

## Limitations of Antinuclear Antibody Tests (HEp-2) Are Overcome with the Autoimmune Target Test (IT-1) in Systemic Lupus Erythematosus

To the Editor:

Controversy concerning the antinuclear antibody (ANA)-positive rates in patients with systemic lupus erythematosus (SLE) has persisted, and many efforts in improving the test quality have been made. After reading the article "Abnormal ANA titers are less common than generally assumed in established cases of SLE" by Sjöwall, *et al*<sup>1</sup>, we would like to express our opinion. In this article, the 95th percentile titer (women 1:200, men 1:80) of healthy blood donors was chosen as the standard in determining the cut-off titer for the ANA test in patients with SLE. As a result, the article reported a fluorescent ANA-positive rate in patients with established SLE as 76%, and it focused on its result of a lower positive rate. However, this finding may result in misunderstanding, by leading investigators to accept a much lower ANA-positive rate than what had been previously reported. Further, it has a potential threat in questioning the basic pathogenesis of SLE, which is destined to possess ANA. Looking at the data from this article, the positive rates were low even when the cutoff titers were similar to the conventional screening titers (84% in 1:50, 80% in 1:100), and the positive rate in healthy blood donors at 1:40 dilution level was as high as 45%. Looking at all these aspects, it would be more reasonable to think that the problem lies with the limitation of the HEp-2 cell line in detecting autoantibody in patients with established SLE than to merely conclude that abnormal ANA titers are less common. These limitations have been recognized in many studies testing the HEp-2 cell line. It would also be hard to deny that such limitations have troubled many investigators in interpreting their data.

Table 1. Prevalence of autoimmune target test in healthy blood donors (n = 234).

Result	N (%)	Pattern	Number
Negative	214 (91.5)		
Positive	20 (8.5)	Speckled	8
		MTOC	8
		Diffuse granular	3
		Nucleolar with fine speckled	1
Total	234		20

MTOC: microtubule organizing center (this is a new pattern that had not been reported in HEp-2 cell line<sup>7</sup>).

Table 2. Immunofluorescence patterns and titers of positive autoimmune target test in negative ANA samples.

Titer ≤ 1:160	N	Titer ≥ 1:320	N
MTOC	116	Speckled	22
MTOC + speckled	43	Diffuse granular	20
Diffuse granular	17	MTOC	7
Cytoskeleton	13	MTOC + diffuse granular	3
MTOC + diffuse granular	6	MTOC + speckled	2
GiM	6	Discrete speckled (centromere)	2
Centriole	4		
Others	8	Others	6
Total	213	Total	62

ANA: antinuclear antibody; MTOC: microtubule organizing center; GiM: granules in macrophage (MTOC and GiM are new patterns that had not been reported in HEp-2 cell line<sup>7,8</sup>).

To overcome such limitations of the HEp-2 cell line, a human macrophage cell line, IT-1, was introduced along with HEp-2000 at the 1994 American College of Rheumatology conference<sup>2,3</sup>. It is currently commercialized and has passed Korea Food and Drug Administration inspection as the autoimmune target (AIT) test. This test is currently used in Korea and it is included as part of the quality control program supervised by the Korean Society for Laboratory Medicine<sup>4</sup>. In 2 trials of ANA tests using the IT-1 cell line, on 208 SLE patients in 1999 and on 588 patients in 2007, all were reported to be positive<sup>5,6</sup>. Also, the ANA test results we obtained from 234 healthy blood donors showed only an 8.5% positive result, which was markedly lower than the result reported using the HEp-2 cell line. Further, the positive rate was even lower, at 5.1%, when new autoantibodies that were not reported with the use of the HEp-2 cell line were excluded<sup>7,8</sup>, showing results near the 95th percentile at 1:40 screening titer (Table 1). For cases of antibody against SSA/Ro antigen, suspected as a main cause of ANA-negative lupus, all showed positive results in 67 patients who were confirmed to be positive by the double-immunodiffusion method<sup>2</sup>. Also, when we performed the AIT test on 570 ANA-negative samples (performed with the HEp-2 cell line), discrepancies were seen in 275 samples<sup>9</sup>. Even when we selected cases with titer ≥ 1:320 to exclude any interlaboratory or intertest difference, 62 cases showed positive results, and when we excluded new autoantibodies, 55 (9.6%) cases showed positive results (Table 2).

We would like to point out that the basic reason for the continuing controversy concerning SLE and the autoantibody detection rate lies in the use of the HEp-2 cell line. The evolution from cryostat sections to cell-line investigations surely upgraded the ANA test one step further. However, the fact that the HEp-2 cell line originated from laryngeal carcinoma cell was the "original sin" that HEp-2 could not escape from. In contrast, the IT-1 cell line possesses ideal requirements by being originated from nonmalignant cells and also by being a macrophage that plays a pivotal role in the immune response mechanism. Indeed, authors' data prove all this. If cells were mistakenly chosen in the beginning, there should now be an ANA substrate breakthrough that can lead us out of the HEp-2 trap. This could be the new starting point in resolving our old grudge toward autoantibody testing. In addition, we think that the autoantibody detected in healthy controls should not be considered just a false-positive result, but instead be approached as a preclinical disease state. Although they might currently be free of symptoms, it should be taken into account that autoantibody can be formed many years before the onset of autoimmune disease<sup>10</sup>. Autoantibody detection using the macrophage cell line is expected to help in proving this matter as well.

LA-HE JEARN, MD; DUCK-AN KIM, MD; THINK-YOU KIM, MD,  
Department of Early Arthritis / Laboratory Medicine, The Hospital for  
Rheumatic Diseases, Hanyang University Medical Center, 17  
Haengdang-Dong, Seongdong-Gu, Seoul, 133-792 Republic of Korea.  
Address reprint requests to Prof. T.Y. Kim; E-mail: tykim@hanyang.ac.kr

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