**MICROTEST's FEEDBACK ON NICE ASSESSMENT REPORT**

**Summary of feedback**

We believe there is adequate data to support a claim of 80-95% agreement among SPT, ImmunoCAP, ISAC and Microtest independently of which pair of methods is compared (Williams, Konradsen, Onell, see also references 16-18, 23-30 in Willliams et. al. Appendix 2). Furthermore we did not find any studies using microarray platforms produced the last 4 years that questions the agreement or accuracy of microarrays compared to physician's diagnosis or traditional tests. More importantly, several studies report additional clinical utility for certain types of patients, especially in poly-sensitized patients.

On this basis, we believe there is no reason to delay approval of microarray testing as an alternative test method for poly-sensitized patients. We believe that microarrays provide a more efficient diagnostic process as a function of the information gained and from a cost perspective. Nevertheless, we concur with the recommendation to develop a consensus document over time.

If no recommendations for service provision can be made at this stage it would be of value if the report specifies the major concerns and what additional information is required for a future recommendation of the service. For example,

1) Is the accuracy of ISAC/Microtest a major concern? What data would be needed to overcome this issue? Should sensitivity and specificity data be based on physician's diagnosis, challenge data, ImmunoCAP and/or SPT? Do all allergen sources on the chip need to be covered for ISAC/Microtest?
2) Is the clinical value of ISAC/Microtest a major concern for a recommendation? What type of additional information is required to overcome this issue?
3) Is the cost-effectiveness of ISAC/Microtest a major concern if the test is reimbursed for patients where >X (X can for example be 10) allergens needs to be tested?

Key messages and questions:

A. Is there a doubt on the validity and value of sIgE testing as illustrated in the flowchart on p.93?
B. The positive/negative agreement between SPT and ImmunoCAP is generally reported to be within 80-98% depending on allergen and population studied. Several microarray technologies have shown agreements in the same range as the agreement between STP and ImmunoCAP. Further, we could not find any publication from the last 4 years questioning the accuracy or reliability of the microarrays assessed. If there was a problem with the performance this would have come across in some of the studies in the reference list.

C. Is there a doubt on the validity and reliability of the microarray technology as such? Or is the doubt mainly in the way the technology may be implemented and interpreted?
D. The health economics and cost effectiveness values of microarray platforms have yet to be proven short or long-term for the majority of defined target patients. One very relevant question is what proportion of patients seeking 2nd or 3rd level care is poly-sensitized with/without cross-reacting protein families such as profilins, PR-10 proteins or storage proteins? According to a review article in Allergy 2014, 4:16 by Migueres et al. "*more than 50% of patients consulting for respiratory allergies are poly-sensitized*." At a certain point, if a large number of allergens must be tested for optimal patient care, and if allergen components are needed to resolve potential cross-reactivity, the microarray technology is, logically, a good alternative approach from a cost, data gained, and sample blood volume optimization perspective.

**Feedback topics according to DAP27 DAR comments table:**

1. Microtest description
2. Updated/new Microtest & ISAC references
3. Table 1
4. Study design: Accepted methods to calculate the sensitivity and specificity of microarray allergy tests in absence of a gold standard.
5. Reliability of sIgE test results. Flowcharts on p.93
6. Cost-effectiveness
7. The term "patients with difficult to manage allergies" may be difficult to find in the literature due to unclear terminology.
8. Objective 1 and 2
9. Conclusion: Further needs for a recommendation
10. Study by Heaps et al. regarding false positive results

For more detailed information on the above feedback topics please read more below and in Appendix 1-3.

---

**More details on the feedback topics**

1. **Microtest information**
   a. **The microarray descriptions on p.22-23:** Should preferably include accurate and similar content information for both microarrays (see Appendix 2 as an examples).

2. **and 4. Updated Microtest and ISAC references/publications**

**Accuracy (sensitivity, specificity data) of Microtest and ISAC.** P.39 *"The protocol stated that diagnostic accuracy studies would in included only where such studies reported both the accuracy (sensitivity and specificity) for the*

*prediction of clinical reactivity and the number and details of participants for whom multiplex testing provided addition information. No studies of this type identified*." There is one new study that fulfills this specification outlined above: Ref [155], see Appendix 1 - References below. This study presents data on both sensitivity/specificity results as defined by SPT and physician diagnosis, as well as the added value information provided by microarray testing.

Remark: The main reason to the big lack of microarray accuracy studies is the costs involved. Assume a multiplex test containing 20 allergens. In order to assess the accuracy of such test one must consider the resources for challenge testing, SPT or sIgE test to a broad panel of allergens. This will be costly, time consuming and unpractical or unethical for certain allergens and patients.

**New publication available on Microtest and ISAC [210] (Appendix 1)** including the positive/negative concordance of Microtest and ISAC compared to both ImmunoCAP and SPT for 10 common allergens applied on a group of patients with difficult to diagnose/manage allergies.

**3. Table 1. p.24.**

The Table lack the following information about the methods:

a) ISAC: Measures IgE abs to allergen components only, 112 in parallel.
b) Microtest: Measure IgE abs to allergen components and extracts, 26 in parallel.
c) ImmunoCAP: Measure IgE abs to components and extract, one at the time.
d) SPT: Measures skin reaction to allergen extracts only.
e) Challenge: Foods, one at the time.

5. **The value of blood tests:** We believe there are no data in the literature to support any claim of meaningful clinical difference in diagnostic accuracy or significance between SPT and sIgE titre measurement. International guidelines do not support the flowchart on page 93 that indicates that sIgE tests cannot be used to confirm allergy like SPT when there is a supportive history.
6. **Cost-effectiveness:** Testing a panel of allergens as well as testing for allergen components offers opportunities for improved characterization and has been suggested to be useful in poly-sensitized patients.

The number of allergens to be tested, the amount of information required to increase the diagnostic certainty/resolution and the serum volume needed will influence the decision that determines whether microarray testing or single testing will offer a more efficient diagnostic pathway.

One alternative could be to support microarray testing when some minimal number of allergens need testing AND allergen components are included in that list. Under these circumstances, it may be more efficient from a cost, sample volume and information point of view to utilize microarray testing.

If some food challenges can be reduced, this is an extra bonus that saves costs.

**7. About studies including patient with allergies difficult to manage:**
The definition “patients with difficult to manage allergies” is unclear. Does it mean patients where it is difficult to identify the offending allergen and hence there symptoms persist?

We believe some/many of the studies included in the reference list do include patients with difficult to manage allergies although it may not be stated clearly in the manuscript since this terminology is not commonly used. Thus, the studies by Heaps (idiopathic analphylaxis), Luengo (severly multi-sensitized food and inhalation allergies), Konradsen (children with problematic asthma and poly-sensitization including both food and inhalation allergens), Hong (patients with analphylactic reactions to peanut) do all include patients with difficult to diagnose and manage allergies. See Appendix 3 for additional studies demonstrating additional value provided by Microarray testing (Gassner, Onell)

## APPENDIX 1 – Microarray descriptions

### 2.2 Intervention technologies - Microarray technologies

The aim of allergen microarrays is to assess the presence of multiple antibodies in a single blood test. Microarray technology enables miniaturized immune-assay platforms. One application of the technology allows measurement of hundreds of allergens simultaneously using only a small sample volume and tiny amounts of allergens spotted on a chip.

The use of the microarray technology may provide more detailed information about individual sensitization profiles when compared to the single IgE testing. In particular, it has been suggested that microarrays are especially useful in poly-sensitized patients or patients with complex allergies such as those with inconsistent case histories, unsatisfactory response to treatment or idiopathic anaphylaxis. These are people with severe or unclear allergic disease, who test positive to a range of allergens but in whom the true cause of symptoms can be difficult to identify.

Therefore, it is claimed that the use of microarray tests could improve health outcomes by providing an equally good or more efficient way of testing in certain types of patients who a) require testing to a broad panel of allergens to identify the IgE profile of the patient and b) where component testing is required to resolve the true cause of the offending allergen(s). This may lead to decreased time to diagnosis and a refined diagnosis that, in turn, may lead to an improved allergy management and a more appropriately targeting specific immunotherapy. These improvements could also lead to potential savings to the NHS from reducing the number of tests, reduced number of patient visits and avoiding the use of unnecessary immunotherapy.

This assessment includes two microarray technologies: ImmunoCAP ISAC and the Microtest allergy system (described below). The main differences between the immunoCAP ISAC and the Microtest systems are:

a) ImmunoCAP ISAC measure 112 allergen components (derived from 51 allergen sources) using 30 ul of sample. Microtest measures 19 allergen extracts + 16 allergen components (derived from 22 allergen sources) using 100 ul of sample.

b) ImmunoCAP ISAC is a manual assay. Microtest is an automated assay.

c) ImmunoCAP has been on the market for a longer time, and used in more studies than Microtest.

Table 1 summarizes the key characteristics of the multiplex allergen tests ImmunoCAP® ISAC and Microtest, compared to comparator tests which are currently used in the standard diagnostic work- up of patients with difficult to manage allergic disease.

### 2.2.1 ImmunoCAP® ISAC

ImmunoCAP® ISAC 112 is a molecular diagnostic test that can simultaneously test for IgE antibodies to 112 components from 51 allergen sources. The Immuno Solid-phase Allergen Chip (ISAC) is a miniaturised immunoassay platform that uses a single sample (30µl) of serum, plasma or capillary blood to test for IgE antibodies to multiple allergens. ImmunoCAP® ISAC is a two-step assay. IgE antibodies from the patient sample bind to immobilized allergen components spotted in triplets on polymer coated slides. Each slide contains four microarrays giving results for four samples per slide. The results are measured using a biochip scanner (confocal laser scanning devices, in particular the CapitalBio LuxScan 10k microarray scanner are recommended), and evaluated using proprietary software produced by the same company, Phadia Microarray Image Analysis software (MIA). ImmunoCAP® ISAC is a semi-quantitative test and results are reported in ISAC standard units (ISU) giving indications of specific IgE antibody levels; the operating range is 0.3 to 100 ISU-E. This range approximately corresponds to a concentration range of 0.3 -100 kilo international units of allergen specific antibody per unit volume of sample (kUA/L) of IgE (1 kUA/L is equal to 2.4 ng/mL). The assay takes a total of four hours, including sample processing and incubation time.

### 2.2.2 Microtest

The Microtest system is a microarray system with allergen components and extracts immobilized on the chip. The test can simultaneously measure IgE abs to 16 allergen components and 19 allergen extracts covering the following allergens: Cat, dog, horse, house dust mite (Dermatophagoides pteronyssinus), German cockroach, birch, olive, timothy grass, rye grass, cultivated rye grass, Bermuda grass, Alternaria, egg, milk, peanut, hazelnut, wheat, soy, cod, shrimp, latex, bee venom. The Microtest Allergy system is a semi-automated assay that uses 100µl serum or plasma sample to test for IgE antibodies to multiple allergens. The immobilized allergens on the chip react with specific IgE in the patient sample. An enzyme labeled antibody detects the sIgE-allergen complex and a detection solution is used to develop the fluorescence. The fluorescent signal is processed in the Microtest software. Each Microtest slide contains one microarray. Microtest is a semi-quantitative test and results are calculated in kU/l and reported in IgE classes (Class 0: <0.35 kU/l, Class 1: 0.35 – 1 kU/l, Class 2: 1.01-15 kU/l and Class 3: >15kU/l) giving indications of specific IgE antibody levels. The operating range is 0.3 to 100 kU/l (1 kU/l is equal to 2.4 ng/mL). The assay takes about 4 to 5 hours depending on how many samples are processed at the same time. Up to 5 samples can be processed in parallel per run.

## APPENDIX 2 – MICROTEST REFERENCES

In order to avoid duplication of study references published e.g as multiple conferences abstract and a publication we suggest to prioritize journal publications if available. If the study has not been published in a journal, conference abstracts may be included once per study.

**Updated Ref [210]**

Evaluation of a novel automated allergy microarray platform compared with three other allergy test methods.
Williams P, Önell A, Baldracchini F, Hui V, Jolles S, El-Shanawany T.
Clin Exp Immunol. 2015 Oct 5. doi: 10.1111/cei.12721. [Epub ahead of print]
PMID: 26437695

Summary of study: This is the same study as referred to in Ref [210]. The concordance between 4 different diagnostic methods (ISAC, Microtest, SPT and ImmunoCAP) on 103 patients with difficult to manage allergies was investigated in this study. Egg, milk, peanut, hazelnut, mite, cat, dog , birch and timothy were tested using all 4 test methods and compared. The positive/negative concordance range between 81% (ImmunoCAP vs SPT) to 88% (ISAC vs ImmunoCAP). The quantitative agreement between the 3 blood tests was also analysed. Microtest and ISAC demonstrate comparable results to ImmunoCAP and SPT.

**Updated Ref [155]**

J Konradsen, B Nordlund, A Winkler, F Baldracchini, G Mazzoleni, A Önell, G Hedlin, H Grönlund
Evaluation of Microtest Allergy System compared to three established diagnostic methods. Allergy Sept 2015; Volume 70, Issue Supplement S101 no. 379 P.173

Summary of study: Manuscript to be submitted in Dec. Data collection (including Drs diagnosis, clinical history, SPT, ImmunoCAP, ISAC and Microtest), as well as the Methods and Result manuscript parts have been finalized and may be shared confidentially.) This study focuses on patients with difficult to manage allergies (children with problematic asthma, n=71, and the majority are poly sensitized, n=46). The 71 children included in the study were all tested with the Microtest, ISAC; ImmunoCAP and SPT for 10 common allergens (egg, milk, peanut, cod, cat, dog, mite, birch, timothy, Alternaria). The positive/negative concordance between methods was 90-92% independently of which two methods compared. All 4 methods gave the same positive/negative indication in 83.5% of the 710 observations. The accuracy of the tests in terms of sensitivity and specificity compared with Drs diagnosis varied between 0.77-0.88 and 0.97-0.99 respectively. The microarray tests showed sensitivity and specificity values between SPT and ImmunoCAP.

## APPENDIX 3 – ADDED VALUE REFERENCES

See below list of additional references that show the added value provided by microarray testing compared to traditional testing. (There are more than these two. Some are in the reference list of the report but the manuscripts may not clearly define either the added value or the type of patient's studies).

### Exploring the temporal development of childhood IgE profiles to allergen components.

*Onell A, Hjälle L, Borres MP.*
Clin Transl Allergy. 2012 Dec 19;2(1):24. doi: 10.1186/2045-7022-2-24.
Comment: This study shows both the reliability/accuracy of microarrays to identify triggering allergens in poly-sensitized children. It also shows that IgE profiling provides additional information regarding early identification of IgE sensitization (prior to clinical symptom onset), cross-reactivity, true co-sensitization and unexpected triggers in allergic children.

64 children included in the study and they were classified and analsyed:
Accuaracy: Out of 82 triggering allergens causing symptoms as defined by doctors diagnosis, 76 (93%) were identified by the ISAC chip.
Additional information provided by the microarray:
**Early food, inhalation and multi-sensitized sensitized children (n=10)**
By resolving co-sensitization for cross-reactivity and by identifying unexpected triggers prior to symptom development, the ISAC chip provided valuable information in 8 out of 10 children in the multi-sensitized group.
**Late inhalation sensitized group (n=20)**
Nine of 20 children showed relatively simple IgE profiles, with only one or two species-specific allergens (typically mono-sensitized to birch, grass, or cat). The remaining 11 children displayed a multi-sensitized IgE profile, involving cross-reacting allergens (often PR-10 proteins) with concomitant sensitization to at least 2 species-specific components. For these more complex, multi-sensitized children the ISAC results gave new, relevant information not easily available from SPT or case histories.
**Only early food (n=2)**
This group of patient was not studied further due to the limited number of individuals.
**Non-sensitized group (No IgE) n= 22**
The ISAC results did not add new, relevant information relative to traditional diagnostic methods, except for delivering a rapid and reliable answer that the child is non-sensitized to a broad spectrum of allergens.

There are also two patient case conference abstracts describing the added value on the individual level for 2 of the children.)

### Hay Fever as a Christmas Gift.

*Gassner M, Gehrig R, Schmid-Grendelmeier P.*
N Engl J Med. 2012 Dec 21.
Comment: This study demonstrates the ability of ISAC to detect unexpected triggering allergen. Neither clinical history, SPT or ImmunoCAP testing were able to identify the true cause to the clinical symptoms around Christmas time of children in the village. ISAC does (if you have the knowledge to interpret the information provided as they had in this study).