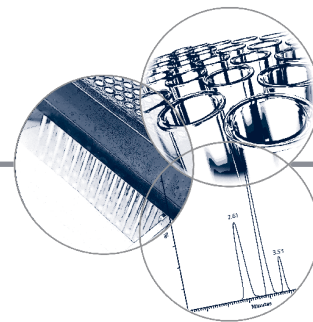




Is there a future for multiplexed technologies in the point-of-care setting?

“Early and objective diagnostics allow for an earlier intervention and onset of treatment resulting in significant healthcare cost savings...”

Keywords: antigen analysis ■ autoimmunity ■ diagnosis ■ immunoglobulin analysis ■ *in vitro* diagnostics tests ■ molecular pathology ■ multiplexed microarray ■ personalized medicine ■ rheumatoid arthritis

Despite the hopes (and predictions) of diagnostic scientists and researchers, like myself, the use of multiplexed technologies in the point-of-care (POC) setting has not achieved the level of growth and universal adoption in the last several years that might have been reasonably expected based on recent technological advances. In fact, notwithstanding the adoption of rapid testing in the POC setting, the use of multiplex technologies in the POC setting is rare, that is, almost no multiplex testing is performed in the POC setting.

Furthermore, multiplexed microarray tests are rarely used in the routine laboratory setting. The reasons for this include both technical and implementation issues, however, none are insurmountable. In *Clinical Chemistry*, Ellington *et al.* argue that multiplex platforms need further analysis before routinely becoming used in the clinical setting. Variation in results, which the authors attribute to variability of assay manufacturing processes and incomplete calibration of quantitative immunoassay processes, among other issues are blamed [1]. This, despite a Kalorama prediction in 2008, that by 2015, many *in vitro* diagnostic analyses for both the lab and POC would be based on miniaturized and multiplexed (e.g., ‘chip’) technologies [101].

For many scientists and startup companies who expected to see a major change in diagnostic procedures and an increased demand for multiplexed tests by this point in time, the lack of progress is confounding. The question remains to be asked: ‘Why?’, and following that: ‘Is there a future for multiplexed technologies in the POC setting?’

Diagnostic industry changes are occurring

Unsurprisingly, the diagnostic industry is undergoing great changes, with new emerging technologies creating the possibility to analyze more variables more quickly and more accurately. For example, specific immunoglobulin analysis is today routine in the diagnosis of allergic and autoimmune diseases. However, using standard sequential assay analysis methods (singleplex), we cannot easily determine the crosseffects of a multitude of different antigens for S-IgE (allergy) or S-IgG (autoimmunity). The need for more indepth and simultaneous information has encouraged us to search for new possibilities.

In recent years, diagnostics companies, such as Phadia, have developed the ability to simultaneously analyze more than 100 different antigens with a minute amount of patient sample. After reviewing a large number of assay technologies, we now have the possibility to use multiplexed technologies; ImmunoCAP ISAC [2] as the basis for new laboratory systems. In addition, for several years now we have had an assay format (ImmunoCAP Rapid) [3] that allows the analysis of ten different allergens to be completed in approximately 15 min. These enabling technologies allow for the development of new information in immunology, leading to the possibility to develop true personalized medicine approaches – finding the correct treatment for unique individuals based on precise multiplexed measurements. The goal is to find more cost-effective and clinically effective treatments for individuals who do not respond to traditional treatments



Per Matsson

Phadia AB, PO Box 6460, SE-751 37
Uppsala, Sweden
Tel.: +46 1816 5000
Fax: +46 1816 6275
E-mail: per.matsson@phadia.com

**FUTURE
SCIENCE**

part of
fsg

(e.g., the approximate third of rheumatoid arthritis [RA] patients who cannot be effectively treated with anti-TNF treatments). But more than that, it is about being able to treat patients based on a combination of numerous identifiable markers before symptoms progress to the advanced stages.

Cost benefits & improved outcomes

Like other *in vitro* diagnostics tests, the use of multiplexed technologies has the potential to lead to significant cost benefits as well as to achieve more proactive treatments to improve patient outcomes. Looking at the case for immunological diseases, such as RA or celiac disease, we can see the benefits.

For example, in the diagnosis of RA, clinicians typically must see at least four of the presenting symptoms from a standard list that includes: morning stiffness for more than 1 h, swollenness in more than three joints, swollenness in hands, symmetric joint involvement, typical joint destruction by x-ray, rheumatoid nodules and presence of rheumatoid factors in serum. Such symptoms, of course, typically present after destruction of the joints has occurred. There is a clinical and cost advantage to the accurate, early diagnosis and treatment of such diseases before more expensive, invasive and painful surgeries or therapies are needed. In one study, serum samples from clinical RA patients in Sweden showed CCP-2-positive markers up to 10 years before the onset of the first clinical symptoms [4].

This becomes obvious if you compare the present average timepoint for a physician's diagnosis of RA to the timepoint for early identification based on molecular pathology. Using RA markers, as outlined in the *Lancet* by Klareskog *et al* [5], the early identification makes more patients eligible for treatment before the disease progresses to the advanced stages and chronic destruction of joints.

Furthermore, healthcare spending in the field of RA is enormous. The anti-TNF drugs alone cost approximately US\$20 billion per year and, as stated, the drugs work well on only approximately two-thirds of the patients. Through multiplex testing, we have shown that we can differentiate the RA patients into a number of subgroups and optimize treatment on a more personalized level, based upon objective measures. This is possible by having successfully developed a microarray for the simultaneous detection of multiple RA-associated autoantibodies

and singling out citrullinated proteins/peptides (ACPA), which are highly specific and predictive of RA development [6].

In the case of molecular allergology, microarray peanut allergy studies show that we can identify a combination of multiple markers to determine which patients are dangerously allergic to peanuts, and have a potential for life-threatening reactions, and which are not.

The Nicolaou peanut allergy study in 2010 shows that not all peanut-sensitized children develop allergic reactions on exposure [7]. In fact, the study demonstrates that the current methods of assessment of peanut sensitization do not accurately predict peanut allergy, because IgE directed against pollen allergens may bind to components of the whole peanut extract, resulting in a false-positive peanut allergy. Microarray technology, which allows the detection of IgE to individual allergens of peanut and other potentially crossreacting allergens including grass pollen, reveals a marked difference in the pattern of allergic detection. Thus, the majority of children in the general population with a positive skin test or measurable serum IgE to peanut do not have a clinical peanut allergy. Using a microarray test can eliminate the need for costly, oral challenge testing and give parents more assurance that protection against all accidental exposure is indeed warranted.

What are the reasons of slow adoption?

The benefits in terms of cost savings and proactive patient care are obvious. So why is adoption slow? I have my theories, and these include:

- Physicians are hesitant to use new methods. Instead they rely on their patient's history and empirical experience;
- Traditionally, most testing has been done in the central clinical laboratory rather than in the primary care setting and there is a reluctance to change this venue;
- Regulators are unclear about how to regulate tests that include multiplexing and computer algorithms for interpretation;
- The cost pressure against new spending is high and the introduction of new technology is hampered by lack of funding;
- The burden on commercial developers becomes too high, that is, they can not justify the investments in the development projects nor the significant amount of clinical trials needed.

Understanding how multiplex technology works

The key to multiplex microarray testing lies in microchip technology. As I stated in a 2008 article [8] for *Clinical Laboratory International*, component-resolved diagnostics is already in use and will, in all likelihood, be an essential part of allergy diagnostics in the future.

However, in order to run component-resolved diagnostic assays rapidly and cost effectively, some form of multiplexing technology is necessary. This allows labs to process a large number of allergenic components in a single step, using only a small amount of blood. Multiplexing has already been used for some time in autoimmunity testing and now the technology is also available for allergy, using recombinant or purified allergen components in a biochip-based allergy test. In this way, a 20 µl sample can be used to analyze more than 100 different allergen components or antigens.

As part of the process, picoliter quantities of each allergen preparation are deposited on spatially separate locations on the biochip. The resulting addressable microarray allows for many biological affinity reactions to be monitored in parallel (i.e., parallel multiplexing). In a single analytical step, the comprehensive allergen sensitization profile of a patient can be measured using a single serum or plasma sample.

The power of this data, of course, comes with interpretation. Ensuring that it is managed and organized into a database structure that can collect information from multiple technology platforms is essential, and also possible. I believe that in the future it will be possible to seamlessly transfer data between systems and include both simplified and complex data interpretation. These systems will both support the clinical scientist who needs to move the medical diagnostic frontier forward as well as help the practicing physician to treat the patient and cut healthcare costs more effectively.

Conclusion

We expect to see a whole range of new tools to enable early differential diagnosis of patients in the primary care setting, which in combination with quantitative laboratory tests and/or highly resolved component analysis, can shed new light on the management of autoimmune and allergy patients. These new tools and opportunities will allow the monitoring of the whole immune system and lead to new discoveries in

the field of immunological diseases. We have already proven this is possible in the allergy and RA field.

In summary, is multiplex testing viable? I for one, think it is essential to the future of accurate diagnostic medicine. Physicians and clinicians should not fear that multiplex testing usurps their knowledge, but indeed look to it as confirmation of their diagnostic abilities.

From previous studies, it is clear that a patient history and physical examination alone only diagnoses approximately 50% of the cases correctly, whereas the addition of *in vitro* or skin-prick testing significantly increased the percentage of correct diagnoses [9].

Multiplex testing needs to become part of near-patient diagnosis and I believe it will for the reasons summarized below:

- Early and objective diagnostics allow for an earlier intervention and onset of treatment resulting in significant healthcare cost savings;
- We have shown the significant advantages of multiplex testing and molecular pathology for immune-related diseases such as allergy, asthma, RA and other autoimmune diseases. Using these tests during the first visits to the general practitioner will differentiate the right patient to be treated by the right specialist;
- A number of good multiplexed diagnostics tests already exist;
- The prognosis for continuous technological development is excellent and there are numerous new technologies that may be used for multiplexed applications both in the lab and for POC;
- Modern information technology supports interpretation, help and treatment options based on the outcome of the testing in combination with the patient's history.

■■■■■■■
"From previous studies, it is clear that a patient history and physical examination alone only diagnoses approximately 50% of the cases correctly, whereas the addition of in vitro or skin-prick testing significantly increased the percentage of correct diagnoses..."
 ■■■■■■■

The examples discussed above can be generalized since most disease treatments benefit from an early and correct diagnosis, prediction

of prognosis and an objective measurement of treatment outcome. Finally, the modern pharmaceutical industry with the development of biopharmaceuticals will benefit significantly from the possibilities of developing personalized treatments, early patient differentiation and POC methods for monitoring of treatment progression.

Financial & competing interests disclosure

P Matsson is a Chief Technology Officer for Phadia. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

- 1 Ellington AA, Kullo IJ, Bailey KR, Klee GG. Antibody-based protein multiplex platforms: technical and operational challenges. *Clin. Chem.* (56)2, 186–193 (2010).
- 2 Sanz Maria L, Blázquez Ana B, García Blanca E. Microarray of allergenic component-based diagnosis in food allergy. *Curr. Opin. Allergy Clin. Immunol.* 11(3), 204–209 (2011).
- 3 Sarratud T, Donnanno S, Terracciano L *et al.* Accuracy of a point-of-care testing device in children with suspected respiratory allergy. *Allergy Asthma Proc.* 31(2), e11–e17 (2010).
- 4 Rantapää-Dahlqvist S, de Jong BA, Berglin E *et al.* Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 48, 2741–2749 (2003).
- 5 Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. *Lancet* 373, 659–672 (2009).
- 6 Mathsson L, Hansson M, Israelsson L *et al.* A multiplex microarray for the detection of rheumatoid arthritis-associated autoantibodies. *Ann. Rheum. Dis.* 70(Suppl. 2), A1–A94 (2011).
- 7 Nicolaou N, Poorafshar M, Murray C *et al.* Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J. Allergy Clin. Immunol.* 125, 191–197 (2010).
- 8 Matsson P, Harwanegg C. The future of allergy testing: new technologies and opportunities. *Clinical Laboratory International* 6, 26–28 (2009).
- 9 Duran-Tauleria E, Vignati G, Guedan MJA, Petersson CJ. The utility of specific immunoglobulin E measurements in primary care. *Allergy* 59(Suppl. 78), 35–41 (2004).

■ Website

- 101 The worldwide market for *in vitro* diagnostic tests, 6th edition. Kalorama, June 2008. www.kaloramainformation.com