

# Evaluation of a novel point-of-care test for the rapid semi-quantitative detection of allergen-specific immunoglobulin E

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August 2017

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# Evaluation of a novel point-of-care test for the rapid semi-quantitative detection of allergen-specific immunoglobulin E

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## ABSTRACT

**Background:** Allergen-specific IgE levels in human sera support the diagnosis of allergies. However, commonly used laboratory tests are time consuming. The new point-of-care test FastCheckPOC 20 (FCP20) allows the simultaneous and semi-quantitative evaluation of IgE antibodies for 20 aeroallergens and food allergens.

**Methods:** Two hundred patients with allergic symptoms were included in a multicenter performance evaluation using both whole blood and plasma or serum samples. FCP20 results were compared with the skin prick test (SPT) and the ImmunoCAP system (Phadia) which was used as reference for specific IgE values.

**Results:** The concordance between FCP20 and ImmunoCAP was 76% for positive results and 80% for negative results over 20 allergens. Overall agreement (accuracy) of FCP20 with the ImmunoCAP was 80% across all 20 allergens. The concordance between FCP20 and SPT over all 20 allergens was 66% for positive results and 71% for negative results. Overall agreement of FCP20 with the SPT was 70%. Semi-quantitative FCP20 results (Levels 1–5) revealed nearly identical values for whole blood and plasma/serum test results. A good concordance was also observed between FCP20 levels and ImmunoCAP classes.

**Conclusions:** FastCheckPOC 20 is reliable to determine allergen-specific IgE in human whole blood and plasma/serum samples.

## KEYWORDS

Allergy diagnostics; allergen-specific IgE; ImmunoCAP; skin prick test; point-of-care test

## Background

Immediate-type allergies such as asthma, atopic eczema, or several food allergies are among the most common medical disorders, in adults as well as in children, and their prevalence has been on the rise for several decades worldwide.<sup>[1,2]</sup> They are characterized by an increase in circulating allergen-specific IgE (sIgE) and are associated with a variety of symptoms which are also commonly observed in other, non-allergic diseases. The differentiation between allergic and non-allergic conditions can be challenging, but a clear

diagnosis is important for early intervention and prognosis. In addition to a detailed analysis of the patient's medical history and clinical symptoms, the skin prick test (SPT) and the demonstration of sIgE antibodies in symptomatic patients are accepted tools to help distinguish immediate-type, allergic from non-allergic conditions.

The skin prick test (SPT), that allows to assess the patient's hypersensitivity to components of specific allergen extracts by pricking the patient's skin with a needle containing small amounts of the respective allergen, has been and is still the most commonly used in-vivo sIgE test. Although the SPT is considered a reliable method to test for allergies, it has several drawbacks. Interpretation of SPTs may be difficult in children younger than two years because of their reduced reactivity, i.e., their prick test wheals are smaller than wheals of adults.<sup>[3]</sup> The comparison of results from different centers or studies across Europe is difficult<sup>[4]</sup> because different allergen extracts and different testing procedures are used by European allergy centers, a limitation that equally applies to different in-vitro diagnostic assays. Further, the test cannot be performed under some conditions such as skin disorders, and children often do not tolerate multiple skin needle pricks. Finally, despite its high sensitivity for most allergens, the test is less reliable for food allergens than for aeroallergens.<sup>[5,6]</sup> Thus, the measurement of sIgEs in serum has become an additional and widely accepted tool in the diagnosis of allergies, complementary to the SPT.<sup>[7]</sup> Such sIgE antibodies have now been used as biomarkers for more than 40 years.<sup>[8,9]</sup>

Most approaches to evaluate serum sIgE are generally time-consuming laboratory tests and require relatively large amounts of blood plasma or serum for each individual allergen to be tested (60–100  $\mu$ L). For small children, the blood collection of several milliliters, which is required to test the IgE reactivity to several allergens, may be unfeasible. Besides these laboratory tests, point-of-care (POC) tests are increasingly available which allow serum sIgE measurements by health care professionals in their general practice. However, most of these devices include only 10 or 12 allergens with a grading of only up to three sensitization levels. Because an early diagnosis of the potential allergy is most important for treatment and prevention of disease progression, there is a need for a standardized and easy-to-use POC test that can assess a large number of allergens with a minimal blood volume.

The commercially available rapid POC test FastCheckPOC 20 (FCP20) simultaneously measures serum sIgE of 20 allergens with five sensitization levels. This modified ELISA system requires only small volumes of whole blood (200  $\mu$ L), plasma or serum (67  $\mu$ L), and it can be easily performed in the physician's office allowing health care professionals to generate all relevant parameters needed for the in-house diagnosis of allergic conditions. The test results are available within 30 min, thus eliminating patient revisits and allowing for immediate therapy and treatment. The multiplex test is

manually performed in a simple sequence of steps using the same syringe for all reagents without the need for any other instruments. The test system is fully closed as all waste reagents remain inside the test cassette. The amount of bound sIgE is visualized by an enzymatic reaction that forms purple-stained bands and the intensity of the colored bands correlates with the patient's level of sensitization. Reading the test allows to differentiate between five sensitization levels (FCP20 levels 1–5) hereby facilitating interpretation of the signals and patient's sensitization.

The present FCP20 panel allows the diagnosis of the following 20 aero- and food allergens: common ragweed, mugwort, timothy grass, grass mix (sweet vernal grass, orchard grass, perennial rye grass, and kentucky blue grass), rye, silver birch, ficus/latex mix, cat dander, dog dander, house dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*), egg white, cow milk, cod, wheat, peanut, soy, celery, almond, walnut, and hazelnut. The composition of the allergen panel was based on the most frequently occurring specific allergens in Europe.<sup>[4,10]</sup> As a limited number of allergens can be tested in parallel with the FCP20, some allergens were used as mixtures.

The aim of the present study was to evaluate the performance of the new generation FCP20 test system by comparing FCP20 results to an established laboratory method, the Phadia ImmunoCAP, and the SPT. The ImmunoCAP system is widely used and known to offer accurate and consistent test results.<sup>[11–14]</sup> The SPT, as outlined above, is the preferred method for in-vivo testing for IgE-mediated sensitivity.<sup>[15]</sup>

## Methods

### Study information

This was an intra-individually controlled, multicenter performance evaluation at six sites in Germany including hospitals, adult and pediatric allergy centers, and medical practices (Ludwig-Maximilians University Munich; Charité, Berlin; UZDAA, Berlin; and others). The performance evaluation was done in accordance with DIN EN 13612:2002-08 (Performance evaluation of in vitro diagnostic medical devices) and in compliance with the ethical principles originating in or derived from the Declaration of Helsinki. The evaluation was approved by the applicable ethics committees and a written informed consent was obtained from each patient or the patient's legal representative.

### Study population

The study comprised 200 patients including 176 adults from 18–78 years of age with a median age of 35 years and 24 children with a median age of 2.5

years with 75% being  $\leq 4$  years. All patients were included into this study based on either criteria: (1) new patients with relevant clinical allergic symptoms referred to allergy testing or (2) patients with known allergic reactions in the SPT or with an sIgE level of  $\geq 3.5$  kU<sub>A</sub>/L and an indication for sIgE retesting and optionally an SPT. To increase the percentage of ImmunoCAP positive results the patients were categorized into groups (either indoor inhalant-related allergies, outdoor inhalant-related allergies or food-related allergies) based on the clinical history and symptoms, see section “ImmunoCAP” for details. The number of allergens per skin prick test also varied, see section “Skin prick test” for details.

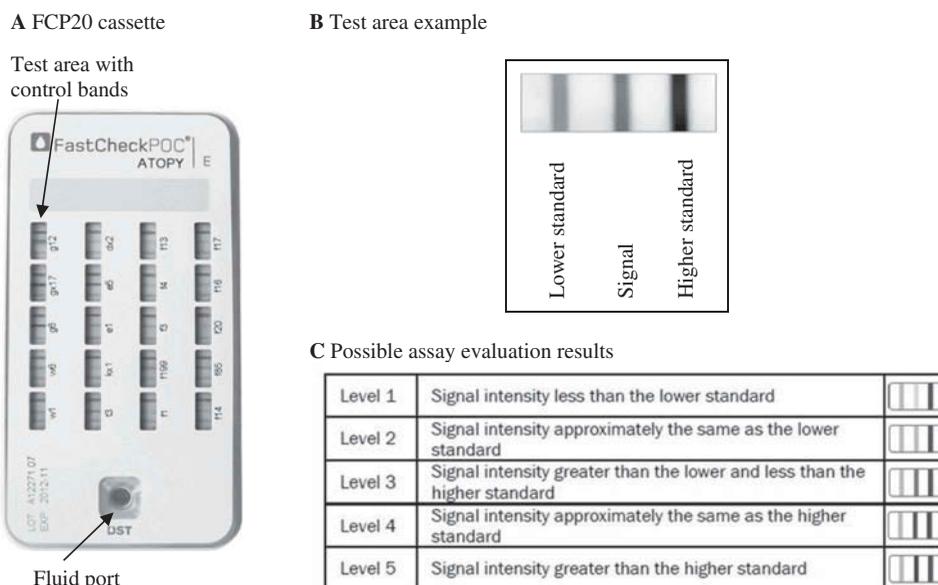
### **Allergy testing**

Study patients were tested with the ImmunoCAP (Phadia AB, Uppsala, Sweden) and SPT for a panel of allergens. The allergen panel for ImmunoCAP was set according to the classification of having an indoor or outdoor inhalant-related or food-related allergy, the panel of allergens used for SPT was selected by the physician based on prior anamnesis. All patients were tested with the FCP20. All tests were performed during routine allergy diagnosis.

### **FCP20 test**

The FCP20 is a modified enzyme linked immunosorbent assay. The fluidics inside the test cassette were optimized via an intricate design of its micro-channels. In turn, this allows reducing sample volumes and minimizes the required incubation times of sample and antibodies. The assay device contains 4 rows with 5 test areas (windows) each, with each test area consisting of a specific allergen and 2 controls (low and high) bound to a membrane (Figure 1). Upon addition of the test blood sample, specific IgE antibodies of the sample bind to the corresponding antigens. The bound human IgE is detected by specific anti-human-IgE antibodies. A secondary antibody coupled to alkaline phosphatase is used for signal amplification. At the end, a color reagent is added which is converted by the antibody coupled alkaline phosphatase to an indigo colored precipitate at sites where IgE is bound. Test results are visible as bands with a color intensity that is proportional to the amount of bound sIgE. Sensitization levels can be read semi-quantitatively with the naked eye for all 20 allergens by comparing the color intensity of the test line with that of the corresponding controls (low, high) in each test area. The correlation between FCP20 level, sensitization, CAP class, and sIgE as determined in previous evaluations is shown in Table 1.

The FCP20 tests were carried out according to the manufacturer’s instructions. The tests were performed using one whole blood sample (200  $\mu$ L) and one plasma or serum sample (67  $\mu$ L) per patient. Five sites applied plasma samples, one site applied serum samples. Former evaluations showed that



**Figure 1.** The FastCheckPOC 20 test system. The test cassette contains 20 test areas with each test area consisting of a specific allergen and two controls (A). The patient's blood sample is transferred to the fluid port. IgE molecules bound to the test area are detected via specific anti-human-IgE antibodies and a secondary antibody used for signal amplification. IgE bands are visualized via alkaline phosphatase coupled to the secondary antibody, which forms a colored precipitate at sites where IgE is present after adding a color reagent (B). The intensity of the color reaction is proportional to the amount of bound IgE. By comparing the color intensity of the test line with the corresponding controls sensitization levels can be obtained (C).

**Table 1.** Correlation between FCP20 level, sensitization, CAP class and sIgE.

FCP20 level	Sensitization	CAP class	sIgE [kU <sub>A</sub> /L]
1	low	0–2	0–3.4
2	moderate	2–3	0.7–17.4
3	high	3–4	3.5–49.9
4	very high	4–5	17.5–100
5	very high	5–6	>50

sIgE = specific immunoglobulin E.

FCP20 results do not differ between plasma and serum (data not shown). The intensity of each test band was classified to one of the five FCP20 levels by visual comparison with the control bands by three independent operators who had only limited training. For the analysis of sensitivity and specificity mean FCP20 data were dichotomized as follows: FCP20 Level  $\geq 2$  = diseased and FCP20 Level  $< 2$  = healthy.

### ImmunoCAP

ImmunoCAP (Phadia AB, Uppsala, Sweden) is a widely used laboratory test system to detect relevant inhalant and food allergens by measuring

the patient's allergen specific IgE levels in plasma or serum. The number and type of allergens tested for specific IgE antibodies depended on the categorization into one or more allergen groups (indoor, outdoor, and/or food), which were assumed to be relevant for the patient. Besides this classification, the choice of allergens within each group was based as well on known cross reactions between inhalant and food allergens. Thus, the IgE reaction to a particular allergen with the ImmunoCAP was measured only for a subgroup of the 200 patients.

The following allergen panels were used: outdoor inhalant: common ragweed, mugwort, timothy grass, perennial rye grass, rye, silver birch, peanut, soy, celery, almond, walnut, and hazelnut; indoor inhalant: ficus, latex, cat dander, dog dander, house dust mites (*D. pteronyssinus* and *D. farinae*); food: egg white, cow milk, cod, wheat, peanut, soy, celery, almond, walnut, hazelnut, mugwort, and silver birch.

The  $kU_A/L$  values measured with the ImmunoCAP system were categorized into CAP classes 0–6 as follows: Class 0:  $<0.35 kU_A/L$ , Class 1:  $\geq 0.35$  to  $<0.7 kU_A/L$ , Class 2:  $\geq 0.7$  to  $<3.5 kU_A/L$ , Class 3:  $\geq 3.5$  to  $<17.5 kU_A/L$ , Class 4:  $\geq 17.5$  to  $<50 kU_A/L$ , Class 5:  $\geq 50$  to  $<100 kU_A/L$ , Class 6:  $\geq 100 kU_A/L$ . For the analysis of sensitivity and specificity ImmunoCAP data were dichotomized as follows: CAP Class  $>2$  = diseased, CAP Class  $\leq 2$  = healthy.

### **Skin prick test**

Skin prick testing with routine allergens was done according to standard clinical practice. The tested allergens depended on the allergy anamnesis and the allergen panels routinely used at the sites. Originally SPT results were reported by wheal diameter or on a “+” scale. To standardize SPT assessments, results were classified into three classes as follows. Class 0 included 0, (+), (++) , or wheal diameter  $<3$  mm (if positive control wheal diameter  $>4$  mm) or  $<2$  mm (if positive control wheal  $\leq 4$  mm); Class 1 included +, ++, or wheal diameter 3–6 mm (if positive control wheal diameter  $>4$  mm) or 2–4 mm (if positive control wheal  $\leq 4$  mm); Class 2 included +++, +++++, or wheal diameter  $>6$  mm (if positive control wheal diameter  $>4$  mm) or  $>4$  mm (if positive control wheal  $\leq 4$  mm).

As SPT procedures as well as the SPT results at the six sites differed widely, we used the following dichotomizations based on the different sizes of the positive controls: for tests with a control wheal diameter of  $>4$  mm, a test wheal diameter of  $>6$  mm was considered diseased and a test wheal diameter of  $\leq 6$  mm as healthy; for tests with a control wheal diameter of  $\leq 4$  mm a test wheal diameter of  $>4$  mm was considered diseased and a test wheal diameter of  $\leq 4$  mm as healthy.

### **Clinical evaluation of the FastCheckPOC 20 test**

The FastCheckPOC 20 panel comprised the following 20 allergens: common ragweed, mugwort, timothy grass, grass mix (sweet vernal grass, orchard grass, perennial rye grass, and kentucky blue grass), rye, silver birch, ficus/latex mix, cat dander, dog dander, house dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*), egg white, cow milk, cod, wheat, peanut, soy, celery, almond, walnut, and hazelnut. The sensitivity (true positive rate), specificity (true negative rate), and accuracy were calculated for the FCP20 compared with the ImmunoCAP and SPT for all 20 allergens as follows (with N = number of samples):

- For the comparison with the ImmunoCAP:

Sensitivity:

$$\frac{\text{N with CAP Class } > 2 \text{ and FCP20 Level } \geq 2}{\text{N with CAP Class } > 2}$$

Specificity:

$$\frac{\text{N with CAP Class } \leq 2 \text{ and FCP20 Level } < 2}{\text{N with CAP Class } \leq 2}$$

Accuracy:

$$\frac{(\text{N with CAP Class } > 2 \text{ and FCP20 Level } \geq 2) + (\text{N with CAP Class } \leq 2 \text{ and FCP20 Level } < 2)}{\text{N of all samples}}$$

- Performance data of FCP20 compared to SPT:

Sensitivity:

$$\frac{\text{N with SPT Class } 2 \text{ and FCP20 Level } \geq 2}{\text{N with SPT Class } 2}$$

Specificity:

$$\frac{\text{N with SPT Class } \leq 1 \text{ and FCP20 Level } 1}{\text{N with SPT Class } \leq 1}$$

Accuracy:

$$\frac{(\text{N with SPT Class } 2 \text{ and FCP20 Level } \geq 2) + (\text{N with SPT Class } \leq 1 \text{ and FCP20 Level } 1)}{\text{N of all samples}}$$

## **Statistical analysis**

For each allergen, the mean FCP20 level of the three independent readers was calculated and the rounded (to the integer) value was used for further analyses.

## **Results**

### **Summary of analyzed data and inter-reader variance for the FCP20 results**

FCP20 whole blood test data of 199 patients and plasma/serum data of 200 patients were analyzed. The analysis of one FCP20 whole blood test failed due to an operating error. The ImmunoCAP test was performed with blood samples from 199 patients and the SPT was performed on 168 patients. For the ImmunoCAP and SPT, patients were tested only for a panel of suspected allergens according to their classification of having an indoor or outdoor inhalant-related or food-related allergy. Thus, the number of patients tested for individual allergens varied, between 57 and 165 for the ImmunoCAP system and between 18 and 161 for the SPT.

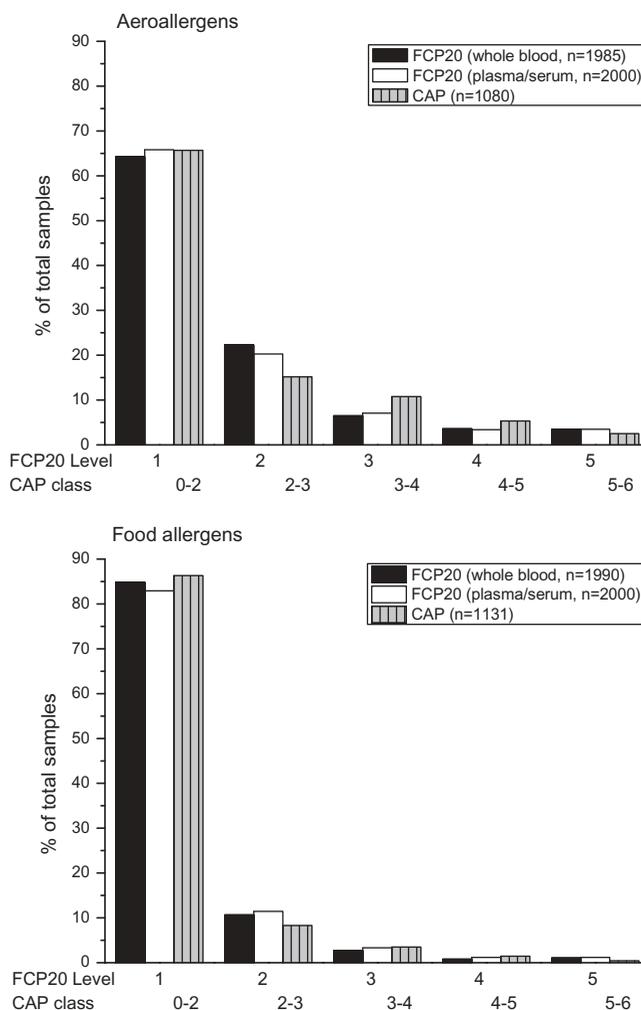
The concordance of the read-outs by the 3 independent operators for FCP20 results was >97%, i.e., FCP20 test results did not differ by more than 1 level from the median FCP20 level of the 3 readers (data not shown).

### **Concordance of FCP20 levels and ImmunoCAP classes**

The distribution of the FCP20 test results versus the ImmunoCAP results by FCP20 level and CAP class for all aeroallergens and food allergens are shown in [Figure 2](#). The great majority of samples were tested negative (FCP20 Level 1) for both aeroallergens (about 65%) and food allergens (85%) with only few subjects showing a high or very high sensitization (FCP20 Levels 3–5). Excellent agreement was observed between the whole blood and serum/plasma FCP20 test results, and between the FCP20 and ImmunoCAP results.

Detailed test results for two individual allergens, common ragweed and mugwort, are summarized in [Table 2](#) including only subjects for whom both tests were performed. The results confirmed the trend seen above with all allergens.

Similarly to the results described for the groups of aeroallergens and food allergens ([Figure 2](#)), for both individual allergens an excellent agreement was observed between FCP20 levels assessed with blood or plasma/serum, and between FCP20 levels and ImmunoCAP classes. For both blood or plasma/serum, 68% (common ragweed) or 77% (mugwort) of the samples that were tested negative in the CAP system (CAP Class 0 or 1) were also tested negative with the FCP20 (FCP20 Level 1 or 2, [Table 2](#)). For positive results, 88% of samples tested CAP Class 3 or higher were also tested positive with



**Figure 2.** Test results by FCP20 level and CAP class. n = number of samples. Comparison of the distributions of FCP20 levels and CAP classes for all tested samples. Samples of which the CAP class is included in two CAP class groups were equally distributed between these groups (e.g., half of the samples with a CAP class 2 result were each assigned to the 0-2 and 2-3 CAP class group).

the FCP20 (FCP20 level >2) for common ragweed in both, blood samples and plasma/serum samples. For mugwort, the concordance was 100% for blood samples and 86% for plasma/serum samples (Table 2). The overall number of samples tested positive with the FCP20 and CAP classes matched results from previous studies.<sup>[16,17]</sup>

### ***Sensitivity, specificity, and accuracy of FCP20 vs. the ImmunoCAP and SPT***

The sensitivity and specificity of the FCP20 system (whole blood) relative to the ImmunoCAP system and SPT are shown in Table 3. Compared with the

**Table 2.** Comparison of FCP20 levels with CAP classes for common ragweed and mugwort. Example (blood): Sensitivity for Ragweed was 88% as determined by 7 true positives when compared to CAP measurements and 1 false negative when compared to CAP measurements.

FCP20 level	Blood samples					Plasma/serum samples					
	N	CAP class				N	CAP class				
		0/1	2	3	4		5/6	0/1	2	3	4
Ragweed	127					128					
FCP20 Level 1		68	13	1	0	0	68	13	1	0	0
2		24	7	3	0	0	24	7	3	0	0
3		2	5	1	2	0	3	4	1	2	0
4		0	0	0	0	0	0	1	0	0	0
5		0	0	0	1	0	0	0	0	1	0
Mugwort	164					165					
FCP20 Level 1		107	8	0	0	0	109	9	2	0	0
2		22	6	8	0	0	22	5	6	0	0
3		7	0	1	0	0	6	0	1	1	0
4		0	0	1	2	0	0	0	1	1	1
5		0	0	0	1	1	0	0	0	1	0

**Table 3.** Sensitivity and specificity of FCP20 (whole blood) versus CAP system and SPT.

Allergen	CAP				SPT			
	Sensitivity		Specificity		Sensitivity		Specificity	
	n	%	n	%	n	%	n	%
Common ragweed	8	88	119	68	1	-	141	69
Mugwort	14	100	150	77	8	63	146	73
Timothy grass	50	92	75	79	40	75	121	64
Grass mix	50	84	59	83	40	68	121	63
Rye	46	83	81	85	30	67	126	71
Silver Birch	70	81	93	52	38	74	117	36
Ficus/Latex mix	2	100	63	92	0	-	79	91
Cat dander	15	80	50	70	19	63	82	71
Dog dander	6	100	53	51	3	-	91	46
House dust mites	21	76	48	81	21	52	136	78
Egg white	2	100	55	93	0	-	34	94
Milk	1	100	56	84	0	-	59	90
Cod	0	-	56	91	0	-	36	92
Wheat	3	67	57	89	1	-	21	76
Peanut	8	50	142	94	1	-	33	91
Soy	2	50	146	81	0	-	58	81
Celery	11	36	137	88	5	60	55	82
Almond	2	50	148	70	1	-	17	82
Walnut	4	25	146	92	0	-	25	100
Hazelnut	55	47	94	82	2	-	36	72
<b>Mean value (weighted)</b>	<b>370</b>	<b>76</b>	<b>1828</b>	<b>80</b>	<b>210</b>	<b>66</b>	<b>1534</b>	<b>71</b>

The weighted mean values were calculated with respect to the total numbers of n per allergen relative to the overall total number.

n = number of tests included in the respective calculation of sensitivity and specificity.

- = not calculated as n was below the minimum number of positive samples.

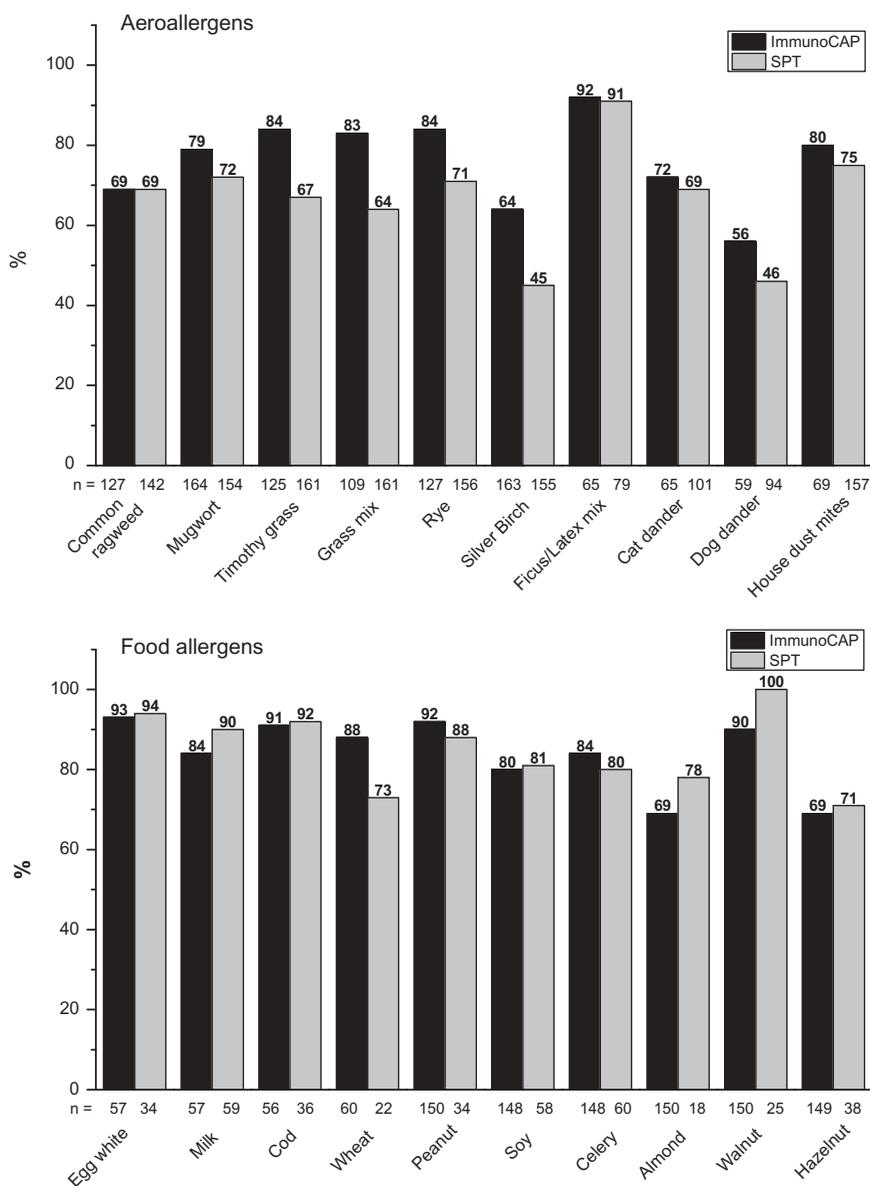
ImmunoCAP system the overall sensitivity and specificity over all 20 allergens was 76% and 80%, respectively. Sensitivity and specificity varied among the 20 allergens. For aeroallergens, the sensitivity varied between 76% and 100% and specificity between 51% and 92% with many being  $\geq 80\%$ . For food allergens, the sensitivity tended to be lower ranging from 25–100%. Similarly to aeroallergens, the specificity of food allergens ranged from 70–94% with most being  $\geq 85\%$ . Compared with the SPT system the overall sensitivity and specificity for all 20 allergens combined was 66% and 71%, respectively, varying between 52% and 75% for sensitivity and 36% and 100% for specificity.

The accuracy of the FCP20 system (whole blood) relative to the ImmunoCAP system and SPT is shown in [Figure 3](#). For all allergens accuracy of FCP20 was high compared with both, the ImmunoCAP and SPT system. The overall accuracy over all 20 allergens was 80% when compared with the ImmunoCAP system and 70% compared with the SPT. Accuracy varied among the 20 allergens with higher accuracies observed for food allergens compared with aeroallergens. For aeroallergens accuracies varied between 56% and 92% and were almost all above 64% (with the exception of dog dander) when compared to the ImmunoCAP with most being above 80%. Compared to the SPT, accuracies of aeroallergens were lower than those observed versus the ImmunoCAP and were between 45% and 91% with most values being above 65%. Accuracy of silver birch and dog dander was comparatively low with both compared with the ImmunoCAP (64% and 56%, respectively) and the SPT (45% and 46%, respectively) due to low specificities. For all food allergens accuracies were above 69% (range 69–93%) when compared with the ImmunoCAP with most being above 80%. Very few patients were tested positive for food allergens with the SPT, nevertheless, the accuracy of each food allergen was similarly high as when compared to the ImmunoCAP with all accuracies being above 70% and most above 85% (range 71–100%). Low accuracies were observed for hazelnut and almond when compared with ImmunoCAP and SPT.

Because FCP20 results are not influenced by the sample used, i.e., whole blood or plasma/serum (see above), sensitivity, specificity, and accuracy for the FCP20 using plasma/serum samples were very similar to those observed with whole blood samples (data not shown).

## Discussion

Allergy diagnostic testing is important to evaluate and manage allergic diseases because in most cases the assessment of the clinical history is not sufficient to clearly identify the specific allergen the patient is sensitized to. Besides data on specific sensitivities, such tests also provide information on the future risk to develop an allergic condition and on the severity and/or



**Figure 3.** Accuracy - comparison of FCP20 (whole blood) versus ImmunoCAP and SPT. n = number of ImmunoCAP or SPT results (patients), SPT = skin prick test. Concordance of FCP20 results relative to ImmunoCAP results (black) and SPT results (grey) depicted for all 20 allergens. Example: Accuracy for Common ragweed (n = 127) was 69% as determined by 88 true positives and true negatives when compared to ImmunoCAP measurements.

persistence of the allergic disease.<sup>[18]</sup> The two most commonly used tests are skin testing and serum sIgE measurements.

The main objective of the present study was to evaluate the performance of a new diagnostic POC testing system, i.e., the FastCheckPOC 20 to determine sensitization to specific aero- and food allergens compared to objective and

well-established in-vivo and laboratory test methods. Specific IgE antibodies to 20 allergen components of the FCP20 test were evaluated. The SPT was used as the clinical method, the ImmunoCAP as the in-vitro test system for comparison. Although there is no accepted reference standard for in-vitro sIgE measurements, the ImmunoCAP system has emerged as quasi standard for laboratory tests.<sup>[13]</sup> It has been validated in a many studies including thousands of subjects, and sensitivities and specificities above 90% were reported with a wide variety of diagnostic standards used (e.g., clinical history, SPT, or combination of both).<sup>[19]</sup>

The present study demonstrated good accuracy of the FCP20 with both the ImmunoCAP system and SPT. In addition, FCP20 results were independent from the sample used, i.e., whole blood, plasma, or serum. Across all 20 allergens, accuracy of positive and negative results of the FCP20 with the ImmunoCAP was 80%, and 70% with the SPT. The accuracy values for individual allergens varied as observed with other diagnostic modalities.<sup>[7,20,21]</sup> For food allergens the accuracies tended to be higher than for aeroallergens, particularly when the FCP20 was compared to the SPT. The accuracy of the FCP20 and the ImmunoCAP was comparatively low for hazelnut, birch, almond, and dog dander. For hazelnut, this is most probably due to the fact that in the FCP20 system pure hazelnut extract is used while in the ImmunoCAP system hazelnut extract is used that is spiked with coral, an allergen that correlates primarily with the pollen of the hazelnut tree but to a much lesser extent with the nut. For birch, FCP20 detected a higher number of samples as sIgE positive compared with the ImmunoCAP system. For almond and dog dander, current productions of FCP20 have been modified to achieve higher accuracies.

In addition to the binary “all or nothing” assessment, the FCP20 allows an easy semi-quantitative classification into four positive levels (2–5) which was in good concordance with ImmunoCAP classes. This classification is extremely valuable for the health care professional because a quantitative approach allows a higher diagnostic precision as shown in previous studies.<sup>[22]</sup> A high level, i.e., a high IgE concentration, implies a higher degree of sensitization and a higher probability of clinical allergic reactivity. A high level, therefore, may allow an allergy diagnosis without further procedures, while lower levels may need additional assessments.

The performance of the FCP20 is good considering that it is an easy-to-use, simple and rapid test that does not require complex laboratory testing. The results compare very favorably to other rapid immunotests. Sensitivities for the ImmunoCAP Rapid system were recently reported to be between 51% and 80% when compared with the ImmunoCAP and between 19% and 71% when compared with the SPT for a variety of aeroallergens.<sup>[23]</sup> In comparison, for aeroallergens sensitivities reached with the FCP20 were between 76% and 100% vs. the ImmunoCAP and between 52% and 75% vs. the SPT.

Sensitivities for dog dander were also comparatively low with the ImmunoCAP Rapid indicating that dog dander might be a difficult allergen in membrane-based rapid tests. Food allergens were not tested with the ImmunoCAP Rapid. Finally, tests with a sensitivity and specificity of  $\geq 70\%$  are considered to be meaningful for in-vitro allergy diagnosis.<sup>[24]</sup>

Based on these results the FCP20 is a more accurate screening test for the rapid detection of allergen-specific IgE than the ImmunoCAP Rapid. In addition, the FCP20 allows the simultaneous evaluation of 20 allergens, including food allergens, while the ImmunoCAP Rapid is restricted to 10 aeroallergens. The possibility to obtain semi-quantitative results is a further advantage of the FCP20 compared with the “all-or-nothing” assessment with the ImmunoCAP Rapid.

One limitation of the study was the low prevalence of sensitization to food allergens in samples assessed with the SPT which may bias the results. Overall, the agreement between the FCP20 and the SPT was lower than compared with the ImmunoCAP, but this was not unexpected. Literature data show a higher correlation of the ImmunoCAP with skin testing, i.e., 87–95%,<sup>[19]</sup> compared with the observed correlation of 70% of the FCP20 vs. the SPT. For food allergens, the accuracy obtained for the FCP20 compared with the SPT were in most cases above 80%, and half of the tested food allergens were above 85%. Further, the SPT was not standardized in this study which may negatively affect the results. Inter-center differences in SPT techniques, grading and interpretation are of particular concern in this respect.<sup>[25]</sup>

Overall, this study demonstrates that the FCP20 test is a valuable tool in routine allergy diagnostics. It can be used with whole blood samples, plasma or serum samples. The results obtained with this rapid simple POC were in good agreement with the ImmunoCAP, a technically advanced laboratory test, and the SPT. Noteworthy, the personnel performing the tests did receive no or only limited training showing that the FCP20 can be easily performed and evaluated. Thus, the new FCP20 serves as a fast, easy-to-use and reliable alternative to time-consuming laboratory sIgE testing.

### ***Abbreviations used***

FCP20	FastCheckPOC 20
POC	point-of-care
SPT	skin prick test
sIgE	specific immunoglobulin E

### ***Availability of data and materials***

The data involved in the current study are available upon request.

## Acknowledgments

We thank Annette Rempel, FGK Clinical Research GmbH, for supporting the writing of the manuscript on behalf of DST Diagnostische Systeme & Technologien GmbH.

## Ethics approval and consent to participate

The performance evaluation was done in accordance with DIN EN 13612:2002-08 and in compliance with the ethical principles originating in or derived from the Declaration of Helsinki. A written informed consent was obtained from each patient or the patient's legal representative. Further, approval of the study was obtained from the Ethics Committee of the Federal Association of Medical Doctors in Bavaria (reference number 12123) and the Medical Ethics Committee at the Ludwig-Maximilian University Muenchen (reference number 534-12).

## Funding

The present research described within this manuscript was funded by DST Diagnostische Systeme & Technologien GmbH.

## Competing interests

The authors are employees of DST Diagnostische Systeme & Technologien GmbH.

## Notes on contributor

MDA designed the study and acted as coordinating investigator. RR performed the analysis, interpreted the data and wrote the manuscript. All authors evaluated the results and discussed the conclusions and actively participated in the elaboration of the manuscript. All authors read and approved the final manuscript.

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