1. Massenburg D, Oldenberg J, Sell A, Krause T, Wells A.F. **An Antigen Microarray to Rule-out Systemic Lupus Erythematosus, the SLE-Key® Rule-out Test, Performs Well as an Aid in Clinical Practice.** ACR 2016 meeting. Arthritis Rheumatol. 68 (suppl 10) 2016.
Conclusion: While the incidence of SLE is relatively low, ANA testing and the subsequent referrals of patients with positive ANA and/or ENA to tertiary care specialists creates an unnecessary burden on the health care system resulting in increased waiting time for rheumatology consultations. The SLE-key® rule out 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 a multisite cohort, SLE was ruled out in 65% of 159 ANA positive patients. In our 55 patient cohort, the SLE-key® test provided actionable clinical information, leading to termination of evaluation for SLE or initiation of therapy. Multi-center experience is warranted to further validate the clinical advantages of this serologic multi-analyte test.

2. Putterman C, Safer P, Tamir I, Jakobi K, Sorek R, Gilkaite I, Ferber K, Wallace S, Harris Altice A, Batty S, and Cohen I.R. **Autoantibody Reactivities Correlated with SLE Disease Activity Identified By the SLE-key® iCHIP® Platform.** ACR 2016 meeting. Arthritis Rheumatol. 68 (suppl 10) 2016.
Conclusion: The iCHIP® platform can be used to successfully separate between SLE patients with high and low SLEDAI scores. Our analysis suggests that in addition to being useful for the development of a single MAAA test (Multianalyte Assays with Algorithmic Analyses) the iCHIP® can also provide information regarding the mechanisms related to disease activity and a window to support development of therapeutic options for these patients.

3. Cohen IR, **Antigen-Microarray Profiling of Antibodies in SLE: A Personal View of Translation from Basic Science to the Clinic.** Lupus Open Access October 1:118, 2016.
Conclusion: A review of the development of the first iChip® product – the SLE-key® Rule Out test - describes how basic observations and a philosophical notion of how the immune system functions led to the development by a company to a clinically useful product for dealing with complex diseases, the first of which is SLE, and why SLE, like other complex medical problems, can be better managed using immune profiling.

4. Putterman C, Safer P, Jakobi K, Sorek R, Gilkaite I, Wallace S, Harris Altice A, Batty DS, Cohen I. **SLE-key® iCHIP® Platform Identifies a 'Lupus Autoantibodies Signature' Early in Disease Which Persists Independent of Disease Duration and Activity.** Lupus 2016 meeting. Venice; October 5-8, 2016
Conclusion: The SLE-key® microarray test that we previously reported for reliably ruling out SLE detects a lupus signature close to diagnosis which persists independent of disease activity or duration. This signature is stable, and even patients with inactive disease are clearly differentiated from healthy individuals.

5. Putterman C, Wu A, Reiner-Benaim A, Batty DS, Sanz I, Oates J, Jakobi K, Petri M, Safer P, Gerwien R, Sorek R, Blumenstein Y, Cohen IR. (2016). **SLE-key® Rule-Out Serologic Test for Excluding the Diagnosis of Systemic Lupus Erythematosus: Developing the ImmunArray iCHIP®,** The Journal of Immunological Methods, 2016, 15, 30069-7.

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Conclusion: The iCHIP® autoantibody detection platform combined with a multi-analyte-based assay with an algorithm is able to overcome the complexity and variability inherent in SLE to enable clear discrimination between affected and unaffected individuals. The SLE-key® rule-out test device achieves a sensitivity of 94% and a specificity of 75% and NPV of 93%.

6. Madi A, Bransburg-Zabary S, Maayan-Metzger A, Dar G, Ben-Jacob E, Cohen IR. (2015). **Tumor-associated and disease-associated autoantibody repertoires in healthy colostrum and maternal and newborn cord sera.** The Journal of Immunology, 2015; 11, 5272-81.
Conclusion: Antigen microarray analysis was used to detect binding to a selection of 295 self-Ags, compared with 27 standard foreign Ags. The magnitude of binding to specific self-Ags was found to be not less than that to the foreign Ags. As expected, each newborn shared with its mother a similar IgG repertoire-manifest as early as the 24th week of gestation. IgM and IgA autoantibody repertoires in cord sera were highly correlated among the newborns and differed from their mothers' repertoires; the latter differed in sera and milk. The autoantibodies bound to self-Ags known to be associated with tumors and to autoimmune diseases.

7. Fattal, I., Shental, N., Ben-Dor, S., Molad, Y., Gabrielli, A., Pokroy-Shapira, E., Oren, S., Livneh, A., Langevitz, P., Zandman-Goddard, G., Sarig, O., Margalit, R., Gafter, U., Domany, E. and Cohen, I.R. (2015). **Guanine polynucleotides are self-antigens for human natural autoantibodies and are significantly reduced in the human genome.** Immunology 2015, 146, 401-10.
Conclusion: In the course of investigating anti-DNA autoantibodies, IgM and IgG antibodies to poly-G and other oligonucleotides in the sera of healthy persons and those diagnosed with systemic lupus erythematosus (SLE), scleroderma (SSc), or pemphigus vulgaris (PV) were examined; an antigen microarray and informatic analysis were used. All of the 135 humans studied, irrespective of health or autoimmune disease, manifested relatively high amounts of IgG antibodies binding to the 20-mer G oligonucleotide (G20); no participants entirely lacked this reactivity.

8. Cohen, I. R., Putterman, C., Jordan, N., Jakobi, K., Sorek, R., Blumenstein, Y., & Batty, S. (2015). SAT0590 **SLE-key® Rule-Out Serologic Test for SLE Using the ImmunArray ICHIP®.** *Annals of the Rheumatic Diseases*, 74 (Suppl 2), 875-875.
Conclusion: The SLE-key® multiplex test can be used to assist physicians in ruling out serologically a diagnosis of SLE with a sensitivity of ≥90%. Work comparing this testing performance in direct comparison to standard serologic testing is ongoing.

9. Batty S, Cohen IR, Putterman C, Jordan N, Jakobi K, Sorek R, Blumenstein Y, Safer P, Pisetsky D. (2015). **SLE-KEY® Rule-Out test to assess lupus in anti-nuclear antibody positive subjects using the ImmunArray iCHIP®.** ACR. San Francisco, CA, USA; November 7-11, 2015.

Conclusion: The SLE-key® Rule-Out test can be used as a decision-support tool for physicians in ruling out a diagnosis of SLE with a sensitivity of 94%, specificity of 75% and NPV of 93%. In the validation study, we were able to successfully rule out the diagnosis of SLE in 67% of ANA+ subjects with the LDA classification model. A structured RUO study with community-based rheumatologists shows good correlation between the referring rheumatologist's clinical impression and SLE-key® Rule-Out results for both the ANA+ (95% agreement) and ANA- populations (100% agreement). These initial

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findings suggest that the iCHIP® technology can be applied to develop an even more refined classification to rule out SLE in the ANA+ population, presently an important unmet need.

10. Putterman C, Batty S, Cohen IR, Jordan N, Rybak D, Rubinstein T, Jakobi K, Sorek R, Reiner-Benaim A, Blumenstein Y, Safer P. (2015). **Serological diagnosis of human neuropsychiatric lupus using the ImmunArray iCHIP®.** ACR. San Francisco, CA, USA; November 7-11, 2015.
Conclusion: In a proof of concept study, classification methods based on autoantibody profiles from the ImmunArray iCHIP® were able to successfully distinguish between lupus patients with and without neuropsychiatric symptoms. Our preliminary results based on 38 patients are very promising and warrant additional validation in a larger cohort of NPSLE patients.

11. Cohen IR, Putterman C, Jordan N, Jakobi K, Sorek R, Blumenstein Y, Batty S. (2015). **SLE-Key® Rule-Out serological test for SLE using the ImmunArray iCHIP®**. Lupus 2015 meeting. Vienna; September 2-4, 2015.
Conclusion: The SLE-key® Rule-Out serologic test can be used as a decision support tool for physicians in ruling out a diagnosis of SLE with a sensitivity of 94%, specificity of 75% and NPV of 93%. A structured observational study with community-based rheumatologists shows good correlation between referring Rheumatologist's clinical impression and SLE-key® Rule-Out results, supporting the use of SLE-key® Rule-Out as an objective tool for decision support in the Rule-Out of a diagnosis of SLE.

12. I. Cohen, C. Putterman, N. Jordan, K. Jakobi, R. Sorek, Y. Blumenstein, S. Batty. (2015). **SLE-KEY® Rule-Out serologic test for SLE using the ImmunArray iCHIP®,** Vol EULAR 15-6213. Rome; June 10-12, 2015.
Conclusion: SLE is associated with a large spectrum of autoantibodies, but currently there is no serologic diagnosis.
Each autoantibody individually fails to discriminate with sufficient specificity and/or sensitivity SLE patients from healthy controls or from subjects afflicted with other autoimmune diseases. ImmunArray has developed the previously described iCHIP® as an effective SLE rule-out diagnostic test by profiling with an antigen microarray multiple, distinct autoantibody reactivities in the sera of SLE patients compared to healthy controls and by using informatics analysis to rule out a diagnosis of SLE. All classification methods explored have differentiated SLE patients from healthy subjects with a sensitivity of greater than 90% and specificity of greater than 70% using selected informative auto-antigens. These represented some known to be associated with SLE, as well as some not previously known to have an association with the disease. The SLE-Key® multiplex test can be used to assist physicians in ruling out serologically a diagnosis of SLE with a sensitivity of >90%.

13. Wu A., Cohen I. R., Putterman C., Jakobi K., Sorek R., Batty D. S., & Field E. (2015). **SLE-key® Rule-Out Serologic Test for Excluding the Diagnosis of SLE.** Abstract presented at AACC July 26-30 2015, Atlanta, Georgia.
Conclusion: The SLE-key® Rule Out serologic test can be used as a decision support tool for physicians in ruling out a diagnosis of SLE with a sensitivity of 94%, specificity of 75% and NPV of 93%. Community-based rheumatologists are participating in a structured observational study and the results

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of post hoc analysis show good correlation and provides objective data to substantiate the referring Rheumatologist's clinical impression. The SLE-key® may also show utility in evaluating ANA (+) otherwise healthy patients after further confirmatory studies are performed. Work comparing the testing performance of the SLE-key® in direct comparison to standard serologic testing in SLE patients is ongoing.

14. Fattal I, Shental N, Molad Y, Gabrielli A, Pokroy-Shapira E, Oren S, Livneh A, Langevitz P, Pauzner R, Sarig O, Gafter U, Domany E, Cohen IR. (2014). **Epstein–Barr virus antibodies mark systemic lupus erythematosus and scleroderma patients negative for anti-DNA**, Immunology, 2014, 141(2), 276-285.

    Conclusion: Many SLE patients who were negative for autoantibodies to dsDNA manifested abnormal antibody responses to Epstein–Barr virus (EBV): these subjects made IgG antibodies to EBV antigens to which healthy subjects did not respond or they failed to make antibodies to EBV antigens to which healthy subjects did respond. This observation suggests that SLE may be associated with a defective immune response to EBV. The Scleroderma patients shared many of these serological abnormalities with SLE patients, but differed from them in increased IgG autoantibodies to topoisomerase and centromere B. Hence an aberrant immune response to a ubiquitous viral infection such as EBV might set the stage for an autoimmune disease.

15. Cohen, I. R. (2013). **Autoantibody repertoires, natural biomarkers, and system controllers**. *Trends in immunology*, *34*(12), 620-625.

    Conclusion: This review describes the use of antigen microarrays and informatics to profile the repertoires of autoantibodies in health and disease. Autoantibody profiling provides an insight into the biomarkers used by the immune system in its dialog with the body. HSP molecules and peptides can be viewed as natural regulators because the immune system itself deploys them to modulate inflammatory reactions. The discovery of such natural biomarkers paves the way towards control.

16. Bransburg-Zabary, S., Kenett, D. Y., Dar, G., Madi, A., Merbl, Y., Quintana, F. J, Tauber A. I., Cohen I. R., & Ben-Jacob, E. (2013). **Individual and meta-immune networks**. *Physical biology*, *10*(2), 025003.

    Conclusion: The use of network science conceptual and methodological approaches is prevalent in a variety of scientific disciplines. In our works we presented a new approach to investigate antigen microarray data of autoantibody reactivity of IgM and IgG isotypes present in the sera of 10 mothers and their newborns. We were able to show that using advanced network analysis tools, mainly developed in physics, new findings regarding immune development can be uncovered. We were able to show the immune repertoire, which represents both antibody reactivity to antigens, and can be treated as a network.

17. Madi, A., Bransburg-Zabary, S., Kenett, D. Y., Ben-Jacob, E., & Cohen, I. R. (2012). **The natural autoantibody repertoire in newborns and adults**. In *Naturally Occurring Antibodies (NAbs)* (pp. 198-212). Springer New York.

    Conclusion: The recent development of antigen microarray chip technology for detecting global patterns of antibody reactivities makes it possible to study the immune system quantitatively using network analysis tools. Here, we review the analyses of IgM and IgG autoantibody reactivities of sera of mothers and their offspring (umbilical cords) to 300 defined self-antigens; the autoantibody reactivities present in cord blood represent the natural autoimmune repertories with which healthy

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humans begin life and the mothers' reactivities reflect the development of the repertoires in healthy young adults.

18. Fattal, I., Shental, N., Mevorach, D., Anaya, J. M., Livneh, A., Langevitz, P., Zandman-Goddard G., Pauzner R., Lerner M., Blank M., Hincapie M. E., Gafter U., Naparstek Y., Shoenfeld Y., Domany E., & Cohen, I. R. (2010). **An antibody profile of systemic lupus erythematosus detected by antigen microarray**. *Immunology*, *130*(3), 337-343.
    Conclusion: Our antigen microarray and informatic views of SLE differ considerably from the standard ways of characterizing antibodies in lupus and from the ways in which others have deployed antigen microarrays to study SLE. The data appear to be meaningful: the signal generated by the microarray was prominent and highly significant statistically. Moreover, the subjects were recruited from three different centres on two continents, and the core reactivity profile was robust in being able to detect subjects in remission for as long as 30 years, as well as one subject who was diagnosed with clinical SLE more than 1 year after her serum sample was collected and tested. Microarray technology and informatic analysis thus provide a promising entry into immunomics – a global view of a subject's immune state.

19. Quintana, F. J., Merbl, Y., Sahar, E., Domany, E., & Cohen, I. R. (2006). **Antigen-chip technology for accessing global information about the state of the body.** *Lupus*, *15*(7), 428-430.
    Conclusion: The state of the body appears to be encoded by the immune system in collectives of reactivities and not by single reactivities. Here we describe our use of microarray technology and informatics to develop an antigen chip capable of detecting global patterns of antibodies binding to hundreds of antigens simultaneously. The patterns fashion diagnostic signatures.