ImmunoCAP® Rapid

A near-patient test for qualitative detection of
specific IgE antibodies
against inhalation allergens in human whole blood

manufactured by Phadia AB, now Thermo Fisher Scientific Inc,
Uppsala, Sweden

Report from an evaluation
under standardised and optimal conditions
and in primary health care
organised by SKUP

Evaluated at the request of Phadia, Denmark

SKUP/2013/68

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A detailed list of previous SKUP evaluations is included in the attachments.
Attachments with raw data are only included in the copy to Phadia.

1. Summary

The evaluation of ImmunoCAP Rapid was performed on the request in 2007 of the market company in Denmark, Phadia ApS. The evaluation was performed in the Department of Clinical Biochemistry, Odense University Hospital and Hillerød Hospital. Thirteen primary health care centres and one specialised clinic for allergic diseases represented the intended users.

The aim of the evaluation

In primary health care

- Determination of the sensitivity and the specificity of house dust mite (d1), cat epithelia (e1), birch pollen (t3), dog epithelia (e5), mugwort pollen (w6) or timothy grass pollen (g6), and *Alternaria alternata* (m6) with ImmunoCAP Rapid compared to skin prick test and Phadia 250.
- Number of samples/persons included in the evaluation should be at least 100 positive and 100 negative results with skin prick test for at least two of the following antigens: house dust mite (d1), cat epithelia (e1), birch pollen (t3), dog epithelia (e5), mugwort pollen (w6) or timothy grass pollen (g6), and *Alternaria alternata* (m6).
- Count the fraction of positive results for cockroach (i6), olive pollen (t9), wall pellitory (w21) with ImmunoCAP Rapid.
- Determination of the "intra-person agreement in testings" by letting the same person perform repeated tests with the same sample.
- Determination of the "inter-person agreement in readings" by letting two persons perform readings on the same test cassette on 20% of the tests
- Evaluation of user-friendliness of ImmunoCAP Rapid

In the hospital laboratory

- Compilation of facts about the test system
- Repeat approximately 1/5 of the tests from primary health care, but using heparinised venous whole blood with ImmunoCAP Rapid.
- Evaluation of user-friendliness of ImmunoCAP Rapid

The results were compared with: 1. Skin prick test and 2. Phadia 250.
ImmunoCAP Rapid is using the same antigens as Phadia 250. According to internal studies made by the manufacturer ImmunoCAP Rapid has a level of detection of 1-2 kIU/L for all antibodies. In this evaluation 0-1,49 kIU/L with Phadia 250 is considered as negative and concentrations above 1,49 kIU/L are considered as positive when compared to ImmunoCAP Rapid. This is higher than the threshold (0,35 kIU/L) for positive results on Phadia 250. The serum results of Phadia 250 were also compared to the skin prick test using 0,35 kIU/L as the cut-off.

Materials and methods

ImmunoCAP Rapid was evaluated with capillary whole blood samples from 300 patients from 13 primary health care centres and one specialised clinic for allergic diseases. Standard skin prick tests (10 allergens) were performed on all participants with Soluprick, ALK Abello, Denmark. For each patient a serum sample were analysed for six allergens on Phadia 250. About 1/7 of the samples were analysed using heparin whole blood with ImmunoCAP Rapid in hospital.

Quality goals in the evaluation of ImmunoCAP Rapid

	Comparison **Skin prick test**	Comparison **Phadia 250**
Sensitivity *	>85%	>95%
Specificity	>85%	>95%
Fraction of technical errors	2% or less	
User-friendliness	satisfactory	

*the sensitivity is expected to be much lower than 85% for timothy and dog dander

Results

Sensitivity, specificity, percentage of positive skin prick test and positive and negative predictive value of ImmunoCAP Rapid compared to skin prick test (in percent) is given in the table below. The sensitivity and specificity figures are printed in green if the results fulfil the quality goals, yellow if the results are inconclusive or red if the results don't fulfil the quality goals.

Allergen	Name	Percentage of positive skin prick test	Sensitivity	Specificity	PPV	NPV
	Cat epithelium and dander	22,2	**71,2**	**99,6**	97,9	92,4
	Common silver birch (pollen)	31,6*	**69,1**	**97,5**	92,9	87,2
	Mugwort	17,5	**59,6**	**96,7**	79,5	91,9
	Timothy	36,7*	**59,6**	**98,4**	95,6	80,8
	Dog dander	26,6	**19,0**	**98,6**	83,3	77,1
	House dust mites** *Dermatophagoides pteronyssinus* *Dermatophagoides farinae*	31,6	**70,2**	**95,6**	88,0	87,4
	Mould** *Alternaria alternata* *Cladosporium hebarum*	9,1	**59,3**	**99,3**	88,9	96,1

* The inclusions were random until 85 patients with positive skin prick tests for common silver birch and grass (timothy) were enrolled, then patients positive for birch or grass in skin prick test were chosen prior to others. This was agreed in order to finish the evaluation, however; the percent of positive skin prick test for birch and grass did not change. ** These results refer to the sum of skin prick tests positive for either one or both of the two allergens.

Sensitivity, specificity, percentage of positive Phadia 250 test and positive and negative predictive value of ImmunoCAP Rapid compared to Phadia 250 (in percent) is given in the table below. The sensitivity and specificity figures are printed in green if the results fulfil the quality goals, yellow if the results are inconclusive or red if the results don't fulfil the quality goals.

Allergen	Name	Percentage of positive Phadia 250 test	Sensitivity	Specificity	PPV	NPV
	Cat epithelium and dander	10,9	**75,0**	**90,8**	50,0	96,7
	Common silver birch (pollen)	22,4*	**80,3**	**92,5**	75,7	94,2
	Mugwort	9,2	**51,9**	**90,6**	35,9	94,9
	Timothy	22,1*	**76,9**	**92,6**	74,6	93,4
	Dog dander	4,4	**53,8**	**96,1**	38,9	97,8
	House dust mites** *Dermatophagoides pteronyssinus* *Dermatophagoides farinae*	21,4	**79,4**	**89,2**	66,7	94,1
	Mould** *Alternaria alternata* *Cladosporium hebarum*	5,1	**80,0**	**97,8**	66,7	98,9

* The inclusions were random until 85 patients with positive skin prick tests for common silver birch and grass (timothy) were enrolled, then patients positive for birch or grass in skin prick test were chosen prior to others. This was agreed in order to finish the evaluation, however; the percent of positive skin prick test for birch and grass did not change. ** These results refer to the sum of skin prick tests positive for either one or both of the two allergens below.

The fraction of positive results for cockroach (i6), olive pollen (t9), wall pellitory (w21) with ImmunoCAP Rapid was expected to be low. This was also the case: 1,3%, 2,3 and 2,0% of the 298 results were positive. These allergens are therefore not evaluated as the statistical basis is too small.

Intra-person reading agreement: >99,9%
Inter-person reading disagreement: 14 of 1280 test readings were not in accordance with each other ~1,1%
Disagreement, capillary samples / heparin samples: 17 of 780 heparinised whole blood sample results analysed with ImmunoCAP Rapid were not in accordance with the corresponding capillary sample results ~2,2%.

Technical errors: There were technical errors in two test cartridges but both errors just occurred in one of two test windows. The fraction of technical errors was ~0,3% and less than the quality goal <2,0%

The user friendliness was evaluated by more than 10 individuals and they were in general terms pleased with the test. Some concerns were present among the evaluators regarding the interpretation of the test for low concentration of allergens, since the distinction between positive and negative responses was difficult. There was great satisfaction with the fact that the test can be performed even if the patient is on medication for rhinitis symptoms, and that it was possible to perform the test using venous heparinised whole blood after the patient left the clinic.

Overall, ImmunoCAP Rapid showed good user friendliness, and the evaluators expressed that ImmunoCAP Rapid was very easy to operate.

Conclusions

Compared to the skin prick test

The quality goal for sensitivity was >85%. The sensitivity of ImmunoCAP Rapid was expected to be low for timothy (60-70%) and even lower for Dog Dander. The found sensitivities were about 60% and 19%, respectively. All sensitivity results, including confidence intervals are lower than 85% and therefore the quality goal for sensitivity was not fulfilled.
The quality goal for specificity was >85%. This quality goal was reached for all components when compared to skin prick test.

Compared to Phadia 250

The quality goal for sensitivity was >95%. A positive result on ImmunoCAP Rapid should correspond to about 1,50 kIU/L on Phadia 250.
The found sensitivity was lower than the quality goal for all components, lowest for dog, 53,8%, and highest for common silver birch, 80,3%.

The quality goal for specificity was >95%. This quality goal was reached for dog dander and mould. For common silver birch (92,5%) and timothy (92,6%) the confidence interval of the specificity results included the 95% goal. The specificity for cat epithelium and dander (90,8), mugwort (90,6%) house dust mites (89,2%) did not fulfil the goal.

The user-friendliness was satisfying for the manual, time factors and operation facilities. The evaluators found the test easy to use. They think it is an improvement that the patients can be tested during medication for rhinitis, but it was mentioned that the reading of test results can be difficult, as it is sometimes difficult to determine whether a test is positive or negative

Technical errors: There was 0,3% technical errors. The quality goal <2,0% was fulfilled.

2. Abbreviations

CI	Confidence Interval
C-NPU	Committee of Nomenclature, Properties and Units
CV	Coefficient of Variation
DAK-E	Danish Quality Unit of General Practice
DEKS	Danish Institute of External Quality Assurance for Laboratories in Health Care
EQA	External Quality Assessment
EQUALIS	External quality assurance in laboratory medicine in Sweden
GP	General Practitioner
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
ImmunoCAP Rapid	ImmunoCAP® Rapid specific IgE antibodies system
IUPAC	International Union of Pure and Applied Chemistry
NPV	Negative predictive value
NOKLUS	Norwegian Quality Improvement of Primary Care Laboratories
PPV	Positive predictive value
SKUP	Scandinavian evaluation of laboratory equipment for primary health care

3. Quality goals

To qualify for an overall good assessment in a SKUP evaluation, the measuring system must show satisfactory analytical quality as well as satisfactory user-friendliness.

There are no generally recognised analytical quality goals for near-patient measurements of allergen specific IgE antibodies. Quality goals are often based on biological variation [1]. However, healthy individuals are not supposed to have antibodies to allergens in high concentrations, and biological variation of the individual antibodies is therefore not described in the Westgard database [2].

3.1. Analytical quality goals

Quality goals for measurements of antibodies are closely related to the definitions of when the skin prick tests with specific allergens are positive. Therefore the definition of a positive skin prick test and the definition of the specific allergens are described together with the quality goals for ImmunoCAP Rapid in this chapter. The analytical quality of ImmunoCAP Rapid is assessed in a comparison with Skin Prick Test and with Phadia 250.

Evaluated parameters with quality goals in this evaluation:

- Sensitivity: The fraction of positive results on ImmunoCAP Rapid in proportion to the positive results with the comparison test: 1) Skin Prick Test and 2) Phadia 250.
  Quality goal: The result for ImmunoCAP Rapid should not differ significantly from the comparison method Phadia 250 test results. Compared to the skin prick test the sensitivity is expected to reach about 85% for most allergens except timothy and dog dander. The response to timothy pollen can originate from several species of the timothy grass, and the combination of allergens in the reagents varies considerably. The differences are even bigger for dog dander. Reagents are not identical and none of them cover all dog races. The response for one reagent can vary for dogs with long hair and dogs with short hair. The manufacturer expected the sensitivity to be about 60-70% for timothy and even lower for dog dander.

- Specificity: The fraction of negative results on ImmunoCAP Rapid in proportion to the negative results with a comparison test (Skin Prick Test or Phadia 250).
  Quality goal: The result for ImmunoCAP Rapid should not differ significantly from the comparison method Phadia 250 test results. Compared to the skin prick test the specificity is expected to reach about 85% for most allergens.

- Count the number of positive results for cockroach (i6), olive pollen (t9) and wall pellitory (w21). The allergens used in Europe differ across the countries. In Scandinavia it is not common to be allergic to cockroach, olive pollen or wall pellitory. Expectation: no cross reaction from other allergens and therefore a low number of positive results.

- Intra-person testing disagreement: The fraction of all results with the evaluated system, which is in disagreement in a repeated test read by the same evaluator. No quality goal.

- Inter-person reading disagreement: The fraction of all results with the evaluated system, which is in disagreement when read by different persons. No quality goal.

- Is the test positive at the time specified by the manufacturer? Quality goal: Reading at the specified time should give the best agreement with the comparison methods.

- Robustness. Is the test positive when the reading time deviates from the time specified by the manufacturer? Quality goal: Minor time differences should not influence on the results. Can the test be saved as documentation for the results?

For sensitivity and specificity the calculation of a 90% confidence interval (CI) is described in chapter 5 and in attachment 4.

The manufacturer expects that ImmunoCAP Rapid has a specificity of >95% for house dust mite (d1), cat epithelia (e1), birch pollen (t3), dog epithelia (e5), mugwort pollen (w6) and grass pollen (g6) if compared to Phadia 250.

Percentage of positive skin prick test, Positive and negative predictive values
Positive and negative predictive values are dependent on the percentage of positive skin prick test. Before the evaluation the percentage of positive skin prick test of the specific antigens were estimated in Fyn and in a hospital in the southern of Sweden. The percentage of positive skin prick test of the antigens in this evaluation is expected to be similar to the percentage of positive skin prick test in one of these two places (attachment 4). The percentage of positive skin prick test of positive skin prick tests and positive Phadia 250 results is calculated (chapter 5).
It was a wish from the client that the results for positive predictive value and negative predictive value were mentioned in the conclusion of the report. There were no goals for the positive and negative predictive values.

Limit for allowable technical errors
It is a wish from the National Danish Committee for General Practice Laboratory Testing, that the percentage of "tests wasted" caused by technical errors should not exceed 2%. User errors due to wrong handling are not included in "tests wasted" in section 3.3.

Quality goals in Denmark, Norway and Sweden:
The Scandinavian countries have not yet defined analytical quality goals for specific IgE antibodies against inhalation allergens for neither skin prick tests nor test strip devices [3-5].

3.1.1. *Definition of positive skin prick test*
Wheals more than 3 mm in diameter (an area of 7 mm^2 or more) are defined as positive [6], see also attachment 2.

3.1.2. *Definition of positive and negative results with the method Phadia 250*
Negative results are defined as samples with concentrations of specific allergens (IgE) less than 0,35 kIU/L for the component. Positive results are defined as samples with concentrations of specific allergens (IgE) of 0,35 kIU/L or more for the component [7].

3.1.3. *Definition of positive and negative results with the ImmunoCAP Rapid*
ImmunoCAP Rapid is calibrated to give positive results when Phadia 250 gives results above 1,49 kIU/L. ImmunoCAP Rapid is using the same antigens as Phadia 250.

3.1.4. *Definition of clinical interpretation*

True negative results for allergy are defined as negative skin prick test and no relevant history of allergy for the component.
True positive results for allergy are defined as positive skin prick test and a relevant history of symptoms for the component.
A sensibilized patient is defined as a patient with either a positive skin prick test or a relevant history of symptoms for the component.

Examples: A patient with a positive skin prick test for grass and no symptoms in the summer is sensitive to grass, but not allergic to grass. A patient with a negative skin prick test and strong symptoms of grass allergy in the summertime might be sensitive to a species of grass, but it is not confirmed that grass is the allergen. The reason for this might be lack of the right grass antigen in the test.
Clinical interpretation is described in table 1.

Table 1. Clinical interpretation, a sum of symptoms and test results

Result Skin prick test	**Result Questionnaire**	**Clinical interpretation for the patients**
Negative	No allergy	No allergy and not sensitive
Negative	Yes allergy	Sensitive?
Positive	No allergy	Sensitive
Positive	Yes allergy	Atopic allergy

3.2. Evaluation of user-friendliness

The evaluation of user-friendliness is carried out by asking the evaluating persons (end-users) to fill in a questionnaire divided into four sub-areas, see table 21-24.

3.3. SKUP's quality goals in this evaluation

It was attempted to apply the definition in Table 1 'clinical interpretation' in the report. In the protocol it was assumed, that there would be few patients with negative skin prick test and positive Questionnaire for allergy. However, there were many patients with negative skin prick test who had very convincing seasonal-related allergic symptoms in the evaluation. One reason for this may be that there are few tests available, while there is the possibility of thousands of individual allergens. If these patients are counted as positive, the sensitivity drops and if they are regarded as negative, the specificity decrease. It was therefore decided to assess the results from the evaluation of ImmunoCAP Rapid against 1) the comparison method Skin Prick Test and 2) the comparison method Phadia 250. The quality goals are shown in table 2.

Table 2. Quality goals in the evaluation of ImmunoCAP Rapid

ImmunoCAP Rapid compared with	**Skin prick test**	**Phadia 250**
Sensitivity *	>85%	>95%
Specificity	>85%	>95%
Fraction of technical errors	2% or less	
User-friendliness	satisfactory	

*the sensitivity is expected to be much lower than 85% for timothy and dog dander

If the upper CI limit of the achieved result is below the quality goal, the quality goal is not fulfilled.

4. Materials and methods

4.1. Definition of Allergen specific IgE

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Union of Pure and Applied Chemistry (IUPAC) work in a joint Committee on Nomenclature, Properties and Units (C-NPU). The descriptions of clinical laboratory tests are listed in the ”NPU database” [8]. In the database the recommended name is given for the measurand together with which unit the result should be reported in. The quantities being measured by allergens are described in table 3 for both the skin prick test, the measurements on Phadia 250 and for the measurement with the ImmunoCAP Rapid test.

Table 3. Summary of the measured properties, with NPU codes, in the evaluation

	ImmunoCAP Rapid	**Phadia 250**	**Skin prick test**
Name of allergen	**Arbitrary concentration (neg/pos)**	**Arbitrary substance concentration** (x 10^3 int.unit/l)	**Diameter of papule (mm)**
House dust mite			NPU22197 Skin(spec.)—House dust mite(Derm. farinae) induced papule; diam.(proc.) = ? mm
House dust mite	NPU11327 P—Dermatophagoides pteronyssinus antibody(IgE); arb.c.(NCCLS/d1; proc.)	NPU10881 P—Dermatophagoides pteronyssinus antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/d1; proc.)	NPU22198 Skin(spec.)—House dust mite(Derm. pteronyssinus) induced papule; diam.(proc.)
Cat epithelia	NPU11474 P—Cat epithelium antibody(IgE); arb.c. (NCCLS/e1; proc.)	NPU11028 P—Cat epithelium antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/e1; proc.)	NPU22210 Skin(spec.)—Cat epithelium induced papule; diam.(proc.)
Mugwort pollen	NPU11382 P—Mugwort antibody(IgE); arb.c.(NCCLS/w6; proc.)	NPU10936 P—Mugwort antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/w6; proc.)	NPU22176 Skin(spec.)—Mugwort pollen induced papule; diam.(proc.)
Timothy grass pollen	NPU11345 P—Timothy grass antibody(IgE); arb.c.(NCCLS/g6; proc.)	NPU10899 P—Timothy grass antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/g6; proc.)	NPU22182 Skin(spec.)—Grass pollen induced papule; diam.(proc.)
Dog dander/ epithelia	NPU11414 P—Dog dander antibody(IgE); arb.c.(NCCLS/e5; proc.)	NPU10968 P—Dog dander antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/e5; proc.)	NPU22195 and NPU22196 Skin(spec.)—Dog dander and epithelium induced papule; diam.(proc.)
Birch pollen	NPU11288 P—Birch antibody(IgE); arb.c.(NCCLS/t3; proc.) = ?	NPU10842 P—Birch antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/t3; proc.)	NPU22171 Skin(spec.)—Birch pollen induced papule; diam.(proc.)
Mould	NPU11260 P—Alternaria alternata/tenuis antibody(IgE); arb.c.(NCCLS/m6; proc.)	NPU10814 P—Alternaria alternata/tenuis antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/m6; proc.)	NPU22162 Skin(spec.)—Alternaria alternata induced papule; diam.(proc.)
Mould			NPU22178 Skin(spec.)—Cladosporium herbarum induced papule; diam.(proc.)

Name of allergen	ImmunoCAP Rapid	Phadia 250	Skin prick test
Horse dander			NPU22193 Skin(spec.)—Horse dander induced papule; diam.(proc.)
Olive pollen	NPU11545 P—Olive antibody(IgE); arb.c.(NCCLS/t9; proc.)	NPU11099 P—Olive antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/t9; proc.)	
Wall pellitory pollen	NPU11638 P—Parietaria judaica antibody(IgE); arb.c.(NCCLS/w21; proc.)	NPU11192 P—Parietaria judaica antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/w21; proc.)	
Blatella germanica Cockroach	NPU11458 P—Cockroach antibody(IgE); arb.c.(NCCLS/i6; proc.)	NPU11012 P—Cockroach antibody(IgE); arb.subst.c.(IRP 75/502; NCCLS/i6; proc.)	

4.1.1. *Definition of the tests on the ImmunoCAP Rapid system*

ImmunoCAP Rapid is a test that detects ten different specific Immunoglobulin E inhalation allergens. Results are produced on an ordinal scale, which in this case means that there are only two possible results of the test:

- 0 arbitrary unit or 'Negative'. The tested specific IgE antibody is not detected in the sample.
- 1 arbitrary unit or 'Positive'. The tested specific IgE antibody is detected in the sample.

The tests on ImmunoCAP Rapid are defined according to NPU as shown in table 3.
According to internal studies made by the manufacturer ImmunoCAP Rapid has a level of detection of 1-2 kIU/L for all antibodies. In this evaluation 0-1,49 kIU/L with Phadia 250 is considered as negative and concentrations above 1,49 kIU/L are considered as positive when compared to ImmunoCAP Rapid.

4.1.2. *Definition of the tests on the Phadia 250 system*

The concentrations of specific immunoglobulins have previously been reported in "classes". The relation between classes and kIU/L is described in table 4. In this evaluation 0-0,34 kIU/L (Class 0) is negative and all higher concentrations are positive [7] when compared to skin prick test. The analyses on Phadia 250 are defined according to NPU as shown in table 3.

Table 4. Conversion of kIU/L to classes

kIU/L	Class	Description
0 - 0,34	0	Negative
0,35 - 0,7	1	Low positive
0,7 - 3,5	2	Moderate positive
3,5 - 17,5	3	High positive
17,5 - 50	4	Very High
50 - 100	5	Very High
>100	6	Very High

4.1.3. *Definition of the skin prick tests*

The skin prick tests are defined according to NPU as shown in table 3. Specific antigens are injected in the skin of the patient and, if the patient reacts to the antigen injected, the result is defined according to the size of the wheal produced.

4.1.4. *Definition of 'clinical interpretation'*

In the attempt to use 'clinical interpretation' as a 'comparison method' or 'truth' as described in table 1 all patients completed a questionnaire in which they were asked about their symptoms of allergy (rhinitis), season for symptoms, medication and what allergens the patients thought to be allergic to. After seeing the results of skin prick test, patients had the opportunity to reassess their views of what they are allergic to.

The questionnaire was made for this evaluation to assure that all patients were asked the same questions, see attachment 5 and 6.

4.2. The ImmunoCAP Rapid device

The text and pictures in section 4.2. regarding the ImmunoCAP® Rapid specific IgE antibodies system are derived mainly from the manufacturer's information material.

Description of the ImmunoCAP Rapid device
ImmunoCAP Rapid is a device for determination of allergen specific IgE antibodies in either capillary- or heparinised whole blood. It is intended as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings.

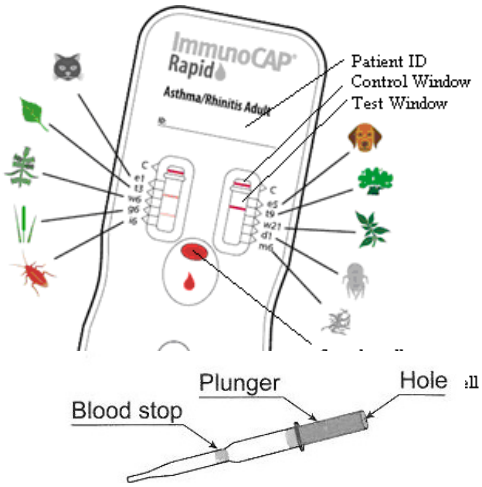


Figure 1. The ImmunoCAP rapid device, showing the allergens. Below the blood sample collector.

The ImmunoCAP® Rapid specific IgE antibodies system consists of the ImmunoCAP Rapid test, the heparinised blood sample collector, a pipette, and the Developer Solution. The test including the blood sampling device is shown in figure 1. An explanation of the different allergens is shown in table 5. Positive and negative controls can be purchased via Phadia. The tests are packed individually in foil and sold with blood sampling devices, pipettes, and Developer Solution. The tests and the Developer Solution must be kept at 2 - 8 °C. When unpacked, the tests must be used within one hour. Both Developer Solution and tests must reach room temperature before analysis and therefore must be taken out of the refrigerator at least five minutes before use.

Processing a sample should be initiated immediately after the capillary puncture and all steps of the procedure should be done continuously thereafter.

Table 5. Allergens on ImmunoCAP Rapid including their Latin, English, Danish, Norwegian, and Swedish names

Allergen	Code	Latin name	English	Danish	Norwegian	Swedish
	e1		Cat epithelium and dander	Katteepitel og skæl	Katt (epitel)	Kattepitel och -mjäll
	t3	*Betula verrucosa syn Betula pendula*	Common silver birch syn white birch (pollen)	Vortebirk	Hengjebjørk	Vårtbjörk (pollen)
	w6	*Artemisia vulgaris*	Mugwort (pollen)	Gråbynke	Burot	Gråbo
	g6	*Phleum pratense*	Timothy (pollen)	Eng-rottehale, Timoté	Timotei	Timotej
	i6	*Blatella germanica*	Cockroach	Kakerlak	Kakerlakk	Kackerlacka
	e5		Dog dander	Hundeskæl	Hund (epitel)	Hundmjäll
	t9	*Olea europaea*	Olive (pollen)	Oliven (pollen)	Oliventre (pollen)	Olivträd (pollen)
	w21	*Parietaria judaica*	Wall pellitory (pollen)	Nedliggende springknap	Vanlig blidnesle	Grenig väggört
	d1	*Dermatophagoi-des pteronyssinus*	House dust mite	Husstøv-mide	Midd	Huskvalster
	m6	*Alternaria alternata*	Mould	Skimmel-svamp - alternaria	Muggsopp	Alternariamögel

4.2.1. *Analysing a patient sample*

A short version of the procedure for analysing capillary blood on ImmunoCAP Rapid is shown in figure 2. The illustrations were found in the Danish version of the instrument guide supplied by Phadia [9]. Capillary whole blood as well as venous heparin whole blood may be used.

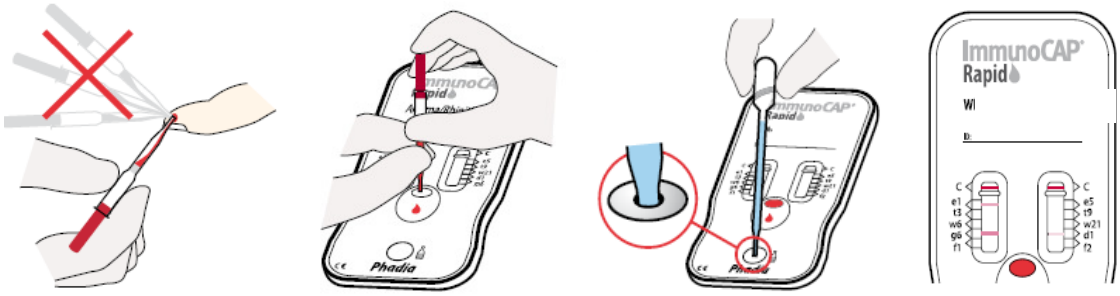


Figure 2. Analysing a patient sample

- Capillary blood is drawn from a fingertip and 110µL is collected with the capillary blood sample collector
- The capillary sample is placed in the sample well by pushing the plunger
- A timer is set to five minutes and when the five minutes are up, 500 µL of the Developer Solution is placed in the Developer Solution well
- A timer is set to fifteen minutes and hereafter the results may be read and interpreted. The test is interpretable until two hours after analysis. Thereafter it must be discarded.

4.2.2. *Measuring principle*

ImmunoCAP Rapid is a lateral flow test. The blood sample is applied to the sample well and the separated plasma portion flows up into the test windows. IgE antibodies present in the sample, specific to any of the allergens in the test, bind to the relevant areas on the strip. Adding of Developer Solution to the designated well, releases the dried gold-anti-IgE conjugate which forms a complex with the already bound antibodies. This complex is visualised as pink-red lines in the test windows. Any form of pink-red line in the test window indicates a positive test.

4.2.3. *Control possibilities with ImmunoCAP Rapid*

Built-in control lines

The assay device has a procedural control. Fifteen minutes after the Developer Solution is added, pink-red lines should appear in the Control windows, indicating that the test has performed correctly.

Internal and external analytical quality control

Internal and external analytical quality control is possible on ImmunoCAP Rapid because heparinised samples with known or unknown concentrations can be used. However, to analyse internal or external control material was not part of the evaluation.

4.2.4. *Product information, ImmunoCAP Rapid*

Technical data from Phadia is shown in table 6. For more details about the ImmunoCAP Rapid System, see attachment 1.

Table 6. Technical data from Phadia

Technical data for ImmunoCAP Rapid	
Sample material	Capillary whole blood or heparinised venous whole blood
Sample volume	110 µL
Measuring time	20 minutes
Measuring range	negative or positive
Operating time	20 minutes
Dimensions	95 mm (L) x 40 mm (D) x 5 mm (H)
Weight	10 g

For name of the manufacturer and the suppliers in the Scandinavian countries, see attachment 3.

4.3. The selected comparison method

The selected comparison method is a fully specified method, which, in the absence of a reference method, serves as the common basis for the comparison of a field method.
The comparison method in this evaluation is skin prick test and specific allergens measured with Phadia 250. In the hierarchy of comparison methods, the skin prick test is regarded as 'gold standard'.

Differences in Scandinavia:
The GP's in Sweden and Norway do not perform allergen testing in primary health care centres. They either send the patients to special clinics where they have the skin prick test performed, or they send a blood sample to a hospital for measurement of specific allergens. In some primary health care centres in Denmark, specialised nurses perform a standard skin prick test with 10 allergens in duplicate. The results are read by the nurse in the following way: she marks the wheals of the skin reaction with a special pencil and transfers a print of the pencil marks on a milimeter paper by help of an adhesive tape. The symptoms and the results are then evaluated by the GP.
A skin prick test can be negative if the patients are taking antihistamine; therefore the patients are not supposed to use medication for rhinitis three days before a skin prick test.

The skin prick tests
The principle in a skin prick test is to "provoke" an allergic reaction in a person by adding a minute amount of allergen. It measures the reactivity of the mast cells/mast cell bound IgE in the person. A small drop of allergen extract is placed in duplicate on the skin, usually the lower forearm, and with a lancet, the skin is pierced. This way a very small amount of allergen is penetrated into the skin. If the patient is allergic, the patient will react with a wheal and flare reaction on the point of skin piercing.
A positive and a negative control are always performed at the same time. The negative control is the solution in which the allergens are contained. The positive control is a skin prick test with histamine, but without allergen. The histamine makes the same physiological reaction as the histamine from the allergic reaction and thus functions as the positive control.

Skin prick tests in this evaluation were performed according to normal Danish procedures [10-12] with duplicates for the standard panel; birch, grass, two house dust mites Dermatophagoides farinae and Dermatophagoides pteronyssinus, cat epithelium, dog epithelium, *Cladosporium hebarum*, *Alternaria alternata*, mugwort and horse epithelium (Soluprick, ALK Abello, Denmark). The diameter of each skin reaction is measured in length and width and the average is used. The skin test is positive if one of the two wheals is 3 mm or more in average (or the total wheal area is >7mm^2). For instructions of skin prick testing, see attachment 2.

Example:

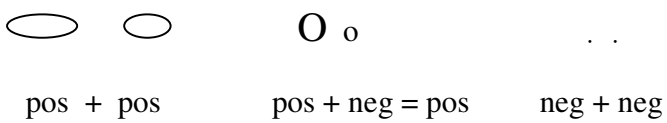


Figure 3. Examples of positive and negative skin prick test

The Phadia 250 method
The Phadia 250 method consists of an 'ImmunoCAP' where to the allergen is coupled. Serum or plasma from a patient is added to the 'ImmunoCAP'. The antibodies in the patient samples are measured with EliA, a FluoroEnzymeImmunoAssay. See attachment 7.

The clinical interpretation
An attempt to use 'clinical interpretation' as one comparison method was made. The assumption was that the patients were 'negative', 'sensitive' or 'allergic' to a given component as described in table 1. The idea of using real and consecutive patients was that all patients were easily placed in one of the three outcomes and that very few would turn out to have a 'grey zone' clinical interpretation. For the purpose 'clinical interpretation' and to describe the patients in the evaluation, all patients filled in a questionnaire in which they were asked which symptoms for allergy they had, the season for the symptoms, the suspected allergens and the actual medication. They also could reassess their views of what they are allergic to after knowing the results of the skin prick test. An example: A patient supposes he is allergic to grass; however he has symptoms all year and the skin prick test is positive to house dust mites and dog dander. He then realizes he is not allergic to grass but to dog dander and house dust mites. The original questionnaire in Danish is shown in attachment 5, and an English copy is shown in attachment 6.

4.4. The evaluation

4.4.1. Planning of the evaluation
The ImmunoCAP Rapid system is produced by Phadia and has been distributed and sold in Sweden since 2007. Phadia, Denmark, applied for an evaluation of the ImmunoCAP Rapid system (inhalation allergens) among the intended end-users in primary health care centres. The components house dust mite (d1), cat epithelia (e1), birch pollen (t3), dog epithelia (e5), mugwort pollen (w6) or grass pollen (g6), cockroach (i6), olive pollen (t9), wall pellitory (w21) and *Alternaria alternata* (m6) were to be evaluated. SKUP accepted to carry out this evaluation on behalf of Phadia.

The planning of the evaluation began in 2005 when Esther Jensen, SKUP, met with Bjarne Kristensen, Phadia, Denmark. Later meetings were with Anders B. Jensen, Phadia. In 2006, Esther Jensen and Per Grinsted wrote a preliminary protocol. This protocol was heard in SKUP and then sent for hearing in Phadia in 2007. The contract was signed in 2007. Data were collected between April 2008 and June 2010.

4.4.2. *Evaluation sites and persons involved*

Persons responsible for the evaluation are shown in table 7. In total two hospitals, 13 primary health care centres and one specialized clinic participated in the evaluation.

Table 7. Evaluation sites and persons involved in the evaluation

Place	Person	Title	Task
Hillerød Hospital, Odense University Hospital	Esther A Jensen	Physician	Author of protocol and report
Odense University Hospital	Nina Brøgger	Biomedical laboratory scientist	Hospital testing and contact person for primary health care
Odense University Hospital	Per Grinsted	General Practitioner	Co-author of and consultant on protocol and report
Hillerød Hospital	Stine Beenfeldt Weber	Cand. Scient.	Co-author of report. Hospital testing and contact person for primary health care
Hillerød Hospital	Inge Lykke Pedersen	Biomedical laboratory scientist	Consultant for primary health care quality
Bogense Lægehus	Anette Ploug Andersen	Biomedical laboratory scientist	Primary health care testing
Lægerne Lægehuset Fåborg	Ulla Thostrup	Biomedical laboratory scientist	Primary health care testing
Lægehuset i Mørkøv	Marianne Madsen	Biomedical laboratory scientist	Primary health care testing
Lægerne Willemoesvej Svendborg	Ditte Helgren	Biomedical laboratory scientist	Primary health care testing
Søndersø Lægehus	Hanne Andersen Bodil Ravn	Nurse Nurse	Primary health care testing
Lægerne Carl Hansens Alle, Ølstykke	Kirsten Poulsen	Biomedical laboratory scientist	Primary health care testing
Arne Agertoft, Odense	Lis Nielsen	Nurse	Primary health care testing
Allergiklinikken, Helsingør*	Susanne Linné Jeanette Lorentzen	Nurse Nurse	Primary health care testing
Torvegade, Odense	Karen Munk	Nurse	Primary health care testing
Glamsbjerg Lægehus	Stine Jacobsen Susanne Kirketorp-Møller	Nurse Nurse	Primary health care testing
Lægerne i Centrum	Ditte Helgren	Biomedical laboratory scientist	Primary health care testing
Lægerne Lærkevej Otterup	Joan Rasmussen Helga Rasmussen	Nurse Nurse	Primary health care testing
Slangerup Lægehus	Vivi Hartvig Christensen	Biomedical laboratory scientist	Primary health care testing
Nivå Lægehus	Gitte Weeke Heidi Dyrberg	Nurse Nurse	Primary health care testing

*specialized clinic for allergy

4.5. The evaluation procedure

4.5.1. *The evaluation model*

The evaluation of ImmunoCAP Rapid in the primary health care centres and the hospital laboratory deals with evaluation of house dust mites (d1), cat epithelia (e1), birch pollen (t3), dog epithelia (e5), mugwort pollen (w6), grass pollen (g6), cockroach (i6), olive pollen (t9), wall pellitory (w21) and *Alternaria alternata* (m6).

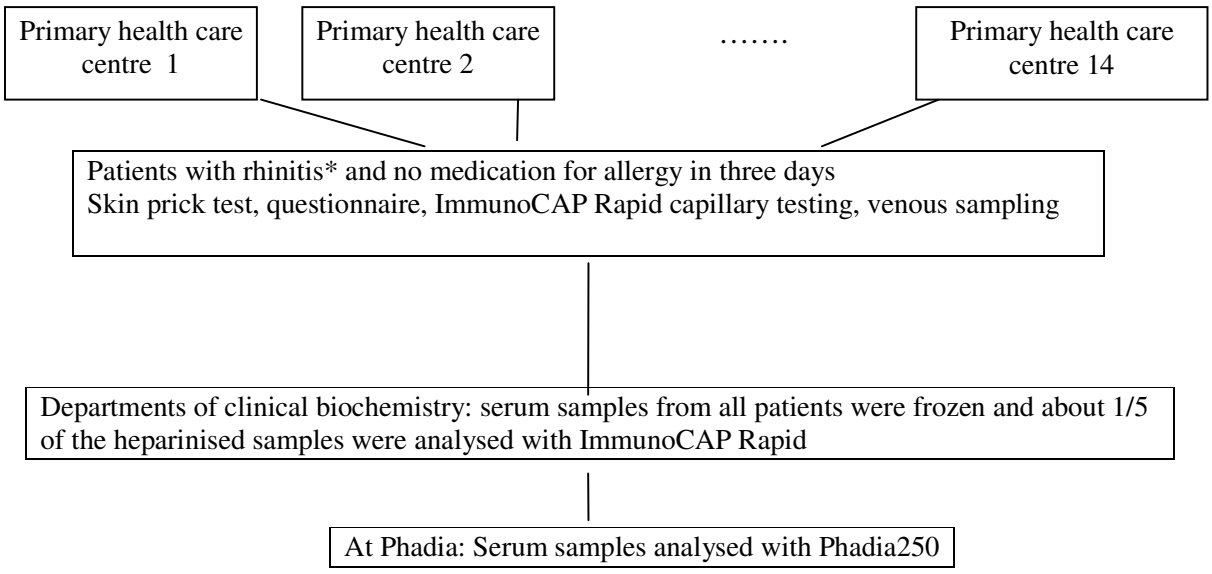
In primary health care

- To collect 100 positive and 100 negative capillary results from skin prick tests for two of the components
- Determination of the sensitivity and specificity of ImmunoCAP rapid for all components
- Repeated reading by the same person ("intra person disagreement")
- Reading by another person ("inter person disagreement") when possible
- Evaluation of user-friendliness of ImmunoCAP Rapid

In the hospital laboratory

- Compilation of facts about the instrument
- Repeat about 1/5 of the samples from primary health care in heparin whole blood.
- Repeated reading by the same person ("intra person disagreement")
- Two evaluators read the same tests to estimate the inter-individual difference
- Evaluation of user-friendliness of ImmunoCAP Rapid
- Serum samples for Phadia 250 were frozen at -80°C

A flow diagram is presented in figure 4 to illustrate the model.



*Enrolling of patients until 100 positive results with the skin prick test were achieved

Figure 4. The model for the evaluation

4.5.2. *Evaluation procedure in the hospital laboratory (standardised and optimal conditions)*

Training

Nina Brøgger and Esther Jensen received practical training in 2007 on how to analyse a blood sample using ImmunoCAP Rapid by Anders Blom Jensen from Phadia. In 2010 Stine Beenfeldt Weber learned to use the ImmunoCAP Rapid test. In all cases, the training lasted less than one hour.

Two multi allergic individuals were measured as a kind of a positive test control before and during the evaluation in Odense. The readings of these samples also demonstrated the variable strength of colour bands that can occur.

Handling of samples and measurements, ImmunoCAP Rapid

About 1/5 of the heparin whole blood samples from the primary health care centres was analysed at random after arriving at the Department of Clinical Biochemistry. The capillary whole blood and the heparinised venous whole blood results were considered duplicate measurements. The evaluators did not know the result of the skin prick test and the capillary ImmunoCAP result performed in the primary health care centres. If possible, two evaluators read the same test to estimate the inter-individual variation in the hospitals.

All serum samples were centrifuged (2000 g) for 10 minutes, aliquoted into 1 mL tubes and frozen at -80^{o}C. The serum samples where collectively send to Phadia in Allerød. Phadia did not know the results of ImmunoCAP Rapid, the skin prick test or the questionnaire.

Analysing with Phadia 250
The serum samples were analysed by Phadia, Allerød. The first half was analysed July 2009 and the second half in July 2010. Phadia did not have access to the evaluation results from the other methods when analysing the samples.

Recording of results
All results were registered and signed for by the evaluator. If a test was invalid, a new measurement was made, if possible, and recorded.

Evaluation of user-friendliness
Stine Beenfeldt Weber and Nina Brøgger evaluated the user friendliness immediately after the hospital evaluation was performed.

4.5.3. *Evaluation procedure in primary health care*
The evaluation of ImmunoCAP Rapid in the primary health care was done by the intended users in the primary health care centres. None of the primary health care centres analyses allergens with capillary samples, but most evaluators are used to handle capillary samples when measuring other parameters. None of the participants had ever used ImmunoCAP Rapid.

Recruitment of primary health care centres
Only primary health care centres that performed skin prick test could participate. Centres that performed more than 20 skin prick test per year were asked to participate. Some centres only participated in a period of the evaluation.

Training
The supplier, Phadia, was responsible for training with ImmunoCAP Rapid. In 2008 Anders Blom Jensen and Nina Brøgger were together in the 11 primary health care centres for training and logistics. Anders B Jensen gave training to the staffs in the primary health care centres. When the evaluation began, the evaluators had to handle ImmunoCAP Rapid on their own without any supervision or correction from the manufacturer/supplier. If there were any questions, these were addressed to SKUP.
In 2010 Stine Beenfeldt Weber learned to use the ImmunoCAP Rapid test. Anders was together with Esther and Stine in additional two primary health care centres and in a special clinic for allergy. In all cases, the training lasted less than one hour.
Nina Brøgger and Stine Beenfeldt Weber were the contact persons for the primary health care centres during the evaluation. They collected data and made sure that the protocol was followed. All results were confidential during the evaluation.

Recruitment of patients
Patients, who were going to have a routine skin prick test performed, were asked to participate in the evaluation. Participation was voluntarily and verbal consent was considered sufficient. A patient could only participate in the evaluation once.
If the patient agreed, the patient was asked to fill in a questionnaire, to donate 110 µl capillary blood and two tubes of venous blood (one Lithium Heparin sample for ImmunoCAP Rapid testing and one serum sample for the Phadia 250 comparison method). Recruitment of patients continued until 100 positives for all the centres combined were found in two individual groups of allergens.

If a patient is medicated for rhinitis, the procedure for a skin prick test is to wait for a medication-free period of three days, because the skin prick test result is reduced by the medication. Medication does not influence results on the ImmunoCAP Rapid test or the Phadia 250 analysis.

Handling of samples and measurements
The patients had one capillary sample taken. The capillary sample was collected according to the protocol and measured on ImmunoCAP Rapid in the same way as the venous sample in the hospital evaluation; please see figure 2. The venous samples were sent either with the routine transportation system for blood samples or by regular mail to either the Department of BFG, Odense University Hospital or Department of Clinical Biochemistry, Hillerød Hospital depending on the time of sampling. The samples from the primary health care centres were measured on various lot numbers.

Analysing with the comparison method skin prick test
Skin prick testing was performed as described in attachment 2. The patients filled in the questionnaires during the consultation.

Recording of results
All results were registered and signed for by the evaluator. If a test was invalid, a new measurement was made, if possible. The errors were recorded. Data was recorded in a form produced by SKUP.

Evaluation of user-friendliness
The evaluators filled in the user friendliness questionnaire after completing the practical work with the evaluation.

4.5.4. *Objectivity of the evaluators*
Several evaluators claimed that the reading of the various allergens on ImmunoCAP often was difficult because of weak colour bands. The evaluators in the primary care centres already knew the results of the skin prick test and the questionnaire when reading the ImmunoCAP Rapid results. This could have affected their interpretation of the ImmunoCAP readings. Some patients are convinced that they are allergic and express their expectation of positive test results. This might influence the evaluators' opinion of the test results.
All results in the hospital laboratory in Odense were blinded. In Hillerød the results of the skin prick test and the questionnaire were known to the evaluators.
At Phadia all results were blinded when measuring the samples on Phadia 250.

5. Statistical expressions and calculations

This chapter is written for this evaluation specifically. The descriptions in section 5.2 are valid for evaluations of qualitative allergen test methods with results on the ordinal scale.

5.1. Statistical terms and expressions

The definitions and formulas in this section originate from the Geigy document [13].

5.2. Statistical calculations

5.2.1. *Statistical calculations*

Sensitivity is true positive/(true positive + false negative)

Specificity is true negative/(false positive + true negative)

Positive predictive value (PPV) is true positive/(true positive + false positive)

Negative predictive value (NPV) is true negative/(true negative + false negative)

Percentage of positive test is 100 × (positive results/all results)

Table 8. Calculated parameters

	Truth		
	Positive	**Negative**	
Evaluated test positive	a	b	PPV = a/(a+b)
Evaluated test negative	c	d	NPV = d/(c+d)
	Sensitivity = a/(a+c)	Specificity = d/(b+d)	

5.2.2. *Calculation of confidence intervals*

Estimation of CI for fractions/proportions is performed according to the formula 772 in Documenta Geigy [13]. For lower (left) and upper (right) confidence limits, see attachment 4 and reference [14].

5.2.3. *Calculation of numbers needed*

See examples in attachment 4.

6. Results and discussion

6.1. Number of samples

In the primary health care evaluation, 300 individuals having skin prick tests performed for a routine allergen specific IgE measurement voluntarily participated with capillary measurements on the ImmunoCAP Rapid test for allergens. The samples were collected from April 2008 to June 2010.

Table 9. Number of tests used on the ImmunoCAP Rapid instruments in the evaluation

The evaluation in the primary health care centres	Number of individuals
Agertoft	60
Allergiklinikken Helsingør	77
Bogense Lægehus	24
Glamsbjerg Lægehus	10
Lægerne i Centrum	9
Lægerne Lægehuset Fåborg	4
Lægerne Lærkevej Otterup	28
Mørkøv	18
Nivå	3
Slangerup	8
Svendborg	3
Søndersø Lægehus	37
Torvegade 1, Odense	5
Ølstykke	14
Patients in total	**300**

The number of allergen measurements for each patient was ten plus two built-in control lines. The results of the 300 patients on ImmunoCAP Rapid were read by the person in charge for the skin prick test (in total 3000 immunoCAP Rapid results plus 300 negative and 300 positive results). For 64 of the 300 patients the ImmunoCAP Rapid cassettes were read by two individuals in the primary health care centres adding up to a total of 3640 individual test readings.
Of the 300 patient samples 39 heparinised whole blood samples were analysed with ImmunoCAP Rapid upon arrival to the hospital laboratory. The samples were chosen by random and the results were read by two individuals adding up to a total of 780 individual test readings.

6.1.1. *Excluded or missing results*

ImmunoCAP Rapid

There are no outliers in an ordinal scale evaluation.
In ID32 and ID34: One half of the cassette performed correctly and is included in the evaluation whereas the other half of the cassette was flawed and the results from this half are excluded (please see figure 1 for the design of the test cassette).
ID114 was discarded because the sample was applied wrong.
17 built-in control lines were not marked as positive and negative on the result form; the evaluators were all certain that the cassettes had been valid with positive and negative results. The results are included.
Three individual results were marked as either positive with a parenthesis around or followed by a question mark. These are counted as positives.

One individual result was marked as a negative followed by a question mark. This result is counted as a negative result.
One result is described as a very weak band; the result is counted as positive.
In the hospital evaluation, there were no failed measurements.

Skin prick test
ID423: skin prick test result form was never received by SKUP.

Phadia 250
ID111, ID116, ID118 and ID428: the four samples are missing.

The total fraction of technical errors in primary health care centres were: (0,5+0,5)/300 x 100 = 0,3%.

Conclusion:
The quality goal for fraction of technical errors <2% was fulfilled.

6.1.2. *Distribution of positive results with skin prick test, ImmunoCAP Rapid and Phadia 250*

Table 10. Distribution of positive results with skin prick test, ImmunoCAP Rapid and Phadia 250

Allergen	Name	Skin prick test	ImmunoCAP Rapid	Phadia 250 n*
	Cat epithelium and dander	66	48	53 (32)
	Common silver birch (pollen)	95	70	88 (66)
	Mugwort	52	39	44 (27)
	Timothy	110	68	94 (65)
	Cockroach	-	4	23 (5)
	Dog dander	79	18	49 (13)
	Olive (pollen)	-	7	40 (14)
	Wall pellitory	-	6	17 (7)
	*Dermatophagoides pteronyssinus*	90	75	86 (63)
	Dermatophagoides farinae	78		
	House dust mites	95 **		
	*Alternaria alternata*	25	18	23 (15)
	Cladosporium hebarum	10		
	Mould	27 **		
	Horse	22	-	-

*gives the number of results ≥0,34 kIU/L and ≥1,50 kIU/L (). ** This result refers to the sum of skin prick tests positive for either one or both of the two allergens above

Comment:
As seen, there is a difference in the number of positives results depending on the test used. The skin prick test always comes out with the highest number of positive results.
According to the manufacturer, ImmunoCAP Rapid should have the same number of positive results as Phadia 250. However, Phadia 250 results are positive at 0,34 kIU/L and ImmunoCAP Rapid results are positive when the Phadia 250 results are 1,5 kIU/L and above.

6.1.3. *Description of the patients regarding rhinitis*

The patients in this evaluation had not previous been diagnosed as having rhinitis. This is a description of the patient population that actually are to be investigated with the purpose to diagnose rhinitis – or to exclude rhinitis.
Below the positive results for the questionnaire are shown. Negatives are not shown.

Table 11. Distribution of answers to the 300 questionnaire forms

Symptoms	***positive answers***
Sneezing	218
Runny nose	210
Itchy eyes	199
Clogged nose	171
Itchy nose	158
Red eyes	135
Lacrimation	126
Itchy palate	111

When do your symptoms appear?

All year	128
Spring	85
Spring and summer	80
Summer	71
Fall	34

Do you believe you have rhinitis? Yes: 161

What gives you symptoms?

Grass pollen……	106
Birch pollen……	92
House dust mites	73
Cat ……	69
Dog……	49
Mould ……	26
Mugwort……	24
Horse ……	23
Hazel pollen……	19

Medication

Do you take medication, when you have symptoms?	180
Yes, Antihistamine, 'rhinitis tablets'	104
Do you take medication throughout the year?	48
Have you taken medication in the last week?	87
Injection with corticosteroids within the last three months	9

Discussion: Only 161 of the patients thought they had rhinitis. With regard to symptoms, some patients reported more than one period in order to tell that they had one type of symptoms in one season, and other symptoms in other periods.
If test results did not confirm patients' expectations for allergies altered about half their opinion about what they were allergic to after having seen and thought about the prick test results. An example, no. 164: the patient believed to be allergic to grass, but he had symptoms all year round and prick test was positive for dust mites and dog dander.
Several patients, who were negative with skin prick test as well as with Phadia 250, claimed to be allergic to allergens. For some of them, it was most unlikely they were allergic to the suspected allergen because the allergen and the period in which they had symptoms did not fit together.

A very high number of the 87 patients that had taken medication within the last week had taken antihistamins. One of the inclusion criteria was 'no medication for three days'. It was tested if sensitivity and specificity changed if the "almost" positive skin prick test, that is, with a diameter of 2 to 2,9 mm were counted as positive. This did not change the results of the evaluation (data not shown).

6.2. Analytical quality of ImmunoCAP Rapid

6.2.1. *External quality control*

External control is possible on ImmunoCAP Rapid. However; to analyse external control material was not part of the evaluation according to the protocol.

6.2.2. *Internal quality control*

Before and during the hospital evaluation samples from two multi-allergic patients were analysed a total of three times using ImmunoCAP Rapid test cassettes. The ten results and the two built in control lines from each test cassette were read independently by two evaluators. In all cases the positive results were the same for the evaluators (data not shown). However, it also became clear for the evaluators that some positive results were very weak

6.2.3. *Analytical quality of the ImmunoCAP Rapid, common silver birch test*

Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": none.
Inter-person disagreement; when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in the hospital: one, the result was negative in primary health care and positive in the hospital laboratory. The skin prick test was positive and so was the Phadia 250 result.
Below in table 12, the percentage of positive skin prick test, sensitivity and specificity are shown for ImmunoCAP Rapid measured against the different comparison methods for common silver birch.

Table 12. Capillary ImmunoCAP Rapid results with common silver birch

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid % (90% CI)
Skin prick test					
	 t3	*Betula verrucosa* Common silver birch (pollen)	297	Percentage of positive skin prick test	31,6 (27,2 - 36,4)
				Sensitivity	69,1 (60,3 - 76,9)
				Specificity	97,5 (94,8 - 99,0)
				PPV	92,9 (85,4 - 97,1)
				NPV	87,2 (82,9 - 90,7)
Phadia 250					
	 t3	*Betula verrucosa* Common silver birch (pollen)	294	Percentage of positive Phadia 250 test	22,4 (18,5 - 26,8)
				Sensitivity	80,3 (70,4 - 87,9)
				Specificity	92,5 (89,0 - 95,2)
				PV-pos	75,7 (65,7 - 83,9)
				PV-neg	94,2 (90,9 - 96,5)

Table 12.a Phadia 250 compared to skin prick test with common silver birch

Skin prick test					
	 t3	*Betula verrucosa* Common silver birch (pollen)	295	Percentage of positive skin prick test	31,9 (27,4 - 36,6)
				Sensitivity	69,1 (60,3 - 76,9)
				Specificity	88,6 (84,1 - 92,0)
				PPV	73,9 (65,0 - 81,4)
				NPV	86,0 (81,3 - 89,8)

The inclusions were random until 85 patients with positive skin prick tests for common silver birch and grass (timothy) were enrolled, then patients positive for birch or grass in skin prick test were chosen prior to others. This was agreed in order to finish the evaluation, however; the percent of positive skin prick test for birch did not change.

ImmunoCAP Rapid, common silver birch test evaluated with skin prick test as comparison method
Comments: The percentage of positive skin prick test of antibodies against birch was as expected (32%). Sensitivity was 69,9%, which was lower than the expected 85%. Specificity (97,5%) fulfilled the goal >95,0%. Five results were positive with ImmunoCAP Rapid and negative with the skin prick test. Four of these five patients had not taken medication the last three days; however they had taken medication within the last week. Three of these five patients had positive results (3,33, 17,7 and 83,3 kIU/L) with Phadia 250. The first patient had symptoms all year, the second suspected birch to give symptoms and the third was bothered mostly when in contact with animals. The result of the third patient was confirmed by two evaluators in primary health care using capillary blood, plus two evaluators in the hospital laboratory using venous heparinised blood.

The reason, these three patient were not positive with skin prick test might be suppressed reaction due to previous medication; however it could also be due to differences in the reagents.
Two of these five patients had negative results with Phadia 250 (0 and 0,02 kIU/L), one believed he/she might respond to birch, the other had relevant symptoms in the spring and summer, which are the seasons for birch pollen.
The two ImmunoCAP Rapid results must be wrong, since ImmunoCAP Rapid is expected to give the same results as Phadia 250 – and the 'true' test, the skin prick test was also negative.
The evaluation is not blinded for the evaluator performing the skin prick test and the reading of the ImmunoCAP Rapid result, therefore a possible bias of the evaluator is also a possibility.

ImmunoCAP Rapid, common silver birch test evaluated with Phadia 250 as comparison method
Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in table 12, the sensitivity was 80,3% and the specificity 92,5%.
17 samples were positive when measured with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250. 13 samples were positive with Phadia 250 and negative with ImmunoCAP Rapid.

Phadia 250, common silver birch test evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same. The sensitivity for Phadia 250 compared to the skin prick test was 69,1% and the specificity was 88,6%. By looking at the sensitivities of ImmunoCAP Rapid and Phadia 250 compared to skin prick test it could be believed that ImmunoCAP Rapid measured exactly the same as Phadia 250. However, it is not the same patients, having the positive results with the two methods.

Conclusion for the ImmunoCAP Rapid, common silver birch test
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >85%, when compared to skin prick test, or the quality goal for sensitivity, >95%, when compared to Phadia 250.
ImmunoCAP Rapid fulfilled the quality goal for specificity, >85%, when compared to skin prick test and might fulfil the quality goal for specificity, >95%, when compared to Phadia 250.

Intra-person disagreement: none
Inter-person reading disagreement: none (64 in primary health care 39 in hospital)
Inter-person reading disagreement, capillary samples / heparin samples: one of 39
Invalid test: none

6.2.4. *Analytical quality of the of ImmunoCAP Rapid, grass test (timothy)*
Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP Rapid capillary sample result": two.
Inter-person disagreement; when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in the hospital: four. Two results were negative in primary health care and positive in hospital and two other positive in hospital and negative in primary health care. The four skin prick tests were positive and so were three of the Phadia 250 result.

Table 13. Capillary ImmunoCAP Rapid results with grass (timothy)

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)
Skin prick test						
	g6	*Phleum pratense* Timothy	294	Percentage of positive skin prick test	36,7	(32,1 - 41,6)
				Sensitivity	59,6	(51,3 - 67,5)
				Specificity	98,4	(95,8 - 99,6)
				PPV	95,6	(88,7 - 98,8)
				NPV	80,8	(76,0 - 84,9)
Phadia 250						
	g6	*Phleum pratense* Timothy	294	Percentage of positive Phadia 250 test	22,1	(18,1 - 26,5)
				Sensitivity	76,9	(66,6 - 85,2)
				Specificity	92,6	(89,0 - 95,2)
				PV-pos	74,6	(64,3 - 83,1)
				PV-neg	93,4	(89,9 - 95,9)

Table 13.a Phadia 250 compared to skin prick test with grass (timothy)

Skin prick test						
	g6	*Phleum pratense* Timothy	295	Percentage of positive skin prick test	36,9	(32,3 - 41,8)
				Sensitivity	72,5	(64,5 - 79,4)
				Specificity	91,9	(87,8 - 94,9)
				PPV	84,0	(76,4 - 89,9)
				NPV	85,1	(80,3 - 89,0)

The inclusions were random until 85 patients with positive skin prick tests for common silver birch and grass (timothy) were enrolled, then patients positive for birch or grass in skin prick test were chosen prior to others. This was agreed in order to finish the evaluation, however; the percent of positive skin prick test for birch did not change.

ImmunoCAP Rapid, grass test (timothy) evaluated with Skin prick test as comparison method
Comments: The percentage of positive skin prick test of antibodies against grass in the material was about 37% as expected in the protocol.
As expected, ImmunoCAP Rapid had a sensitivity of about 60%. The specificity was 98,4% and fulfilled the quality goal, >95,0%.
Three results were positive with ImmunoCAP Rapid and negative with the skin prick test. One of the patients had taken medication within the last week (but not the last three days), the Phadia 250 result was highly positive, (36,3 kIU/L) and the patient believed to be allergic to grass. The other patient with positive Phadia 250 result (2,49 kIU/L) had probably asthma and was only sensibilised while the last patient had no grass related symptoms and negative Phadia 250 result (0 kIU/L).
The reason, the first patient were negative with skin prick test might be suppressed reaction due to previous medication; however it could also be due to differences in the reagents. The third result must be wrong, since the clinical interpretation, Phadia 250 and the skin prick test were negative.

ImmunoCAP Rapid, grass test (timothy) evaluated with Phadia 250 as comparison method
Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in the table above, the sensitivity was 76,9% and the specificity 92,6%.
17 samples were positive when measured with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250. 15 patients were positive with Phadia 250 and not with ImmunoCAP Rapid. It was expected that both the sensitivity and specificity compared to Phadia 250 would be >95%.

Phadia 250, grass test (timothy) evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same . The sensitivity for Phadia 250 compared to the skin prick test was 72,5% and the specificity was 91,9%.
By looking at the sensitivities of ImmunoCAP Rapid and Phadia 250 compared to skin prick test it could be believed that ImmunoCAP Rapid measured exactly the same as Phadia 250. However, it is not the same patients, which have the positive results in the two methods.

Conclusion for the ImmunoCAP Rapid, grass test (timothy
ImmunoCAP Rapid did almost (59,6%) fulfil the quality goal for sensitivity, 60-70%, when compared to skin prick test. Some differences were expected, because the skin prick test and the Phadia methods do not use the same grass antigens. ImmunoCAP Rapid did not (76,9%) fulfil the quality goal for sensitivity, <95%, when compared to Phadia 250. ImmunoCAP Rapid did fulfil (98,4%) the quality goal for specificity, >85%, when compared to skin prick test and might fulfil the quality goal for specificity, >95%, when compared to Phadia 250. The specificity was 92,6% (90% CI 89,0-95,2%).

Intra-person disagreement: none.
Inter-person reading disagreement: two of 113 (64 in primary health care 39 in hospital).
Inter-person reading disagreement, capillary samples / heparin samples: four of 39.
Invalid test: none.

6.2.5. *The Analytical quality of the ImmunoCAP Rapid, house dust mite test*
Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": five.
Inter-person disagreement when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in hospital: one was negative in primary health care and positive in hospital. The skin prick test was positive and so was the Phadia 250 result.

Table 14. Capillary ImmunoCAP Rapid results with *Dermatophagoides pteronyssinus,* house dust mite

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)	
Skin prick test							
	d1	*Dermatophagoi-des pteronyssinus* House dust mite	297	Percentage of positive skin prick test	31,6	(27,2 -	36,4)
				Sensitivity	70,2	(61,5 -	77,9)
				Specificity	95,6	(92,3 -	97,6)
				PPV	88,0	(79,8 -	93,5)
				NPV	87,4	(83,1 -	90,9)
Phadia 250							
	d1	*Dermatophagoi-des pteronyssinus* House dust mite	294	Percentage of positive Phadia 250 test	21,4	(17,4 -	25,7)
				Sensitivity	79,4	(69,1 -	87,3)
				Specificity	89,2	(85,3 -	92,4)
				PV-pos	66,7	(56,6 -	75,6)
				PV-neg	94,1	(90,7 -	96,4)

Table 14.a Phadia 250 compared to the skin prick test with house dust mites

Skin prick test							
	d1	*Dermatophagoi-des pteronyssinus* House dust mite	295	Percentage of positive skin prick test	31,9	(27,4 -	36,6)
				Sensitivity	74,5	(65,9 -	81,7)
				Specificity	92,0	(88,1 -	94,9)
				PPV	81,4	(73,0 -	87,9)
				NPV	88,5	(84,2 -	91,9)

ImmunoCAP Rapid, house dust mites test evaluated with skin prick test as comparison method
Comments: The percentage of positive skin prick test of antibodies against house dust mites was as expected to be about 31,6%. Sensitivity was 70,2% when d1 and d2 were used for comparison. If only d1 was used as comparison, the sensitivity was 73,0%. The sensitivities were lower than the expected 85%. Specificity (95,6%) fulfilled the quality goal, >95,0%.
Eight results were positive with ImmunoCAP Rapid and not with the skin prick test. Of these, four were not positive with Phadia 250 either. Only two of the eight had symptoms all year. One patient with a negative skin prick test had taken medication within the last week.
At least four positive ImmunoCAP Rapid results must be wrong, since ImmunoCAP Rapid is expected to give the same results as Phadia 250 – and the 'true' test, the skin prick test was also negative.

ImmunoCAP Rapid, house dust mites test evaluated with Phadia 250 as comparison method
Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in the table above, the sensitivity was 79,4% and the specificity 89,2%.
25 samples were positive when measured with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250. 13 patients were positive with Phadia 250 and not with ImmunoCAP Rapid.

Phadia 250, house dust mites test evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same . The sensitivity for Phadia 250 compared to the skin prick test was 74,5% and the specificity was 92,0%.

Conclusion for the ImmunoCAP Rapid, house dust mites test
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >85%, compared to skin prick test.
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >95%, compared to Phadia 250. The sensitivity was 73% and 67,1%, respectively.
ImmunoCAP Rapid did fulfil the goal of specificity >85% compared to skin prick test, but not specificity >95% when compared to Phadia 250. The specificity was 95,6% and 89,2%, respectively.
Intra-person disagreement: none.
Inter-person reading disagreement in same capillary result: three of 103 (64 in primary health care and 39 in hospital).
Inter-person reading disagreement, capillary samples / heparin samples: three of 39.
Invalid test: none.

6.2.6. *Analytical quality of the ImmunoCAP Rapid, dog dander test*
Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": four.

Inter-person disagreement when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in hospital: one, the result was positive in primary health care and negative in hospital. The skin prick test was positive and so was the Phadia 250 result (0,59kIU/L).

Table 15. Capillary ImmunoCAP Rapid results with dog dander

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)
Skin prick test						
		e5	Dog dander	298	Percentage of positive skin prick test	26,6 (22,4 - 31,2)
					Sensitivity	19,0 (12,1 - 27,8)
					Specificity	98,6 (96,4 - 99,6)
					PPV	83,3 (61,8 - 95,3)
					NPV	77,1 (72,5 - 81,1)
Phadia 250						
		e5	Dog dander	294	Percentage of positive Phadia 250 test	4,4 (2,7 - 7,0)
					Sensitivity	53,8 (28,6 - 77,7)
					Specificity	96,1 (93,6 - 97,8)
					PV-pos	38,9 (19,9 - 61,0)
					PV-neg	97,8 (95,7 - 99,0)

Table 15.a Phadia 250 compared to the skin prick test with dog dander

Skin prick test						
		e5*	Dog dander	298	Percentage of positive skin prick test	26,4 (22,3 - 31,0)
					Sensitivity	41,0 (31,6 - 51,0)
					Specificity	92,2 (88,4 - 94,9)
					PPV	65,3 (52,5 - 76,5)
					NPV	81,3 (76,7 - 85,3)

**The antigens for ImmunoCAP Rapid (e5 Dog dander) are not the same as the antigens used for skin prick test (e2).*

ImmunoCAP Rapid, dog dander test evaluated with skin prick test as comparison method
Comments: The percentage of positive skin prick test of antibodies against dog dander was about 27% and higher than expected. Sensitivity was 19%, which was low, but a low sensitivity was expected due to the different ways of measuring the allergy response; ImmunoCAP Rapid gets a response dependent of the serum concentration of IgE antibodies against dog dander and the skin prick test uses allergens from dog hair to create the wheal. People sensitive to dogs might be sensitive to either dander or hair – and there might be differences depending on the dog breed. A Specificity of 98,6% fulfilled the goal of >95,0%.
Two of the three patients that were positive with ImmunoCAP Rapid and not with the skin prick test had taken medication within the last week. Their Phadia 250 results were positive. The third patient had negative results with both Phadia 250 and skin prick test. The patient had symptoms in the spring and summer. There was no history of allergy to dogs. The third ImmunoCAP Rapid results must be wrong, since history and comparison methods were negative.

ImmunoCAP Rapid, dog dander test evaluated with Phadia 250 as comparison method
Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in table 15, the sensitivity was only 53,8% and the specificity was 96,1%. 24 samples were positive with ImmunoCAP Rapid, of these, 11 were negative (<1,50 kIU/L) with Phadia 250. 13 samples were positive with Phadia 250, of these six was negative with ImmunoCAP Rapid.

Phadia 250, dog dander test evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same. The sensitivity for Phadia 250 compared to the skin prick test was 41% and the specificity was 92,2%.

Conclusion for the ImmunoCAP Rapid, dog dander test
The sensitivity of ImmunoCAP Rapid compared to the skin prick test was 19%. A low sensitivity was expected, because the skin prick test does not use the same antigens as ImmunoCAP Rapid/Phadia 250. However, ImmunoCAP Rapid is not supposed to have a significantly lower sensitivity than Phadia 250, which had a sensitivity of 41% compared to skin prick test. It was expected that the agreement between Phadia 250 and ImmunoCAP Rapid would be >95%. ImmunoCAP Rapid did fulfil the goal of specificity >85% compared to skin prick test and specificity >95% when compared to Phadia 250.
Intra-person disagreement: none.
Inter-person reading disagreement in same capillary result: three of 103 (64 in primary health care and 39 in hospital).
Inter-person reading disagreement, capillary samples / heparin samples: two of 39.
Invalid test: none.

Analytical quality of the ImmunoCAP Rapid, cat epithelium and dander test
Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": one.
Inter-person disagreement when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in hospital: none.

Table 16. Capillary ImmunoCAP Rapid results with cat epithelium and dander test

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)	
Skin prick test							
	e2	Cat epithelium and dander	297	Percentage of positive skin prick test	22,2	(18,3 -	26,6)
				Sensitivity	71,2	(60,6 -	80,2)
				Specificity	99,6	(97,9 -	100,0)
				PPV	97,9	(90,0 -	99,9)
				NPV	92,4	(89,0 -	94,9)
Phadia 250							
	e2	Cat epithelium and dander	294	Percentage of positive Phadia 250 test	10,9	(8,1 -	14,4)
				Sensitivity	75,0	(59,2 -	86,8)
				Specificity	90,8	(87,3 -	93,6)
				PPV	50,0	(37,3 -	62,7)
				NPV	96,7	(94,1 -	98,4)

Table 16.a Phadia 250 compared to skin prick test with cat epithelium and dander

Skin prick test							
	e2	Cat epithelium and dander	295	Percentage of positive skin prick test	22,4	(18,4 -	26,8)
				Sensitivity	65,2	(54,3 -	74,8)
				Specificity	95,6	(92,6 -	97,6)
				PPV	81,1	(69,9 -	89,3)
				NPV	90,5	(86,8 -	93,4)

ImmunoCAP Rapid, cat epithelium and dander test evaluated with skin prick test as comparison method

Comments: The percentage of positive skin prick test of antibodies against cat epithelium and dander was as expected to be lower than the actual percentage of positive skin prick test of 22%. Sensitivity was 71,2%, which was lower than the expected 85%. Specificity (99,6%) fulfilled the goal of >95,0%. One result was positive with ImmunoCAP Rapid and negative with the skin prick test and Phadia 250. Four results were negative with ImmunoCAP Rapid and positive with both skin prick test and Phadia 250.

The five ImmunoCAP Rapid results must be wrong, since ImmunoCAP Rapid is expected to give the same results as Phadia 250.

ImmunoCAP Rapid, cat epithelium and dander test evaluated with Phadia 250 as comparison method

Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in the table above, the sensitivity was 75,0% and the specificity 90,8%.

24 samples were positive when measured with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250.

32 samples were positive with Phadia 250, of these 32, eight were negative with ImmunoCAP Rapid.

Phadia 250, cat epithelium and dander test evaluated against skin prick tests

The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same. The sensitivity for Phadia 250 compared to the skin prick test was 65,2% and the specificity was 95,6%.

Conclusion for the ImmunoCAP Rapid, cat epithelium and dander test

ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >85%, compared to skin prick test.

ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >95%, when compared to Phadia 250.

ImmunoCAP Rapid did fulfil the goal of specificity >85% compared to skin prick test and did not fulfil specificity >95% when compared to Phadia 250.

The specificity of ImmunoCAP Rapid compared to the skin prick test and Phadia 250 was 99,6% and 90,8%, respectively.

Intra-person disagreement: none.

Inter-person reading disagreement in same capillary result: one of 103 (64 in primary health care and 39 in hospital).

Inter-person reading disagreement, capillary samples / heparin samples: none.

Invalid test: none.

6.2.7. *Analytical quality of the ImmunoCAP Rapid, mugwort test*
Intra-person reading disagreement: none.
Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": none.
Inter-person disagreement when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in hospital: one, the result was positive in primary health care and negative in hospital. The skin prick test was positive and the Phadia 250 result was negative.

Table 17. Capillary ImmunoCAP Rapid results with mugwort

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)
Skin prick test						
	 w6	*Artemisia vulgaris* Mugwort	297	Percentage of positive skin prick test	17,5	(14,0 - 21,6)
				Sensitivity	59,6	(47,2 - 71,1)
				Specificity	96,7	(94,1 - 98,3)
				PPV	79,5	(65,8 - 89,3)
				NPV	91,9	(88,4 - 94,5)
Phadia 250						
	 w6	*Artemisia vulgaris* Mugwort	294	Percentage of positive Phadia 250 test	9,2	(6,6 - 12,5)
				Sensitivity	51,9	(34,6 - 68,7)
				Specificity	90,6	(87,1 - 93,4)
				PV-pos	35,9	(23,2 - 50,4)
				PV-neg	94,9	(92,0 - 96,9)

Table 17.a Phadia 250 compared to skin prick test with mugwort

Skin prick test						
	 w6	*Artemisia vulgaris* Mugwort	295	Percentage of positive skin prick test	17,3	(13,8 - 21,4)
				Sensitivity	58,8	(46,3 - 70,5)
				Specificity	97,5	(95,1 - 98,9)
				PPV	83,3	(69,5 - 92,4)
				NPV	91,9	(88,4 - 94,5)

ImmunoCAP Rapid, mugwort test evaluated with skin prick test as comparison method

Comments: The percentage of positive skin prick test of antibodies against mugwort was 18%, it was expected to be below 20%. Sensitivity was 59,6%, which was lower than the expected 85%. Specificity (96,6%) fulfilled the goal of >95,0%. Seven results were positive with ImmunoCAP Rapid and negative with the skin prick test, of these six were also negative with the Phadia 250. Seven results were negative with ImmunoCAP Rapid and positive with both skin prick test and Phadia 250.

ImmunoCAP Rapid, mugwort test evaluated with Phadia 250 as comparison method
Comments: ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability, however the Phadia 250 is possitive at 0,35 kIU/L and the level of detection is 1-2 kU/l for ImmunoCAP Rapid. In this comparison, ≥1,5 kIU/L is used as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in table above, the sensitivity is 51,9% and the specificity 90,6%.
25 samples were positive when measured with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250.
13 samples were positive with Phadia 250 and negative with ImmunoCAP Rapid. These 38 results are most likely wrong with ImmunoCAP Rapid when measured with both instruments, since ImmunoCAP Rapid is expected to give the same results as Phadia 250. If the 'false positives' and 'false negatives' had been placed near the cut-of concentration 0,35 kIU/L on Phadia 250 the reason could have been problems to identify the exact right cut-off; however; this is not the case (data not shown).

Phadia 250, mugwort test evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same. The sensitivity for Phadia 250 compared to the skin prick test was 58,8% and the specificity was 97,5%.

Conclusion for the ImmunoCAP Rapid, mugwort test
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >85% compared to skin prick test,
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >95% compared to Phadia 250, the sensitivity was 51,9%.
ImmunoCAP Rapid did fulfil the goal of specificity >85% compared to skin prick test and it did not fulfil specificity >95% when compared to Phadia 250.
Intra-person disagreement: none.
Inter-person reading disagreement in same capillary result: none.
Inter-person reading disagreement, capillary samples / heparin samples: one of 39.
Invalid test: none.

6.2.8. *Analytical quality of the ImmunoCAP Rapid, Alternaria alternata and Cladosporium hebarum test*

Intra-person reading disagreement: none.

Inter-person disagreement defined as "the disagreement between two independent evaluators in a primary health care centre when reading the same ImmunoCAP capillary sample result": one.

Inter-person disagreement when reading a capillary sample result in a primary health care centre and a heparin sample from the same patient in hospital: one, the result was positive in primary health care and negative in hospital. The skin prick test was positive and so was the Phadia 250 result.

Table 18. Capillary ImmunoCAP Rapid results with *Alternaria alternata*

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)
Skin prick test						
	m6	*Alternaria alternata*	296	Percentage of positive skin prick test	9,1	(6,5 - 12,4)
				Sensitivity	59,3	(41,6 - 75,2)
				Specificity	99,3	(97,6 - 99,9)
				PPV	88,9	(68,3 - 98,0)
				NPV	96,1	(93,5 - 97,8)
Phadia 250						
	m6	*Alternaria alternata*	294	Percentage of positive Phadia 250 test	5,1	(3,2 - 7,8)
				Sensitivity	80,0	(55,5 - 94,3)
				Specificity	97,8	(95,7 - 99,0)
				PV-pos	66,7	(44,3 - 84,3)
				PV-neg	98,9	(97,1 - 99,7)

Table 18.a Phadia 250 compared to skin prick test with *Alternaria alternata*

	m6	*Alternaria alternata*	296	Percentage of positive skin prick test	7,9	(5,5 - 11,1)
				Sensitivity	65,2	(45,8 - 81,3)
				Specificity	97,4	(95,1 - 98,7)
				PPV	68,2	(48,2 - 84,0)
				NPV	97,0	(94,6 - 98,5)

ImmunoCAP Rapid, mould test evaluated with skin prick test as comparison method
Comments: Comments: The percentage of positive skin prick test of antibodies against mould in the material was 9% and as expected lower than 10%. It was expected that ImmunoCAP Rapid would have a sensitivity of 88%; however, the sensitivity was 59,3% when *Alternaria alternata* and *Cladosporium hebarum* were comparison, if only *Alternaria alternata* was used as comparison, the sensitivity was 64,0%.
Two results were positive with ImmunoCAP Rapid and not with the skin prick test, both were positive with Phadia 250. Both had taken medication within the last week

ImmunoCAP Rapid, mould test evaluated with Phadia 250 as comparison method
Comments: Results from ImmunoCAP Rapid and Phadia 250 are supposed to have equal traceability. However the Phadia 250 is positive at 0,35 kIU/L while the level of detection is 1-2 kIU/l for ImmunoCAP Rapid. In the comparison with ImmunoCAP Rapid, ≥1,5 kIU/L with Phadia 250 is classified as "positive". The supplier expected a sensitivity and specificity higher than 95% as seen in other studies [15-18]. As seen in the table above, the sensitivity was 80,0% and the specificity 97,8%.
Six samples were positive with ImmunoCAP Rapid and negative (<1,50 kIU/L) with Phadia 250.
Three patients were positive with Phadia 250 and not with ImmunoCAP Rapid.

Phadia 250, mould test evaluated against skin prick tests
The best result ImmunoCAP Rapid can possibly achieve will be equal to the results of Phadia 250 compared to the skin prick test. The reason for this is that ImmunoCAP Rapid is using the same antigens as Phadia 250 even if the cut-off for the two methods is not the same. The sensitivity for Phadia 250 compared to the skin prick test was 65,2% and the specificity was 97,4%.

Conclusion for the ImmunoCAP Rapid, mould test
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >85% compared to skin prick test.
ImmunoCAP Rapid did not fulfil the quality goal for sensitivity, >95% compared to Phadia 250.
ImmunoCAP Rapid did fulfil the goal of specificity >85% compared to skin prick test and specificity >95% when compared to Phadia 250.
Intra-person disagreement: none.
Inter-person reading disagreement of the same capillary result: one of 103 (64 in primary health care and 39 in hospital).
Inter-person reading disagreement, capillary samples / heparin samples: one of 39.
Invalid test: none.

6.2.9. *Analytical quality of the ImmunoCAP Rapid, cockroach, olive, wall pellitory tests*

Table 19. Capillary ImmunoCAP Rapid results with cockroach, olive, wall pellitory

Comparison test	Allergen	Name	n	Calculation	ImmunoCAP Rapid %	(90% CI)
Phadia 250						
	 i6	*Blatella germanica* Cockroach	294	Percentage of positive skin prick test	1,7	(0,7 - 3,6)
				Sensitivity	20,0	(0,6 - 66,9)
				Specificity	99,0	(97,3 - 99,7)
				PV-pos	25,0	(0,8 - 76,2)
				PV-neg	98,6	(96,8 - 99,5)
Phadia 250						
	 t9	*Olea europaea* Olive (pollen)	294	Percentage of positive skin prick test	4,8	(2,9 - 7,4)
				Sensitivity	28,6	(10,4 - 54,4)
				Specificity	98,9	(97,2 - 99,7)
				PV-pos	57,1	(22,2 - 87,3)
				PV-neg	96,5	(94,1 - 98,1)
Phadia 250						
	 w21	*Parietaria judaica* Wall pellitory	294	Percentage of positive skin prick test	2,4	(1,1 - 4,5)
				Sensitivity	42,9	(12,7 - 77,8)
				Specificity	99,0	(97,2 - 99,7)
				PV-pos	50,0	(15,0 - 85,0)
				PV-neg	98,6	(96,8 - 99,5)

Table 18.a Horse

Skin prick test						
		*horse*	299	Percentage of positive skin prick test	7,4	(5,9 - 11,6)

It is seen in the table 18 that the numbers of positive samples for cockroach, olive pollen and wall pellitory is low. However; Phadia 250 and ImmunoCAP Rapid did not – as expected, measure the same samples as positive, therefore the low sensitivity. These allergens are not present in Denmark and some of the positive results are supposed to be due to cross-reactions.

6.2.10. *ImmunoCAP Rapid compared to Skin prick test*

Table 20. ImmunoCAP Rapid compared to skin prick test and Phadia 250

		Compared to skin prick test					Compared to Phadia 250¤	
Allergen	**Name**	**Skin prick test % positive**	**Sensi-tivity**	**Speci-ficity**	**PPV**	**NPV**	**Sensi-tivity**	**Speci-ficity**
	Cat epithelium and dander	22,2	71,2	99,6	97,9	92,4	75,0	90,8
	Common silver birch (pollen)	31,6*	69,1	97,5	92,9	87,2	80,3	92,5
	Mugwort	17,5	59,6	96,7	79,5	91,9	51,9	90,6
	Timothy	36,7*	59,6	98,4	95,6	80,8	76,9	92,6
	Dog dander	26,6	19,0	98,6	83,3	77,1	53,8	96,1
	House dust mites** *Dermatophagoides pteronyssinus (d1)* *Dermatophagoides farinae*	31,6	70,2	95,6	88,0	87,4	79,4	89,2
	Mould** *Alternaria alternata* *Cladosporium hebarum*	9,1	59,3	99,3	88,9	96,1	80,0	97,8

¤ the Phadia 250 is considered possitive at ≥1,50 kIU/L *When 85 positive skin prick tests in common silver birch and grass (timothy) were enrolled, it was agreed with the specialised allergy clinic that if only one inclusion could occur, they should choose a patient positive for birch or grass on the skin prick test. This was agreed in order to finish the evaluation. ** These result refers to the sum of skin prick tests positive for either one or both of the two allergens below, Phadia 250 only use d1 and Alternaria.

Discussion

It is seen that the sensitivity is lower than 85% for all components. ImmunoCAP Rapid is not supposed to achieve a better sensitivity than Phadia 250. Phadia 250 did not reach a sensitivity of 85% when compared with skin prick test at any cutoff level.

It was expected, that ImmunoCAP Rapid would reach a sensitivity and specificity of >95% when compared to Phadia 250. However, ImmunoCAP Rapid did not achieve >95% sensitivity, when compared with Phadia 250 and for only two components the specificity was >95%.

The reason for this is unknown.

6.2.11. *Inter-person disagreement*

Inter-person reading disagreement
64 ImmunoCAP Rapids were read by two individuals in the primary health care centres adding up to a total of 1280 individual test readings. In 14 of these cases, the two individuals were not in accordance with each other.

Disagreement, capillary samples / heparin samples
Errors: 39 heparinised whole blood samples were analysed with ImmunoCAP rapid upon arrival to the hospital laboratory. The results were read by two individuals adding up to a total of 780 individual test readings. At least 17 of these (2,2%) were not in accordance with the readings from the primary health care centres.
The disagreement can originate from the different materials: heparinised blood versus capillary blood, from the fact, that individuals do read the same result differently or it could originate from a bias of the evaluator, which also had performed the skin prick test result and had heard of the expectations from the patient.

6.2.12. *Disagreement between ImmunoCAP Rapid and Phadia 250*

ImmunoCAP Rapid is supposed to have about the same sensitivity as Phadia 250. However, according to the manufacturer ImmunoCAP Rapid is positive at the concentration of 1-2 kIU/L on Phadia 250 while Phadia 250 is positive at 0,34 kIU/L. The quality goal for sensitivity >95% was not reached for any component when compared with Phadia 250.

The sensitivity was lower than expected for all components, lowest for dog, 53,8%, and highest for Common silver birch, 80,3%. A possible explanation for the low sensitivities might be a very weak colour band in the ImmunoCAP Rapid device, which gives some false negative results. Another possibility is that the distinction between "positive" or "negative" does not correlate with the concentration 1,50 kIU/L on Phadia 250. A third possibility is that the evaluators have been biased by the skin prick test results or the expectations from the patients.
Further it is demonstrated in figure 5, that it is difficult technical to transfer a result from a quantitative measure in an instrument to an ordinal scale based on lateral flow technology. It has previous been stated [3,19,20], that ‘negative’ and ‘positive’ results in an ordinal scale have to differ from each other with a factor of five in concentration in order to differentiate between ‘negative’ and ‘positive’. This means, that if 0,34 kIU/L is negative, then >1,7 kIU/L can be positive. In the concentration interval 0,35 to 1,69 kIU/L positive as well as negative results may be correct.

Figure 5 demonstrate that there is no obvious cut-off concentration for the seven components in Phadia 250, given that the positive and negative ImmunoCAP Rapid results are correct.

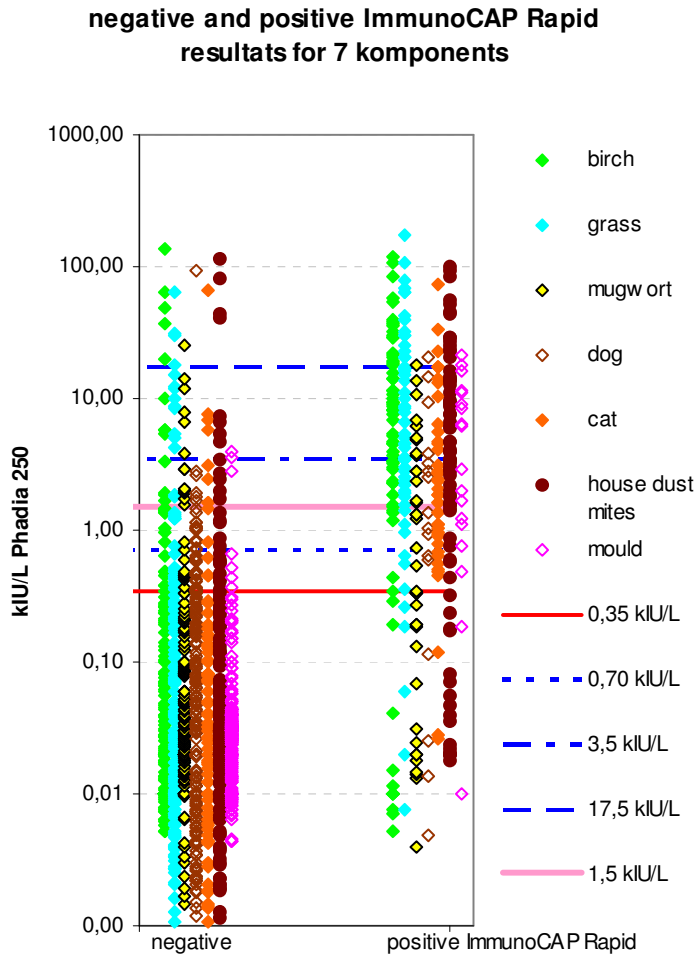


Figure 5. In the x-axis the results for seven allergens on ImmunoCAP Rapid are 'negative' to the left and 'positive' to the right for the same seven allergens. In the y-axis the Phadia 250 results are given. All results lower than 0,35 kIU/L on Phadia 250 are supposed to be negative (red line). In ImmunoCAP Rapid the detection limit is supposed to be 1-2 kIU/L The concentration limits for the old classification is shown with dotted lines.

6.3 Evaluation of user-friendliness

6.3.1 Questionnaire filled in by the evaluators
The most important response regarding user-friendliness comes from the users themselves. The end-users often emphasize other aspects than those pointed out by more extensively trained laboratory personnel.

At the end of the evaluation period, each user filled in a questionnaire about the user-friendliness of the instrument. The questionnaire is divided into four sub-areas:

- Rating of the information in the manual and insert
- Rating of time factors for preparation and measurement
- Rating of performing internal and external quality control
- Rating of operation facilities. Is the system easy to handle?

The questionnaire and the expressed opinions are presented in Table 21 to 24. The first column shows what is up for consideration. The second column shows the rating by the individual users at the evaluation sites. The third to fifth column show the rating options. Coloured frames mark the cells with the overall ratings from all evaluating sites. The last row in each table summarises the total rating in the table. The total rating is an overall assessment of the described property, and not necessarily the arithmetic mean of the rating in the rows. Consequently, a single poor rating can justify an overall poor rating, if this property seriously influences on the user-friendliness of the system.
Unsatisfactory and intermediate ratings will be marked with an asterisk and explained below the table.

In hospital Nina Brøgger, Stine Weber and Esther Jensen read the tests and in the 14 primary health care centres more than 18 individuals read the results. From two centres the evaluation form was not returned and from some centres one form was filled in by two evaluators.

Table 21. Assessment of the information in the manual / insert

Information in manual / insert about:	Ratings	Overall rating		
		Red	**Yellow**	**Green**
General impression	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Table of contents	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Preparations / Pre-analytic procedure	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Specimen collection	G G – Y G G G R -	Unsatisfactory	Intermediate	Satisfactory
Measurement / Reading	R Y – Y G Y G R -	Unsatisfactory	Intermediate*	Satisfactory
Measurement principle	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Sources of error	R R – G Y Y G Y -	Unsatisfactory	Intermediate*	Satisfactory
Troubleshooting	R R – Y Y G G Y -	Unsatisfactory	Intermediate*	Satisfactory
Keyword index	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Readability	G G – G G G G G -	Unsatisfactory	Intermediate	Satisfactory
Available insert in Danish, Norwegian, Swedish	G G – G G G G Y -	Unsatisfactory	Intermediate	Satisfactory
Rating for the information in the manual				Satisfactory

Positive comments:
Negative comments: * ‘Readings can be difficult’, ‘Sources of error? Did not have information about Fault-tracing / Troubleshooting’

Table 22. Assessment of time factors

Time factors	Ratings	Red	Yellow	Green
Time for preparations / Pre-analytical time	G G G G G G G G Y	>10 min	6 to 10 min.	<6 min.
Analytic time*	G G G G R R R Y Y	>20 min	10 to 20 min.	<10 min.
Required training time	G G G G G G G G G	>8 hours	2 to 8 hours	<2 hours
Stability of test, unopened package	? ? G G G ? - - -	<3 months	3 to 5 months	>5 months
Stability of test, opened package	? ? G G Y ? – R -	<14 days	14 to30 days	>30 days
Other comments about time factors (please specify)	- - G G - - G Y -	Unsatisfactory	Intermediate**	Satisfactory
Rating of time factors				Satisfactory

*Analytical time is not filled in because the comparison is the skin prick test, which takes more than 10 minutes.

Positive comments: '*more than 20 minutes OK, because the patient do not have to wait'
Negative comments: '**One has to pay attention to time'

Table 23. Assessment of quality control possibilities

Quality control	Ratings	Red	Yellow	Green
Internal quality control*	G - - - -	Un-satisfactory	Intermediate	Satisfactory
External quality control	- G G -	Un-satisfactory	Intermediate	Satisfactory
Stability of quality control material, unopened	- - G -	<3 months	3 to5 months	>5 months
Stability of quality control material, opened	- - - -	≤1 day	2 to 6 days	>6 days or disposable
Storage conditions for quality control materials, unopened	- - - -	–20°C	+2 to +8°C	+15 to +30°C
Storage conditions for quality control materials, opened	- - - -	–20°C	+2 to +8°C	+15 to +30°C
Usefulness of the quality control	- - - -	Unsatisfactory	Intermediate	Satisfactory
Rating of quality control				Satisfactory

* Internal quality control was not used, the rating is given to the two built-in controls in the test

Table 24. Assessment of the operation facilities

Operation facilities		**Red**	**Yellow**	**Green**
To prepare the test / instrument	G G G G G G G G G	Un satisfactory	Intermediate	Satisfactory
To prepare the sample	G G G G G G G G G	Un-satisfactory	Intermediate	Satisfactory
Application of specimen	G G G G G G G Y G	Un-satisfactory	Intermediate	Satisfactory
Specimen volume	R G G G G G G R G	Un-satisfactory	Intermediate	Satisfactory
Number of procedure step	G G G G G G G Y G	Un-satisfactory	Intermediate	Satisfactory
Instrument / test design	Y G G G G G G G G	Un-satisfactory	Intermediate	Satisfactory
Reading / Interpretation of the test result	R R Y G Y Y Y R Y	Un-satisfactory	Intermediate*	Satisfactory
Sources of errors	R ? Y Y - G G Y -	Un-satisfactory	Intermediate**	Satisfactory
Cleaning / Maintenance	G G G G – G G Y G	Un-satisfactory	Intermediate	Satisfactory
Hygiene, when using the test	G G G G G G G Y G	Un-satisfactory	Intermediate	Satisfactory
Storage conditions for tests, unopened package		–20°C	+2 to +8°C	+15 to +30°C
Storage conditions for tests, opened package		–20°C	+2 to +8°C	+15 to +30°C
Environmental aspects: waste handling	Y Y G G Y G G G G	Special precautions	Sorted waste	No precautions
Intended users	G G G R Y G G R G	Biomedical scientists	Laboratory experienced	GP personnel or patients
Size and weight of package	G G G G G G G G G	Un-satisfactory	Intermediate	Satisfactory
Rating of operation				Satisfactory

*The test has a possibility for false negative results due to a very weak line when results are positive.
**The results did not always compare to the results of the skin test.

Positive comments: 'Very easy to use'. 'A smart principle – that needs some improvement (see below)'. 'Good for primary health care'. 'Does not take long.'

Negative comments: 'The interpretation of the test could be difficult, since there were not always clear lines in the reading chamber'. 'Some test cassettes had vertical lines in the reading chambers.' 'Demands a lot of capillary blood.'

6.3.2 Assessment of the user-friendliness

More than ten evaluators evaluated the ImmunoCAP Rapid and everyone were in general terms pleased with the test.

Some concerns were present among the evaluators regarding the interpretation of the test for low concentration of allergens, since the distinction between positive and negative responses was difficult. Some evaluators thought 110 µL capillary blood was a lot.

There was great satisfaction with the fact that the test can be performed even if the patient is on medication for rhinitis symptoms and that it was possible to perform the test after the patient has left using venous heparinised whole blood.

Overall, the ImmunoCAP Rapid showed good user friendliness, and the evaluators expressed that the ImmunoCAP Rapid was very easy to operate.

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8. The organisation of SKUP

Scandinavian evaluation of laboratory equipment for primary health care, *SKUP*, is a co-operative commitment of NOKLUS[1] in Norway, DAK-E[2] in Denmark, and EQUALIS[3] in Sweden. SKUP was established in 1997 at the initiative of laboratory medicine professionals in the three countries. SKUP is led by a Scandinavian *steering committee* and the secretariat is located at NOKLUS in Bergen, Norway.

The purpose of SKUP is to improve the quality of near patient testing in Scandinavia by providing objective and supplier-independent information on analytical quality and user-friendliness of laboratory equipment. This information is generated by organizing SKUP *evaluations*.

SKUP offers manufacturers and suppliers evaluations of equipment for primary health care and also of devices for self-monitoring. Provided the equipment is not launched onto the Scandinavian market, it is possible to have a confidential pre-marketing evaluation. The company requesting the evaluation pays the actual testing costs and receives in return an impartial evaluation.

There are *general guidelines* for all SKUP evaluations and for each evaluation, a specific *SKUP protocol* is worked out in co-operation with the manufacturer or their representatives. SKUP signs *contracts* with the requesting company and the evaluating laboratories. A *complete evaluation* requires one part performed by experienced laboratory personnel as well as one part performed by the intended users.

Each evaluation is presented in a *SKUP report* to which a unique *report code* is assigned. The code is composed of the acronym SKUP, the year and a serial number. A report code, followed by an asterisk (*), indicates a special evaluation, not complete according to the guidelines, e.g. the part performed by the intended users was not included in the protocol. If suppliers use the SKUP name in marketing, they have to refer to www.skup.nu and to the report code in question. For this purpose, the company can use a logotype available from SKUP containing the report code.

SKUP reports are published at www.skup.nu.

[1] NOKLUS (Norwegian Quality Improvement of Primary Care Laboratories) is an organisation founded by Kvalitetsforbedringsfond III (Quality Improvement Fund III), which is established by The Norwegian Medical Association and the Norwegian Government. NOKLUS is professionally linked to “Seksjon for Allmennmedisin” (Section for General Practice) at the University of Bergen, Norway.

[2] SKUP in Denmark is placed in Hillerød Hospital. SKUP in Denmark reports to DAK-E (Danish Quality Unit of General Practice), an organisation that is supported by KIF (Foundation for Quality and Informatics) and Faglig udvalg (Professional Committee), which both are supported by DR (The Danish Regions) and PLO (The Organisation of General Practitioners in Denmark).

[3] EQUALIS AB (External quality assurance in laboratory medicine in Sweden) is a limited company in Uppsala, Sweden, owned by “Sveriges Kommuner och Landsting” (Swedish Association of Local Authorities and Regions), “Svenska Läkaresällskapet” (Swedish Society of Medicine) and IBL (Swedish Institute of Biomedical Laboratory Science).

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Attachments

1. Specifications and basic facts about ImmunoCAP Rapid
2. Guide to sampling – skin prick test (in Danish)
3. Manufacturer and supplier
4. Expected rate of positives, Odense and Helsingborg (in Danish and Swedish)
5. Questionnaire in Danish
6. Questionnaire in English
7. Information about Phadia 250
8. Raw data, the ImmunoCAP Rapid
9. List of previous SKUP evaluations
10. Comments from the manufacturer?

Attachments with raw data are included only in the report to Phadia and SKUP.

Attachment 1. Specifications and basic facts about ImmunoCAP Rapid

Table 1. Basic facts

Name of the measurement system:	ImmunoCAP® Rapid specific IgE antibodies system
Dimensions and weight:	Width: 95 mm Depth: 40 mm Height: 5 mm Weight: 10 g
Components of the measurement system:	house dust mite (d1) cat epithelia (e1) birch pollen (t3) dog epithelia (e5) mugwort pollen (w6) grass pollen (g6) cocroch (i6) olive pollen (t9) wall pellitory (w21) Alternaria alternata (m6)
Measurand:	Allergen specific IgE
Sample material:	Capillary blood and heparin stabilized whole blood
Sample volume:	110 µL
Measuring principle:	Lateral flow immunoassay
Traceability:	2nd International Reference Preparation (IRP) 75/502 of Human Serum Immunoglobulin E
Calibration:	-
Measuring range:	Positive or negative
Linearity:	-
Measurement duration:	20 minutes
Operating conditions:	ImmunoCAP Rapid are stable for 1 hour at temperature between +18°C and 32°C and relative humidity between 15% and 85%.
Electrical power supply:	-
Recommended regular maintenance:	-
Package contents:	Test cassette, blood collector, Developer solution, user manual
Necessary equipment not included in the package:	Lancets meant for skin prick tests

Table 2. Post analytical traceability

Is input of patient identification possible?	-
Is input of operator identification possible?	-
Can the instrument be connected to a bar-code reader?	-
Can the instrument be connected to a printer?	-
What can be printed?	-
Can the instrument be connected to a PC?	-
Can the instrument communicate with LIS (Laboratory Information System)? If yes, is the communication bidirectional?	-
What is the storage capacity of the instrument and what is stored in the instrument?	-
Is it possible to trace/search for measurement results?	-

Table 3. Facts about the reagent/test strips/test cassettes

Name of the reagent/test strips/test cassettes:	ImmunoCAP® Rapid specific IgE antibodies system
Stability in unopened sealed vial:	Until expiration date at 2-8 °C,
Stability in opened vial:	1 hour at room temperature
Package contents:	Test Cartridge, Blood collector, Developer solution, Manual

Table 4. Quality control

Electronic self check:	-
Recommended check materials and volume:	-
Stability in unopened sealed vial:	-
Stability in opened vial:	-
Package contents:	-

Table 5. Marketing information

Manufacturer:	Phadia AB Marknadsbolag Sverige P.O. Box 6460 SE-751 37 Uppsala Sweden E-mail: marknadsbolaget.sverige@phadia.com Tel: +46 18 16 50 00 Fax: +46 18 16 63 24
Retailers in Scandinavia:	Denmark: Phadia Gydevang 33 DK-3450 Allerød Tel: +45 70 23 33 06 Fax: +45 70 23 33 07 E-mail: info.denmark@phadia.com Norway: Phadia Postboks 4756, Nydalen NO-0421 Oslo Tel: +47 216 732 80 Fax: +47 216 732 81 E-mail: phadia.no@phadia.com
In which countries is the system marketed:	Globally X Scandinavia ☐ Europe ☐
Date for start of marketing the system in Scandinavia:	2007
Date for CE-marking:	2005
In which Scandinavian languages is the manual available:	Danish, Norwegian, Swedish

Attachment 2. Guide to sampling – skin prick test (in Danish)

Allergi i Almen Praksis

Procedure for udførelse af priktest

Formål: At påvise allergi/sensibilisering over for et eller flere allergener
At stille diagnosen type1 allergi.

Ordination: Lægen ordinerer hvilke allergener der skal priktestes med ud fra anamnesen.

Forudsætning: Eksemfrit område på én af underarmenes inderside.

Medicinpause:

Antihistaminer systemisk	3 døgn
Antihistaminer lokalt	ingen
Systemisk steroid 30 mg Prednison/dgl. <1 uge	ingen
Systemisk steroid 10 mg Prednison/dgl.	ingen
Depotsteroid som injektion	ingen
Steroid lokalt, højpotent (gr. 3+4)	2-3 uger
Steroid lokalt, mildt (gr. 1+2)	3 dage
Psykofarmaka	uger

(Specifikt IgE i blod anbefales frem for Priktest)

EMLA Brug af Emla påvirker ikke kvadlen,
men "rødmen" mindskes (paediatri.dk)

Forberedelse: Anafylaksiberedskab.
Allergener opbevares i køleskab ved 2-8^{o} (holdbarhed ½ år).

Materiale:
Allergener
Priklancetter og ikke "kapillærlancetter"
Nummertape
Blank overføringstape
Serviet til aftørring af dråberne
Blanketter til registrering
Pen til aftegning af reaktionerne
Spritsvabs
Kanyleboks
Lineal
Evt. Mepyramin crème 2% til brug efter aflæsning

Information til patienten og pårørende:

Patienten informeres om baggrunden for undersøgelsen, hvorledes denne udføres, at kløe, hævelse og irritation er udtryk for en positiv reaktion, men at dette svinder inden for 30-60 minutter. Evt. udleveres patientinformation om priktest.

Udførelse:

Personen, der skal udføre priktest, vasker hænder, helst håndsprit

1. Huden skal være ren og tør uden creme (evt. afspritte eller afvaske testområdet)

2. Nummertape placeres på underarmens inderside. Ved små børn kan tapen deles til begge arme

3. Afsæt en dråbe af allergenerne på venstre side (”højre håndet” og højre side for de ”venstre håndede”) af nummertapen. Rækkefølgen af allergenerne bør stemme overens med skemaet

4. Med engangslancet prikkes vinkelret gennem dråben 1 mm ned i huden med et konstant og ensartet tryk. (Lancettens spids er 1 mm op til ”skuldrene”)

   Benyt aldrig samme lancet til flere allergener

5. Dobbeltbestemmelse udføres ved at prikke først gennem allergendråber og derefter føre ekstrakt med lancetten til prik på den modsatte side af tapen. Prik først gennem alle allergenerne og til sidst den negative og positive kontrol (positiv kontrol reagerer lidt hurtigere end allergenerne)

6. Fjern overskydende allergenekstrakt med serviet eller lignende (undgå at gnide dråberne sammen)

7. Reaktionen aflæses efter 15 min. hos voksne og 10 min. hos børn. I ventetiden må patienten ikke klø på testområdet

8. Omridset af papelen aftegnes med tynd streg (stregen tegnes på grænsen mellem papel og erythem) og overføres med tape til testblanket. Negativ reaktion registreres med en prik

9. Ved generende kløe smøres evt. med Mepyramin 2% på testområdet

Information:

Ved sikre positive fund orienteres om forebyggelse og behandling af allergien. Der gennemgås og udleveres skriftligt materiale.

Tolkning:

En positiv reaktion er defineret som diameter ≥ 3mm (areal >7mm^2)

Priktestreaktionen på allergenet fortæller alene graden af sensibilisering og intet om den kliniske betydning eller om sværhedsgraden af allergiske symptomer

Hvis **histaminreaktionen er <3mm** kan dette skyldes insufficient teknik eller indtagelse af farmaka, der blokerer histaminreceptorerne

Reaktion >2mm på den negative kontrol kan skyldes for kraftigt tryk med lancetten eller dermografisme (oftest ses reaktion på alle prikker af samme størrelse)
Hvis dobbeltkontroller visuelt afviger væsentligt fra hinanden bør testen gentages indtil der opnås reproducerbare resultater.
Dobbelt-prik udføres som led i kvalitetsvurdering af den tekniske kvalitet

Attachment 3. Manufacturer and supplier

Manufacturer of ImmunoCAP Rapid
Phadia AB Marknadsbolag Sverige
P.O. Box 6460
SE-751 37 Uppsala
Sweden
E-mail: marknadsbolaget.sverige@phadia.com
Tel: +46 18 16 50 00
Fax: +46 18 16 63 24

Suppliers in the Scandinavian countries
Phadia distributes the ImmunoCAP Rapid themselves in Scandinavia via these local addresses:

Denmark:
Phadia ApS
Gydevang 33
DK-3450 Allerød
Denmark
Tel: +45 70 23 33 06
Fax: +45 70 23 33 07
E-mail: info.denmark@phadia.com

Norway:
Phadia
Postboks 4756, Nydalen
NO-0421 Oslo
Norway
Tel: +47 216 732 80
Fax: +47 216 732 81
E-mail: phadia.no@phadia.com

Attachment 4. Statistics and expected rate of positive samples with Phadia 250

Statistical terms and expressions
The definitions and formulas in this section originate from the Geigy document [13]

Statistical calculations
Sensitivity is true positive/(true positive + false negative)

Specificity is true negative/(true negative + false positive)

Positive predictive value (PPV) is true positive/(true positive + false positive)

Negative predictive value (NPV) is true negative/(true negative + false negative)

Percentage of positive test is true positive/(true positive + true negative + false positive + false negative)

Calculated parameters

	Truth		
	Positive	**Negative**	
Evaluated test positive	a	b	PPV = a/(a+b)
Evaluated test negative	c	d	NPV = d/(c+d)
	Sensitivity = a/(a+c)	Specificity = d/(b+d)	

Calculation of confidence intervals
Estimation of CI for fractions/proportions is performed according to the formula 772 in Documenta Geigy [13] for lower (left) and upper (right) confidence limits, probability left and probability right for fractions <0.50:

$$p_1, p_r = (A - B) \mp \sqrt{B(2 - (A - B) - A)}$$

where $A_{P1}, A_{\mathrm{Pr}} = \dfrac{x + 1/2 \mp 1/2 + z^2/4}{N + 1}$ and $B_{p1}, B_{pr} = \dfrac{z^2/2 \cdot (x + 1/2 \mp 1/2)}{(N+1)^2}$
and where z is the standardised deviate of a Gaussian distribution for a certain specified probability. The 90 % Confidence interval were chosen for the calculations

Number of positive and negative tests needed
To assure a high statistical impact in an ordinal scale evaluation 100 true positive and 100 true negative results are needed. With a low number of samples, the confidence intervals become huge. The importance is illustrated below.

The expected percentage of positive tests for birch, house dust mites and grass is about 33% based on all data available at Odense University Hospital in 2005

A top six list for percentage of positive tests of the samples investigated for allergens on Phadia 250 in 2005 in the Odense University Hospital, Denmark is given in the table below. The percentage of positive tests was between 24,4 and 36,8%.

ImmunoCAP250, Odense University Hospital, 2005

komponent	n=	N pos=	% positive		Priktest
S-Derm.farinae(d2)(IgE)	508	187	36,8	husstøvmide, house dust r	1. husstøv
S-Derm.Pteronyssinus(d1)(IgE)	735	251	34,1	husstøvmide	1. husstøv
S-Eng-rottehale(g6)(IgE)	954	323	33,9	græs	2 græs
S-Birk(t3)(IgE)	670	214	31,9	birk, birch	3 birk
S-Hundeskæl(e5)(IgE)	473	118	24,9	hund, dog	4. hund
S-Katteepitel(e1)(IgE)	487	113	23,2	kat, cat epithelium	5. kat
S-Grå bynke(w6)(IgE)	377	92	24,4	Mugwort pollen	

The eight most common inhalation allergens from consecutive patients in Helsingborg, Sweden seeing their doctor for problems which could be allergy are listed in the table below.

Helsingborgs Lasarett. Sweden, data given to Gunnar Nordin, SKUP in Sweden

Antikropp	**n testade**	**n klass >0**	**% klass >0**	**n, positive klass >1**	**% positive klass >1**	**name in english**
Gräspollen-antikroppar (IgE)	296	87	29	69	23	Grass
Kattepitel-antikroppar (IgE)	270	70	26	48	18	Cat
Björkpollen-antikroppar (IgE)	71	43	61	37	52	Birch
D_ Farinae-antikroppar (IgE)	89	48	54	33	37	House dust mites
D_ Pteronyssinus-antikroppar (IgE)	92	36	39	23	25	House dust mites
Hundepitel-antikroppar (IgE)	221	20	9	12	5	Dog
Gråbopollen-antikroppar (IgE)	32	12	38	9	28	Mugwort
Hasselpollen-antikroppar (IgE)	33	13	39	6	18	Hasel nut
Cladiosporium-antikroppar (IgE)	19	3	16	2	11	Mould
Alternaria-antikroppar (IgE)	14	3	21	1	7	Mould
Aspergillus-antikroppar (IgE)	8	0	0	0	0	Mould

The tables were generated to get an idea of The percentage of positive tests of antibodies against the allergens in the Scandinavian population. The table below demonstrates the importance of n and percentage of positive tests for the range of the CI.

The effect of number and percentage of positive tests on the Conficende Interval of sensitivity and specificity

Number of tests	**Percentage of positive test (%)**	**Sensitivity (%) (90% CI)**	**Specificity (%) (90% CI)**
54	50	88,0 (73,2 to 96,9)	95,5 (82,9 to 99,9)
83	33	88,0 (72,5 to 96,3)	95,5 (87,5 to 99,0)
250	33	88,0 (80,4 to 93,3)	95,5 (91,8 to 97,8)
500	20	88,0 (81,2 to 92,9)	95,5 (93,4 to 97,1)

Attachment 5. Questionnaire in Danish

Spørgeskema om høfeber. **Henvisningsnr.:**

Navn:_____ **CPR-nr:**_____.

Tlf. nr.:_____.

Har du en eller flere af nævnte gener:

Næsekløe	ja ☐	nej ☐
Næsen 'løber'	ja ☐	nej ☐
Nysen	ja ☐	nej ☐
Tilstoppet næse	ja ☐	nej ☐
Øjenkløe	ja ☐	nej ☐
Røde øjne	ja ☐	nej ☐
Tåreflåd	ja ☐	nej ☐
Kløe i ganen	ja ☐	nej ☐

Hvornår optræder dine symptomer?
kun et kryds.

Hele året	ja ☐	nej ☐
Forår	ja ☐	nej ☐
Sommer	ja ☐	nej ☐
Forår og sommer	ja ☐	nej ☐
Efterår	ja ☐	nej ☐

Andet, angiv.....

Mener du at lide af høfeber? Ja☐ nej ☐

Hvis ja, Hvad giver dig symptomer, sæt gerne flere kryds?

Husstøv-mider	ja ☐	nej ☐
Græs pollen.....	ja ☐	nej ☐
Birkepollen	ja ☐	nej ☐
Kat	ja ☐	nej ☐
Hund.....	ja ☐	nej ☐
Hest	ja ☐	nej ☐
Hasselpollen	ja ☐	nej ☐
Gråbynke.....	ja ☐	nej ☐
Latex.....	ja ☐	nej ☐
Skimmelsvampe.....	ja ☐	nej ☐

Andet, skriv.....

Medicin

Tager du medicin, når du har gener	Ja☐	nej ☐
Tager du medicin hele året	Ja☐	nej ☐
Har du taget medicin den sidste uge	Ja☐	nej ☐

Hvis ja

Antihistamin, 'Høfeber tabletter' ..... Ja☐ nej ☐
Indsprøjtning med binyrebarkhormon indenfor de seneste 3 mdr.... Ja☐ nej ☐

Andet, Navn.....

Efter resultat af priktest (og evt. 'ny test'). Har du symptoner ved de positive udslag?

Husstøv-mider	ja ☐	nej ☐
Græs pollen.....	ja ☐	nej ☐
Birkepollen	ja ☐	nej ☐
Kat	ja ☐	nej ☐
Hund.....	ja ☐	nej ☐
Hest	ja ☐	nej ☐
Hasselpollen	ja ☐	nej ☐
Gråbynke.....	ja ☐	nej ☐
Latex.....	ja ☐	nej ☐
Skimmelsvampe.....	ja ☐	nej ☐

Attachment 6. Questionnaire in English

Questionnaire regarding rhinitis Reference number

Name:_____ Social security number. _____
Phone: _____

Do you have one or more of the following symptoms:

Itchy nose	yes ☐	no ☐
Runny nose	yes ☐	no ☐
Sneezing	yes ☐	no ☐
Clogged nose	yes ☐	no ☐
Itchy eyes	yes ☐	no ☐
Red eyes	yes ☐	no ☐
Lacrimation	yes ☐	no ☐
Itchy palate	yes ☐	no ☐

When do your symptoms appear? Just one cross, please.

All year	yes ☐	no ☐	other, please specify.....……..
Spring	yes ☐	no ☐	
Summer	yes ☐	no ☐	……..
Spring and summer	yes ☐	no ☐	
Fall	yes ☐	no ☐	……..

Do you believe you have rhinitis? yes ☐ no ☐

If yes, what gives you symptoms? Multiple crosses allowed.

House dust mites	yes ☐	no ☐
Grass pollen……	yes ☐	no ☐
Birch pollen……	yes ☐	no ☐
Cat ……	yes ☐	no ☐
Dog……	yes ☐	no ☐
Horse ……	yes ☐	no ☐
Hazel pollen……	yes ☐	no ☐
Mugwort……	yes ☐	no ☐
Latex……	yes ☐	no ☐
Mould ……	yes ☐	no ☐

Have you changed your opinion after the results of the skin prick test.?

House dust mites	yes ☐	no ☐
Grass pollen ……	yes ☐	no ☐
Birch pollen……	yes ☐	no ☐
Cat ……	yes ☐	no ☐
Dog……	yes ☐	no ☐
Horse ……	yes ☐	no ☐
Wall pellitory……	yes ☐	no ☐
Mugwort……	yes ☐	no ☐
Cockroach…….	yes ☐	no ☐
Mould ……	yes ☐	no ☐

Other, please specify……..

Medication

Do you take medication, when you have symptoms?	yes ☐	no ☐
Do you take medication throughout the year?	yes ☐	no ☐
Have you taken medication in the last week?	yes ☐	no ☐

If yes: Antihistamine, 'rhinitis tablets' yes ☐ which brand ……
Injection with corticosteroids within the last three months yes ☐ ……..
Other, name……..……

Conclusion based on questionnaire and skin prick test: allergy / sensitized?

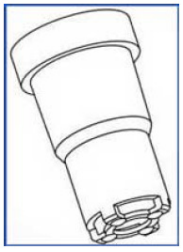
House dust mites	yes ☐	no ☐		
Grass pollen ……	yes ☐	no ☐		
Birch pollen……	yes ☐	no ☐		
Cat ……	yes ☐	no ☐		
Dog……	yes ☐	no ☐		
Horse ……	yes ☐	no ☐		
Wall pellitory……	yes ☐	no ☐		
Mugwort……	yes ☐	no ☐		
Mould ……	yes ☐	no ☐	Other	yes ☐ no ☐

Attachment 7. Information about Phadia 250

(from the manufacturer)

ImmunoCAP description.

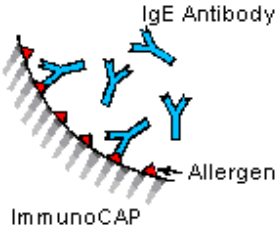
ImmunoCAP are flexible hydrophilic polymer carrier encased in a capsule. The carrier consists of an activated cellulose derivate where to the allergen is coupled.



Principle for the ImmunoCAP 250 Specific IgE method

1. Prewash of ImmunoCAP with washing solution to remove glycerol which is added to protect the coupled allergens to the cellulose sponge.

2. Incubation of patient serum sample to the ImmunoCAP. Allergens are cpuopled to the ImmunoCAP sponge and if there is specific IgE antibodies present in the serum sample an antibody-antigen binding will occur.



3. Wash to remove unspecific IgE.
4. Adding of enzyme-labeled antibodies against IgE. (Conjugate) Forming of complex.

5. After incubation a wash is done to remove any unbound enzyme-labeled anti-IgE (conjugate).
6. Adding of a developing agent and new incubation. Presence of a complex leads to that the enzyme catalyzes a reaction in the development solution so a fluorescent compound is formed.

7. The reaction is stopped by adding Stop solution. The fluorescence in the eluate (in fluid phase) is then being determined. The higher the fluorescence the higher amount of specific IgE is present in the serum sample. For evaluation of the result the response value of the patient sample is being directly compared to the response of the calibrator (standard curve).

Conjugate: B-galactosidase-anti-IgE (mouse monoclonal antibodies)
Development: 4-Methylumbelliferyl-B-D-galactoside
Stop: Natrium karbonat 4%

Attachment 8. Raw data , the ImmunoCAP Rapid

Attachment 9. List of previous SKUP evaluations

Summaries and complete reports from the evaluations are found at www.skup.nu. In addition, SKUP reports are published at www.skup.dk, where they are rated according to the national Danish quality demands for near patient instruments used in primary health care. SKUP summaries are translated into Italian by Centre for Metrological Traceability in Laboratory Medicine (CIRME), and published at http://users.unimi.it/cirme. SKUP as an organisation has no responsibility for publications of SKUP results on these two web-sites.

SKUP evaluations from number 65 and further

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2012/95	Glucose[1]	Mendor Discreet	Mendor Oy
SKUP/2012/94	Glucose[1]	Contour XT	Bayer Healthcare
SKUP/2011/93*	Glucose	Accu-Chek Performa	Roche Diagnostics
SKUP/2012/91	HbA1c	Quo-Test A1c	Quoient Diagnostics Ltd
SKUP/2011/90	CRP	*i*-CHROMA	BodiTech Med. Inc.
SKUP/2010/89*	Glucose	FreeStyle Lite	Abbott Laboratories
SKUP/2010/88*	HbA1c	*Confidential*	
SKUP/2011/86	Glucose[1]	OneTouch Verio	LifeScan, Johnson & Johnson
SKUP/2011/84*	PT-INR	Simple Simon PT and MixxoCap	Zafena AB
SKUP/2010/83*	Glucose	*Confidential*	
SKUP/2010/82*	Glucose, protein, blood, leukocytes, nitrite	Medi-Test URYXXON Stick 10 urine test strip and URYXXON Relax urine analyser	Macherey-Nagel GmBH & Co. KG
SKUP/2010/81*	Glucose	mylife PURA	Bionime Corporation
SKUP/2010/80	PT (INR)	INRatio2	Alere Inc.
SKUP/2010/79*	Glucose, protein, blood, leukocytes, nitrite	CombiScreen 5SYS Plus urine test strip and CombiScan 100 urine analyser	Analyticon Biotechnologies AG
SKUP/2010/78	HbA1c	In2it	Bio-Rad
SKUP/2011/77	CRP	*Confidential*	
SKUP/2009/76*	HbA1c	*Confidential*	
SKUP/2009/75	Glucose	Contour	Bayer HealthCare
SKUP/2009/74	Glucose[1]	Accu-Chec Mobile	Roche Diagnostics
SKUP/2010/73	Leukocytes	HemoCue WBC	HemoCue AB
SKUP/2008/72	Glucose[1]	*Confidential*	
SKUP/2009/71	Glucose[1]	GlucoMen LX	A. Menarini Diagnostics
SKUP/2011/70*	CRP	smartCRP system	Eurolyser Diagnostica GmbH
SKUP/2008/69*	Strep A	Diaquick Strep A test	Dialab GmbH
SKUP/2010/67	Allergens	*Confidential*	
SKUP/2008/66	Glucose[1]	DANA DiabeCare IISG	SOOIL Developement co. Ltd
SKUP/2008/65	HbA1c	Afinion HbA1c	Axis-Shield PoC AS

*A report code followed by an asterisk indicates that the evaluation is not complete according to SKUP guidelines, since the part performed by the intended users was not included in the protocol, or the evaluation is a follow-up of a previous evaluation, or the evaluation is a special request from the supplier. [1] Including a user-evaluation among diabetes patients