Relative value of commercial kits for ANA testing

A.S. Russell[1], C. Johnston[2]

[1]Department of Medicine and [2]Rheumatic Disease Unit Laboratory, University of Alberta, Edmonton, Alberta, Canada. Anthony S. Russell, F.R.C.P.(C), Professor; Carol Johnston, M.L.T.

Please address correspondence to: Dr. A.S. Russell, 562 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, T6G 2S2 Canada.

ABSTRACT

Aims. *We have tested the relative per-formance of 20 commercial ANA test kits along with that of our own labora-tory to assess whether one was clearly superior.*

Methods. *The sera were drawn from 3 pools that had all been pre-tested in our laboratory: patients with definite SLE; patients with non-connective tis-sue diseases (CTD), but where a posi-tive FANA had been found; and normal blood donors. The tests were used in accordance with the recommendations of the specific supplier but in a routine serology laboratory.*

Results. *Sensitivity and specificity ranged between 38 and 100%. While the negative predictive value of 4 ELISA kits was 100%, and most others were close, the HEp-2 kits were 100% in only 1 case. A positive predictive value of 100% was also seen with 1 kit.*

Conclusion. *Some of the tests are clearly better than others, but the choice may differ depending on the clinical needs, e.g. preference for a good positive or negative predictive value. However, the ELISA kits offered better results than the immunofluores-cent technique. Two of them had sensi-tivity/specificity of > 90%.*

Introduction

The diagnosis of SLE and other con-nective tissue diseases can generally be suspected on clinical grounds, but labo-ratory tests can be helpful both to con-firm – or refute – a diagnosis and at times to give an indication of progno-sis, disease activity, or severity (1). Thus, given an appropriate pre-test probability based on clinical criteria,an antinuclear antibody test could for example be used to confirm a clinical suspicion of SLE. Although texts still suggest it may be negative in 5% to 10% of patients, this reflects the tech-niques of an earlier period where a sub-strate of rat liver sections led to a poor detection, particularly of anti-SSA anti-bodies. With modern techniques it would be very uncommon to maintain a diagnosis of SLE in the face of a nega-tive antinuclear antibody test, although it does sometimes occur, especially with complete remissions.

Positive tests may be found in associa-tion with a variety of other diseases, e.g. rheumatoid arthritis, Hashimoto's thyroiditis, etc., but their presence or absence is of no known clinical rele-vance in most of these disorders (2). They are also found in otherwise totally normal, healthy individuals (3-7), and of course in individuals who coinciden-tally may have osteoarthritis, soft tissue pain, etc. The frequency of such results may range from 5% to 30% of normal sera, depending upon the sensitivity of the tests in a given laboratory. Such tests are often termed "false positives" by analogy with the false positive Wasserman reaction in the absence of previous syphilis infection. They do not represent laboratory error. They may in part reflect an increased sensitivity analogous to that described as "techno-logical drift", i.e. improved technical factors and seen even with more specif-ic antibodies, eg. anti-Sm (8). It has been argued (9) that they nevertheless represent an immunologic disorder, but until that has been demonstrated such tests in the absence of any associated relevant disease are clinically unhelpful results.

It is relatively easy to change the per-centage positive seen in normals either by screening at a higher titre, or by reducing the sensitivity of the test sys-tem (6, 10). Thus, the actual cut-off titre used is relatively meaningless. One needs to know the sensitivity – and specificity – of the test at that titre. Sen-sitivity is relatively easy to determine and to manipulate, specificity less so, indeed we were previously not able to increase the specificity of our antinu-clear antibody tests in a clinically important manner, even when we used as a cut-off a higher titre that was found in only 0.5% of normals (4). Commer-cial antinuclear antibody test kits are usually designed for rapid, easy and standard utilization. We have assessed 20 of these to determine how they per-formed in the context of previously defined clinical specimens, i.e. to assess both positive and negative pre-dictive values.

Methods

All kit providers in the study were

approached with the intent of the study and agreed to participate.

We received kits from suppliers either free, or by purchasing them, usually at a reduced evaluation rate. All manufacturer's kit directions were followed and specifications and recommendations complied with. This pertained to dilutions, reagent handling and storage; in addition all results were interpreted according to manufacturer's directions, e.g. a weak positive as negative. If there was still any doubt, results were determined following specific recommendations from company technical service personnel. Nine standard ELISA kits, two EIA membrane kits and 9 standard HEp-2 kits were incorporated in the study together with our own HEp-2 system included for consistency (4).

The sera were not titred, but were used in the screening dilutions recommended for each test, our own being 1:40 (4). All the indirect immunofluorescence assays (IFA) were also at 1:40. The others ranged from 1:20 (Zeus) to 1:200 (Inno-Lia, Seradyn). Our conjugate is goat IgG anti IgG, A and M. Each kit included its own antibody/conjugates. The multiple companies involved were, in alphabetical order, Alexon Trend (Seradyn Color Spot°), Bio-Rad (Bio-Rad°), Biochem Immunosystems (Biochem Pharma° and Zeus*), ESBE Scientific (Immunoconcepts*), Innogenetics (Inno-Lia°), Intermedico (Innova*,°), Nova Century Scientific (Immco*,*), Pharmacia-Upjohn (Varelisa, old and new versions°,°), Phoenix (Biognostic*,°), Qualisys Diagnostics (DiaMedix° and Bion*), Sanofi Diagnostics Pasteur (Kallestad*,°), Somagen (Helix° and Binding Site*).

Test samples

The samples had all been previously screened by our in house test system and comprised known positives, (26 SLE sera), 20 from Red Cross normal donors, mean age 39.5 years (range 17 – 62) (all showing negative FANA, dsDNA and ENA tests), and 50 sera termed "false positives". These latter

_____

* HEp-2 Kit
° ELISA Kit

sera had tested positive at titers of at least 1/640, but the patients, mean age 44 years (range 18 – 72) showed no signs of any CTD when checked clinically by a rheumatologist (4) and remained free of CTD after 5 years of follow up. All these false positive patients had negative anti-dsDNA and anti-ENA results.

Samples were kept frozen between testing. All samples were consistently used amongst kits. If a kit had limited space, normal samples were excluded to allow all patients samples to be run.

All IFA kits were double read by experienced lab personnel, and all ELISA results were checked to ensure accuracy. Evaluation of results, consisted of calculating specificity, sensitivity and predictive value of positives and negatives in the context of our pool of selected sera.

Results

The previously screened sera from blood donors were all reported as negative by all but one of the kits (J). This single kit had 3 positive tests out of all the sera tested. With the lupus sera the ELISA kits seemed significantly more sensitive (mean of 91%) as used, than the Hep-2 (mean 78%) (Table I). Thus, of the 6 ELISA kits that missed one or

two sera, only 4 sera were involved in total and one serum was missed by 4 kits. A review of these specific patients showed no particular features in common except for inactive disease at that time. Similarly, with the Hep-2 kits generally a relatively small number of sera were being missed. One kit (K) read 17 sera as negative that most other kits read as positive, but apart from this only 6 sera were intermittently read as negative, and only 1 serum was missed by some kits of both ELISA and Hep-2 types. As seen in Table I, four of the ELISA kits had an excellent, 100% sensitivity. Thus, the negative predictive value was 100%, and almost all kits were over 95%. Two kits also had a remarkably good positive predictive value (K + B). One was 100% (K), but here the sensitivity was only 38%, and the negative predictive value was only 81%. Of the immunofluorescent tests, our own Hep-2 test (U-41%) had the poorest positive predictive value (although also with 100% sensitivity). The maximum positive predictive value for any Hep-2 test was 68%, and in this kit the negative predictive value was only 87%.

Discussion

The test for antinuclear antibodies is a

Table I.

|		Sensitivity	Specificity	Positive Predictive Values	Negative Predictive Values
ELISA	Kit A	100	74	59	100
	Kit B	92	94	86	97
	Kit C	92	91	83	96
	Kit D	96	79	63	98
	Kit E	96	73	57	98
	Kit E	96	54	44	97
	Kit G	100	71	57	100
	Kit H	92	67	51	96-
	Kit I	100	67	58	100
	Kit J	100	67	55	100
	Kit K	38	100	100	81
Hep-2	Kit L	81	76	55	91
	Kit M	77	80	59	90
	Kit N	65	89	68	87
	Kit O	58	89	65	85
	Kit P	81	76	55	91
	Kit Q	62	89	67	86
	Kit R	62	87	64	86
	Kit S	92	71	55	96
	Kit T	100	52	46	100
	Kit U	100	46	41	100
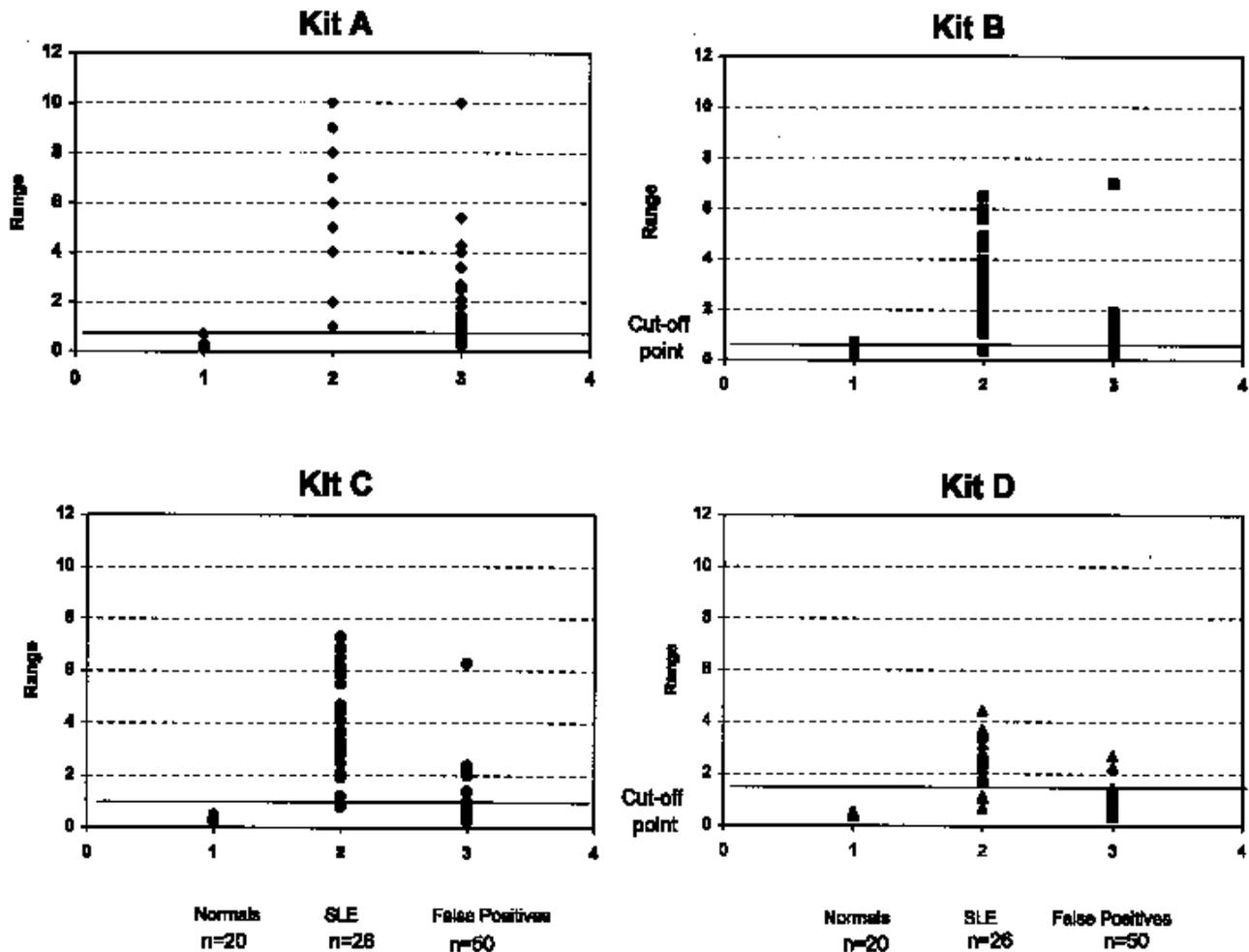
Fig. 1. Performance of four kits in identifying different categories of subjects: normals, SLE patients, and false positives.

commonly ordered clinical test despite efforts to reduce and rationalize its use to confirm – or deny – a clinical impression of a specific disease – usually SLE or a related CTD (4). It is wrongly often used as a screen for any connective tissue disease. When it is being used correctly, as with many tests, the impact of the result on the clinical pre-test probability is important.

Nevertheless, the question commonly asked of test kits is how often they are positive in patients who have a specific disease, and how often they are negative in patients without it, that is, their sensitivity and specificity respectively (12). These are useful measures as they can on the whole be extrapolated to other clinical settings. Unfortunately, the relevant clinical question usually is, given a positive result, what is the probability of the patient having SLE (or a

different CTD), and vice versa for a negative result. This equals the positive (and negative) predictive value of the test, and represents the way a test is used in practice. Here the figures will vary depending upon the admixture of patients tested, that is, the pre-test probability of a given diagnosis.

We selected our groups to represent readily predefined diagnostic categories: patients with SLE (defined according to ACR criteria), patients documented to have no connective tissue disease but with (false) positive ANA tests, and normal blood donors with negative tests. Furthermore, the non-CTD patients not only had a positive FANA documented, but had been subsequently reviewed initially clinically (4) and either by chart or, if there remained uncertainty, by telephone in addition, to ensure that no clinical features to suggest CTD had developed

(13) since the serum was obtained, 5 years earlier.

A clear drawback of this study is that while sensitivity/specificity reflects the properties of the tests themselves, the calculation of post-test probabilities depends on the mix of patients or sera tested. Thus, our selection of 27% of sera to come from SLE patients provides a much higher "pre-test probability" than would be seen in a routine laboratory. Our results therefore provide an enhanced post-test probability for a positive test. Inclusion of large numbers of SLE sera would have been necessary to accurately assess negative predictive values. Nevertheless, the relative performances of the different tests should not be affected by the mix of sera used. Sera from patients with SLE in remission have been known to revert to negative so that a failure to detect 100% positivity in this group is not

479

necessarily a failure of that test kit. All of these patients had established disease. Another drawback is that we excluded other connective tissue diseases and our results cannot therefore be more widely extrapolated.

The results with the pre-screened sera from blood donors were clear, and all but one of the kits had negative results on all sera – the single exception had 3 positive tests out of all the sera tested (J).

Two ELISA kits (B & C) had a remarkably good positive predictive value (as well as a sensitivity and specificity of over 90%). One other (K) had a PPV and specificity of 100%, but here the sensitivity was only 38%. Thus, in this kit, specificity appears as good as is seen with anti-native DNA antibodies or anti-Sm, and sensitivity was not dissimilar. Of the immunofluorescent tests our own HEp-2 (U) test had the poorest positive predictive value (although 100% sensitivity), and a negative predictive value of 100% (this value is artificial and reflects the use of this kit to select the sera for testing). The maximum positive predictive value for any HEp-2 test was 68% and in this kit the negative predictive value was only 87%.

Our study differs from that of Tan et al. (12) partly because we studied a larger range of kits and kit types, but also because we looked at sera from patients with clinical diagnoses rather than antibodies of predefined specificities. Thus, we were able to assess positive and negative predictive values. These, however, will always vary depending on the pre-test likelihood of SLE in that particular population. Our population was designed to represent two major clinically relevant categories of ANA positive individuals – i.e. SLE (for connective tissue diseases) and false positives – i.e. non CTD (in our case usually OA or fibromyalgia). Normals shown to be negative by our own prior testing were included primarily as a reference point. There are too few to give a realistic background prevalence of positive FANA tests. The statistical approximation of the "rule of 3" suggests that if there are no positives out of X specimens, then the frequency of positives is likely to be less than 1 in $X/3$, ($p < 0.05$) (13, 14). Thus, none out of 20 seen, (with all but 1 kit) would represent at the most a 14% prevalence. No test combined 100% sensitivity and specificity, nor 100% positive or negative predictive values. Thus, compromises or combinations must continue to be made, as at present. Griner et al. (11) point out that to confirm a disease one would use the test with highest specificity and to exclude a disease, the test with the highest sensitivity is appropriate. Some tests unfortunately were not especially good from either perspective, and one or two were technically too complex for comfortable routine use.

Thus, as has been previously indicated, the value of the test depends on what it is being used for, but the information we obtained, together with costs, should help choose the best tests for a given purpose.

Acknowledgement

Kit Information

Kit A   Helix Diagnostics

Kit B   Recombi ANA Screen (Pharmacia Upjohn)

Kit C   Varelisa ANA Rheuma Screen (Pharmacia Upjohn)

Kit D   Kallestad Quantafluor (Biorad)

Kit N   Kallestad Hep-2 (Biorad)

Kit O   Immunoconcepts (ESBE Scientific)

Kit Q   Zeus (Biochem Immunosystems Inc.)

Kit R   NCS = Immco (Nova Century Scientific)

Other specific kit information is available on personal request.

References

1. MADDISON PJ: Autoantibody profile. *In* MADDISON PJ, ISENBERG DA, WOO P, GLASS DN (Eds.): *Oxford Textbook of Rheumatology*, 2nd ed., UK, Oxford University Press 1998, Chapter 4.5.
2. COMBE B, DOUGADOS M, GOUPVILLE P *et al.*: Prognostic factors for radiographic damage in early rheumatoid arthritis. *Arthritis Rheum* 2001; 44: 1736-43.
3. SHIEL WC, JASON M: The diagnostic associations of patients with antinuclear antibodies referred to a community rheumatologist. *J Rheumatol* 1989; 16: 782-5.
4. VAILE JH, DYKE L, KHERANI R *et al.*: Is high titre ANA specific for connective tissue disease? *Clin Exp Rheumatol* 2000; 18:433-8.
5. SLATER CA, DAVIS RB, SHMERLING RH: Antinuclear antibody testing: A study of clinical utility. *Archive Int Med* 1996; 156: 1421-5.
6. TAN EM, FELTKAMP TEW, SMOLEN JS *et al.*: Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum* 1997; 40:1601-11.
7. EMLEN W, O'NEILL L: Clinical significance of antinuclear antibodies. Comparison of detection with immunofluorescence and enzyme linked immunosorbent assays. *Arthritis Rheum* 1997; 40: 1612-15.
8. LOCK RJ, UNSWORTH DJ: Antibodies to extractable nuclear antigens. Has technological drift affected clinical interpretation ? *J Clin Pathol* 2001; 54: 187-90.
9. MENARD HA: Antinuclear antibodies, the medium is the message. *Clin Exp Rheumatol* 2000;18: 429-30.
10. NAKAMURA RM, PEEBLES CL, RUBIN RL, MOLDEN DP, TAN EM (Eds.): *Autoantibodies to Nuclear Antigens*. Chicago, American Society of Clinical Pathologists Press,1985.
11. GRINER PF, MAYEWSKI RJ, MUSHLIN AI, GREENLAND P: Selection and interpretation of diagnostic tests and procedures. In: I) Principles of test selection and use; and 2) Principles of test interpretation. *Ann Intern Med* 1981; 94: 559-563 and 565-570.
12. TAN EM, SMOLEN JS, McDOUGAL JS *et al.*: A critical evaluation of enzyme immunoassays for detection of antinuclear antibodies of defined specificities. *Arthritis Rheum* 1999; 42: 455-64.
13. HANLEY JA, LIPPMAN-HAND A: If nothing goes wrong, is everything all right ? *JAMA* 1983; 249: 1743-1743.
14. YAZICI H, BIYIKLI M, van der LINDEN S, SCHOUTEN HJA: The "O patient" design to compare the prevalences of rare diseases. *Rheumatology* 2001; 40: 121-2.