



## Using the SLE-key® Rule-Out Test in Clinical Practice

Donald Massenburg<sup>\*</sup>, Justine Oldenberg, Amanda Sell, Tristan Krause and Alvin F. Wells

Rheumatology and Immunotherapy Center, Franklin, WI, USA

**\*Corresponding author:** Donald Massenburg, Rheumatology and Immunotherapy Center, 4225 Oakwood Park Court, Franklin, WI 53132, USA, Tel: 414-435-0026; E-mail: [don.massenburg@rheumatologyitc.com](mailto:don.massenburg@rheumatologyitc.com)

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### Abstract

**Objectives:** The patient referred to a rheumatology clinic for workup of suspected Systemic Lupus Erythematosus (SLE) often presents a difficult diagnostic problem; until recently, there have been no objective tests validated to rule in or rule out SLE and the diagnosis is based on a list of criteria that may be open to interpretation.

**Methods:** To approach this problem, a serologic rule out test for SLE was developed based on antigen microarray profiling of multiplex antibody reactivities. This SLE-key® test was developed by ImmunArray and, using stored serum samples from recognized academic centers, was validated to rule out SLE with 94% sensitivity, 75% specificity and a negative predictive value (NPV) of 93%. In clinical practice, however, patients are referred one at a time from peripheral clinical units, often with incomplete documentation.

**Results:** We report here the usefulness of the SLE-key® test in aiding the management of a cohort of suspected SLE patients in a large clinical rheumatology practice. We compared the diagnosis and disposition of 163 referrals in whom we used the SLE-key® Rule-Out test to our typical experience with referrals before the test was available. This paper shows that the SLE-key® test provided actionable clinical information and helped us with patient management in several ways; in some patients we were able to definitively rule out a diagnosis of SLE, saving time and evaluation costs; in other patients, we were able to accelerate the diagnosis of SLE and the initiation of therapy.

**Conclusions:** The SLE-key® Rule-Out test increased efficiency in saving undue concern, time and resources both to the patient and to the healthcare system.

**Keywords:** Autoantibody profiling; Diagnosis; iCHIP; Antigen microarray; Systemic lupus erythematosus; Clinical practice; SLE-Key; Antibody repertoires; Multiplex assays

### Introduction

The patient with systemic lupus erythematosus (SLE) challenges the front-line rheumatologist: symptoms and signs of disease are varied; diagnostic criteria may be open to interpretation; referring physicians may not be experienced; patients are readily misinformed when consulting the Internet; the diagnostic workup can be long, not conclusive and costly; the differential diagnosis can be complicated; treatment should be timely and yet can be costly in undesirable side effects and health care dollars; and no single serologic test is diagnostic [1-3]. Since April 2015, the SLE-key® Rule-Out test, an antigen microarray assay, has become available to selected physicians; this test is based on a precisely fashioned, disposable device combined with advanced bio-informatics analysis and requires microliter samples of patient blood serum. Validation of the test on stored specimens of SLE patients and healthy controls obtained from specialized academic centers demonstrated 94% sensitivity in ruling out the likelihood of SLE; the specificity was 75%; and the NPV was 93% [1]. Furthermore, the signature has been demonstrated to persist independent of time post diagnosis. The SLE-key® Rule-Out test adds a degree of objective clarity to what can be a perplexing clinical entity. The question addressed here is how the SLE-key® Rule-Out test help might be used

to facilitate patient management in a clinical setting in which patients are referred to a busy clinic and managed individually?

Here we report the usefulness of the SLE-key® Rule-Out test on 163 consecutive subjects who were referred to our clinic because of suspected SLE.

We classified our impression into three clinical categories- SLE-likely, other disease, and uncertain before ordering and receiving the results of the SLE-Key® test. We then analyzed the effects of the SLE-key® Rule-Out test results on our subsequent diagnostic workups, our final clinical diagnosis, and the subsequent disposition of each patient. We found that the SLE-key® Rule-Out test afforded us and our patients considerable savings in effort, cost, time and diagnostic resources. The SLE-key® Rule-Out test has streamlined our evaluation process and has enabled us to improve our practice throughput; no less important, many subjects have been spared pain, uncertainty, and delayed or unnecessary treatments.

### Materials and Methods

#### Patient samples:

The patients were studied at our Rheumatology and Immunotherapy Center, in Franklin, WI, USA; the clinic is served by two board-certified rheumatologists. Around 3000 patients annually are referred to our clinic where we diagnose and treat patients with a wide variety of autoimmune, rheumatologic and other related

disorders. Serum samples were collected from 163 males and females (see Table 1) with informed consent in a manner compliant with the HIPPA; the sera were sent to Immunarray’s CLIA-certified laboratory, Veracis (Richmond, VA) for SLE-Key® iCHIP® testing and evaluation.

Clinical and Demographic Data (N=163)	
<b>Gender</b>	
Female	N=150
Male	N=13
Age in years Mean (+SD)	44.3 (13.5)
<b>Ethnic Category</b>	
Afro American	N=35
White Non-Hispanic	N=102
White Hispanic	N=23
Indian/Asian/Middle Eastern	N=3
<b>SLICC Criteria at Diagnosis</b>	
Mean (+SD)	5.1 (2.5)

**Table 1:** Clinical and demographic data.

**iCHIP® preparation, spotted antigen array, and serum testing:**

iCHIP® were prepared and sera were tested as described previously for IgG and for IgM antibodies binding to each of the spotted antigens

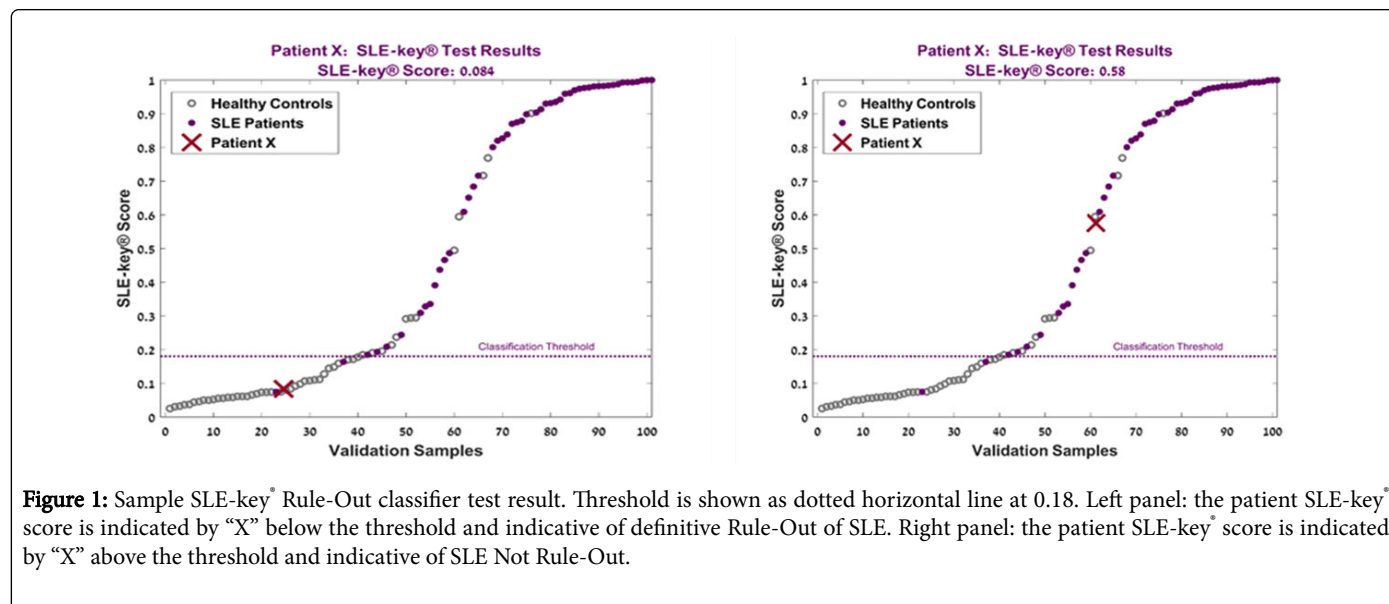
[1]. Positive and negative sera were included in each run for calibration and quality control.

**Data analysis and reporting:**

The slides were scanned using an Agilent scanner with lasers at two wavelengths (532 nm and 633 nm) and the data were recorded and analyzed as described previously [1]. Figure 1 shows two representative SLE-key® test results. SLE-key® Rule Out classifier threshold for SLE is represented by the dotted horizontal line at 0.18. The left panel shows a patient whose SLE-key® score is indicated by the “X” below the horizontal line – this subject with a score of 0.084 is classified as “SLE ruled out” ; the right panel shows an SLE-key® score above the horizontal threshold (indicated by the “X”) with a score of 0.58– such a score indicates “SLE not ruled out”.

**Results**

Figure 2 shows a flowchart of our standard work-up and disposition of patients referred for SLE before the availability of the SLE-key® test. Referrals who lacked symptoms specific for SLE (left branch of the chart) were subject to repeated testing and repeated clinical evaluations. Referrals who presented with more specific SLE symptoms (right branch on the chart) were diagnosed as suffering from SLE if they satisfied the ACR/SLICC criteria for SLE diagnosis and definitive SLE therapy was initiated. Referrals with indeterminate findings were evaluated with extensive serologic testing and repeated clinical examinations until a definitive diagnosis of SLE or some other disease could be made. These patients were followed extensively, with a mean time from the initial visit until disposition of about 6 years [4,5]. The total cost incurred per standard SLE workup – including testing – can run as high as \$2900.



**Figure 1:** Sample SLE-key® Rule-Out classifier test result. Threshold is shown as dotted horizontal line at 0.18. Left panel: the patient SLE-key® score is indicated by “X” below the threshold and indicative of definitive Rule-Out of SLE. Right panel: the patient SLE-key® score is indicated by “X” above the threshold and indicative of SLE Not Rule-Out.

Of the 163 patient serum samples sent to Veracis for SLE-key® testing, the test ruled out a diagnosis of SLE in 92 of these patients; in 71 patients SLE was not ruled out.

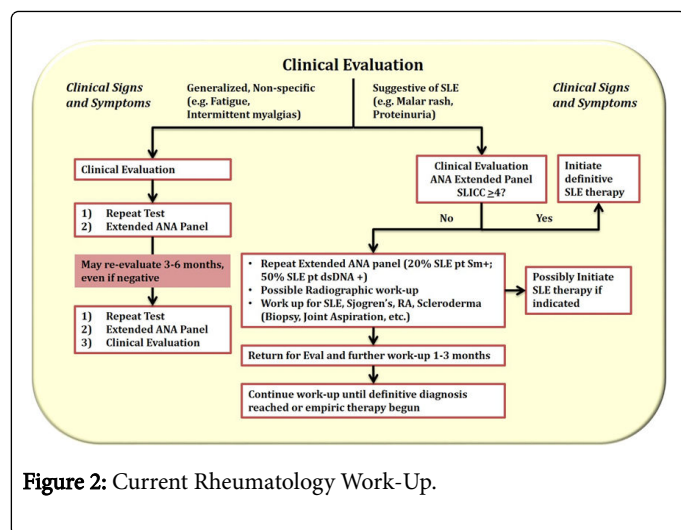


Figure 2: Current Rheumatology Work-Up.

### Patients where SLE is Ruled Out

The 92 patients in which SLE was ruled out fell into 3 groups; for 77 patients, we reached a definitive diagnosis of 'Not SLE'. In 9 cases, our clinical diagnosis was SLE in spite of the results of the SLE-key® test, and in the 6 remaining cases we were unable to reach a conclusive diagnosis and are still considering a diagnosis of SLE.

Our initial clinical impression in 50 of the 77 cases was that these patients had rheumatologic disorders other than SLE. All of these patients were minimally symptomatic at the time of referral. The SLE-key® test confirmed our initial impressions and we were able to definitively rule out a diagnosis of SLE and focus our work-ups on diagnoses including Sjogren's disease (3 patients), discoid lupus (4 patients); and 1 each with cutaneous lupus, vasculitis, polymyositis and spondyloarthropathy. A large fraction of the patients were diagnosed with various forms of fibromyalgia, joint pain and arthritis (both osteo & rheumatoid) and treated accordingly. In this group of 50 patients the SLE-key® Rule-Out test was effective in assisting us with the classification of patients with symptoms that can lead to false diagnosis of SLE.

In the remaining 27 out of 77 'Not SLE' patients, SLE had been considered along with other possible diagnoses prior to SLE-key® testing and these patients would have otherwise entered the repeat testing and re-evaluation cycle typical of suspected SLE patients. These patients were all minimally symptomatic at the time of referral. Four (4) of the 27 patients were subsequently diagnosed with Sjogren's disease and 1 with vasculitis. The remaining 22 patients were diagnosed with various forms of fibromyalgia, joint pain and arthritis (both osteo & rheumatoid) and treated accordingly.

In the 9 cases where, our clinical diagnosis was SLE in spite of a SLE-key® test result, SLE had been considered as part of the differential diagnosis prior to SLE-key® testing and all 9 patients had ACR scores >4.

### Patients where SLE is Not Ruled Out

SLE was Not Ruled Out in 71 patients tested with the SLE-key® test. Note that the SLE-key® test was developed to rule out a diagnosis of SLE compared to healthy controls. Thus, a test result that does not rule out SLE cannot be equated with a diagnosis of SLE. We confirmed the

diagnosis of SLE in 40 of these patients. SLE remained under consideration in 21 patients. In the remaining 10 cases, our final diagnoses included Cutaneous Lupus (2), Psoriasis (1), MCTD (1) and various forms of fibromyalgia, joint pain and arthritis (both osteo & rheumatoid) (6). Among the 40 patients that were definitively diagnosed with SLE, 34 had ACR scores >4. Of the remaining 6 patients, 4 had been considered as possibly suffering from SLE despite an ACR score <4. In the last 2 cases, while we considered other diagnoses, we ultimately adjudicated these patients as suffering from SLE given their symptomatology and clinical presentation. For these 6 patients, the SLE-key® test results led to an acceleration of the initiation of therapy.

Of the 21 patients where SLE remained under consideration, 10 had ACR scores >4, although 5 were ANA negative in our clinic. Ten patients had ACR scores <4 and again 4 of these were ANA negative. No ACR score was available for the remaining patient, who was ANA negative.

### SLE RuleOut in ANA positive patients

ANA testing is commonly used to triage patients suspected of SLE, this in spite of the difficulties associated with these tests and testing methodologies [6]. In our 163 patient cohort, 87 patients were referred to our clinic with positive ANA test results. We diagnosed SLE in 37 cases and SLE remained under consideration as part of the differential diagnosis in 12 cases. Of the remaining 38 patients, 31 were Ruled Out using the SLE-key® test.

Overall, the SLE-key® test was particularly effective in assisting with a definitive Rule Out of both generalized minimally symptomatic patients as well as patients where we did suspect SLE – in many cases, confirming our initial clinical impressions, but also in assisting with the disposition of patients in whom we were initially uncertain of the diagnosis. In cases where a diagnosis of SLE is Not Ruled Out, combining this observation with additional clinical input led to an acceleration of time to diagnosis and therapy. This is especially critical in SLE patients where optimal patient management is directly correlated with improved outcomes. Availability of the SLE-key test allowed us to modify our current approach to the patient work up as can be seen in (Figures 3A and 3B), leading to a more rapid disposition of patients and a more efficient use of clinical resources.

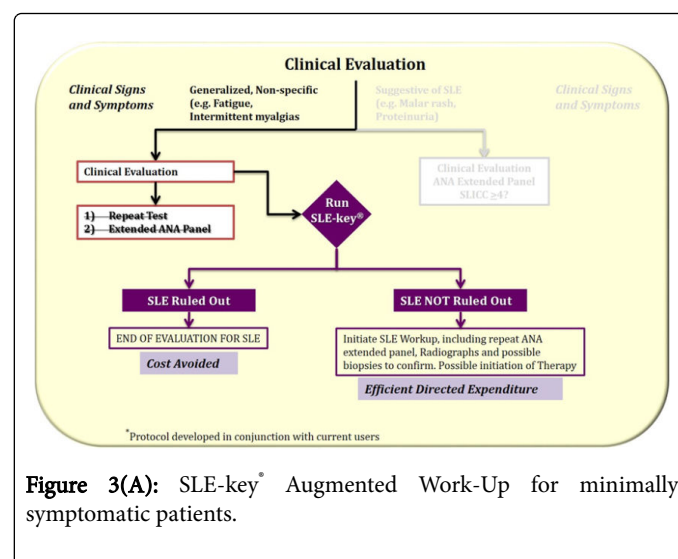
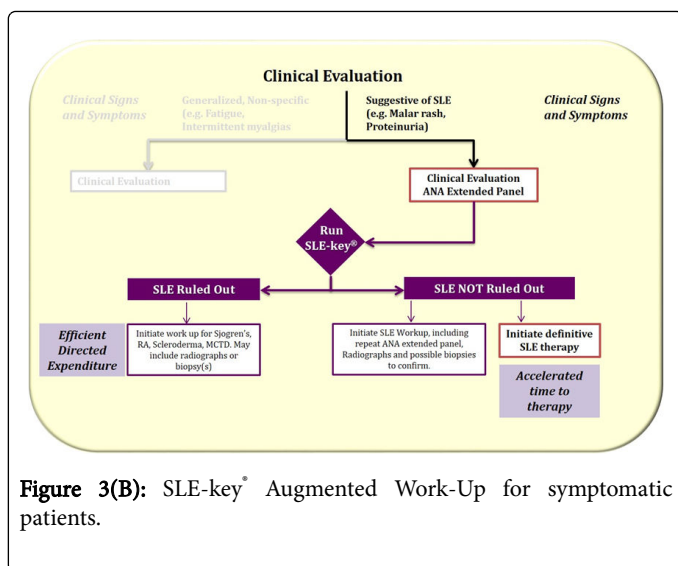


Figure 3(A): SLE-key® Augmented Work-Up for minimally symptomatic patients.



**Figure 3(B):** SLE-key® Augmented Work-Up for symptomatic patients.

## Discussion

The diagnosis of SLE is intrinsically difficult [7,8]. Nosological categories for general disease diagnosis are established largely by three factors – the majority of subjects with the disease manifest essentially similar signs, symptoms and natural histories; physicians tend to agree that there are clear criteria for making a diagnosis; and there exist objective tests (serology, blood chemistries, cellular or radiological deviations, etc.) that confirm the diagnosis. SLE is deficient in all three factors; patients express highly diverse signs, symptoms and natural histories, many of which overlap with other ‘SLE mimic’ connective tissue disorders; diagnostic criteria can be open to interpretation; and in spite of the use of classical serology including measurement of complement levels, serum dsDNA antibody, and erythrocyte sedimentation rate, no objective test exists to reassure both the physician and the patient of the accuracy of the diagnosis. Indeed, the variety of clinical expressions are so great that many patients influenced by neighbors and the Internet and some community physicians continue to be concerned about possible SLE despite inconclusive evidence. The cost of “suspected SLE” in time, resources, worry, and real dollars is considerable; inappropriate treatment adds to the SLE problem. The problem is compounded by the standard ANA serology test which can be positive in diseases that are not SLE and even in significant numbers of healthy persons [9,6]; Reliable and objective diagnostic tools are urgently needed.

It had been known for some time that the antigen-recognizing repertoires of the immune system encode information about the state of the body – the so called immunological homunculus [10,11]. Furthermore, it was shown to be possible to profile antibody and autoantibody repertoires using a suitable antigen microarray chip and informatics analysis [12,13]. These findings provided ImmunArray with the basis for development of a clinical-grade instrument for an objective evaluation of SLE. It was decided to begin with an SLE rule out microarray test because of the intrinsic variability and uncertainty of the SLE diagnosis. In cases where SLE is suspected, the use of the SLE-key® Rule-Out test was designed to shorten the time to reach a final diagnosis.

The present use of the SLE-key® rule out in a busy rheumatology clinic shows that the test provides a laboratory aid to improve

diagnosis and to increase the efficiency of disposition; thus saving undue concern, time and resources to both patients and the healthcare system. In our 163-patient cohort, the SLE-key® test provided actionable clinical information, leading to termination of evaluation for SLE or accelerated initiation of therapy. Multi-center experience is warranted to further validate the clinical advantages of the SLE-Key® serologic multi-analyte test. We think that the SLE-Key® test will be able to help other clinics that diagnose SLE and other rheumatologic diseases. In the future, the SLE-key® test may be useful as part of the procedure for referral to the specialist – allowing even more rapid diagnosis by the rheumatologist.

More detailed study will be necessary to compute the exact economic benefit provided by the SLE-Key® test, but we believe that this test may enable significant cost savings to the healthcare system based on the potential savings from a classic evaluation series (including 2 office visits, joint X-rays (or other procedures) and comprehensive ANA panel testing), which currently costs between \$2000 to \$2900.

## Acknowledgements

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## Disclosures

None of the authors has a financial interest or any conflict of interest in the study or in its results.

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