug induced hepatitis	1	1	100%
Autoimmune hepatitis type 2	3	3	100%
Viral hepatitis	3	0	0%
Cirrhosis	1	0	0%
Primary sclerosing cholangitis	1	0	0%
Autoantibody positive controls	108	11	10%
Normals	163	3	1.8%

CONCLUSIONS

INOVA Diagnostics Inc. QUANTA Lite™ Actin ELISA for the detection of autoantibodies to the main contractile protein of smooth muscle produces similar results to existing commercial immunofluorescent–based products for identification of these antibodies. The kit shows good clinical sensitivity and specificity. The ELISA format is objective and should be easier to standardize and automate.

REFERENCES

1. Anderson P, et al. Studies on the specificity of smooth-muscle antibodies. *Clin Exp Immunol* 26:57-66, 1976.
2. Bottazzo GF, et al. Classification of smooth muscle autoantibodies detected by immunofluorescence. *J Clin Pathol* 129 129:403-410, 1976.
3. George, J and Shoenfeld Y. *Actin Autoantibodies*, p10-12 in Autoantibodies by JB Peter and Y Shoenfeld eds. Elsevier, Amsterdam, 1996.
4. Bretherton L., et al. Elisa assay for IgG autotantibody to G-actin: comparison of chronic active hepatitis and acute viral hepatitis. *Clin Exp Immunol* 51: 611-616, 1983.
5. Dighiero G, et al. Sera with high levels of anti-smooth muscle and anti mitochondrial antibodies frequently bind to cytoskeletal proteins. *Clin Ept Immunol* 82:52-56, 1990.
6. Fagraeus A and Norberg R. Antiactin antibodies. *Curr Top Microbiol Immunol* 82:1-13, 1978.
7. DeScheerder I, et al. Post-Cardiac injury syndrome and an increased humoral immune response against the major contractile proteins (actin and myosin). *Am J Cardiol* 56:631-633, 1985.
8. Leibovitch L, et al. Anti-actin antibodies in sera from patients with autoimmune liver diseases and patients with carcinomas by ELISA. *Immunol Letters* 48:129-132, 1995.
9. Kurki P, et al. Smooth muscle antibodies of actin and nonactin specificity. *Clin Immuno Immunpathol* 9:443-453, 1978.
10. Toh BH, et al. Viral infections and IgM autoantibody to cytoplasmic intermediate filaments. *Clin Exp Immunol* 37:76-82, 1979.
11. Alvarez F, et al. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. *J of Hepatology* 31:929-938, 1999.
12. Toh BH. Smooth muscle autoantibodies and autoantigens. *Clin Exp Immunol* 38:621-628, 1979.
13. Fusconi M, et al. Anti-actin antibodies: a new test for an old problem? *J Immunol Methods* 130:1-8, 1990.

14. Czaja A, et al. Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. *Hepatology* 24:1068-1073, 1996.

15. Homberg J, et al. Chronic active hepatitis associated with antiliver/kidney microsome antibody type 1: a second type of "autoimmune" hepatitis. *Hepatology* 7:1333-1339, 1987.

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