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Determination of Multiple Allergen-Specific IgE by Microfluidic Immunoassay Cartridge in Clinical Settings

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Abstract

1). Shyur SD, Jan RL, Webster JR, Chang P, Lu YJ,4 and Wang JY

2). Determination of Multiple Allergen-Specific IgE by Microfluidic Immunoassay Cartridge in Clinical Settings

3). Pediatr Allergy Immunol

4). **Background**: Our aims were to evaluate the performance of an automated microfluidic immunoassay system for measuring allergen-specific IgE (sIgE) in sera against an established in vitro assay and to assess the system's diagnostic accuracy against objective clinical criteria for identifying sensitization to specific allergens in daily practice of allergy clinics.

Methods: Using both the automated microfluidic–based immunoassay system (BioIC®) and ImmunoCAP®, we measured sIgE in serum samples from 212 children who visited allergic clinics in two medical centers. Outcomes of skin prick tests (SPT) served as the clinical comparison method.

Results: The assay results of targeted allergen of BioIC have a good correlation with ImmunoCAP in the diagnosis of allergen-sensitivity by patients' clinical history. When in comparison with the test results of the sIgE against over-all allergens, the agreement in either two tests among the three assays performed showed high percentage of agreement between BioIC and ImmunoCAP (77.8%. 95%

CI:72-83.3%), but not to SPT respectively (BioIC 64.9%, CI:58-72%, and ImmunoCAP 67.5%, CI:61-74%). Using ROC analysis and SPT as quasi standard, BioIC and ImmunoCAP have nearly the same performance of sensitivity and specificity in the confirmation of SPT results. The total and within one class agreements of each allergen test result between BioIC and ImmunoCAP, ranged between 55.2% and 99.5% with an overall average of 80.9%.

Conclusion: Laboratory testing for sIgE can be performed on a fully automated, microfuidic cartridge system with advantages of low sample volume, simultaneously tested allergens, and with diagnostic accuracy for representative allergens equivalent to the semi-automated CAP technology.

5) **Key words**: microarray; microfluidic immunoassay; allergen-specific IgE; allergy

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Allergic diseases such as asthma, allergic rhino-conjunctivitis, and atopic eczema, as well as other immediate-type allergies, are characterized by an increase in circulating allergen-specific IgE (sIgE) antibodies (1). The prevalence of IgE-mediated allergic diseases has increased dramatically in industrialized as well as in developing countries (2). This increase has created a greater need for early diagnosis to direct early intervention that may prevent disease progression and the development of chronic illness (3-5).

In the clinical setting, the mainstay of diagnosing allergy is obtaining patients' detailed histories which give important information about the type of allergen eliciting the diseases and the symptom severity (6). Previously, the routine diagnostic procedures in clinical practice consist of *in vivo* tests (skin prick and/or intra-dermal tests, SPTs) and are confirmed by *in vitro* measurements of allergen-specific immunoglobulin E (sIgE) against natural allergen extracts (6). For over thirty years of development, *in vitro* testing of sIgE has more benefit to patients and clinicians alike as an alternative to invasive skin prick testing (6-8). It has been demonstrated in primary care that the determination of specific IgE antibodies improves the management of patients with symptoms possibly related to allergy (8). Such tests allow allergists to accurately monitor immunotherapy techniques as well as screen adults, infants and small children for allergic sensitivities (9). Originally described in

1967 by Wide et al. (10), the radioallergosorbent test (RAST) became the first routine technique for the determination of sIgE antibodies in serum. Subsequent second-generation methods (11) had improvements such as greater speed, higher binding capacity, and use of nonisotopic labels, as well as reporting of sIgE concentrations in a continuous scale (kIU/L) standardized to the WHO International Reference Preparation for IgE (2nd IRP 75/502) (12).

In the absence of a recognized reference method for in vitro sIgE measurement, the Pharmacia second-generation ImmunoCAP® technology has become a quasi-standard because of its widespread use, analytical reliability, and the generally adequate correspondence of its results with the results of skin testing (7-11). Nonetheless, second-generation test systems for sIgE have limitations with regard to sample handling, turnaround time, laboratory integration, and personnel requirements. In addition, limitations of the solid-phase immobilization of allergens have been addressed (13, 14).

There has been increased interest in the use of microarrays for the analysis of allergen-specific IgE levels in serum. The advantages of microarrays for allergen screening are the ability to provide a high density multi-target screening technique (50 +) while using minimal serum (25-100 µl). This is even more critical for allergen screening in small children where large blood draws are difficult and serum supply is

limited. Schweitzer's group used microarrays and rolling circle amplification to detect specific IgE (15, 16). Signal amplification using streptavidin-HRP conjugate and a fluorescent substrate was later employed in a seven allergen panel using a microarray (17). Cutoff levels achieved on the microarray were arbitrarily defined as 1 kIU/L (1 IU IgE is equivalent to 2.4 ng). A house dust mite and food extract microarray was also demonstrated using a streptavidin-CY3 conjugate with clinical limits of detection near 1 kIU/L (18). Recombinant proteins have also been demonstrated on microarrays for grass and tree pollen specific IgE detection using a fluorescently labeled anti-IgE conjugate for detection (19, 20).

However, several problems must be addressed before so called allergen chips can be used for routine testing. Protein-microarray assays are generally prone to produce artificial signals, even if experiments are conducted with utmost care, because defects of the glass substrate, accumulation of dust particles on the surface, and partial or complete dehumidification may give rise to artificially increased signals and such defects cannot be simply visualized by the operator (21). Moreover, the performance of sIgE detection on microarrays, in general, is 60 minutes in each step. Therefore total reaction times for these assays were between 2-3 hours per case that render this microarray assay difficult to be adopted in automated laboratories in the present clinical setting.

While progress has been made in reducing reaction times for allergen microarray analysis, little has been done to improve the automation of microarray analysis outside the employment of large robotic workstations. The so called lab-on-chips, microfluidic devices, provide much promise in the area of automation for microarray analysis and have potential to speed up analysis times due to high surface to volume ratios and active mixing (22). Such devices have performed HIV sub-typing using RT-PCR followed by microarray analysis (23), performed DNA purification and real-time PCR with the Taq-Man probe for infectious disease detection (24), and performed DNA amplification followed by capillary electrophoresis analysis (25,26). While microfluidic devices have been utilized to perform automated analysis of nucleic acids, few have applied such devices to protein based diagnostics particularly on sIgE detection.

In this paper, we report the clinical performance of a microfluidic cartridge for the automated analysis of specific IgE (sIgE) using multiple allergen extracts. After a pilot study performed in the allergy clinic in one medical center for the stability and reliability of the test results, we have collected a cohort of 212 allergic patients in another medical center to assess the clinical relevance and matched results obtained with the microfuidic cartridge immunoassay and the immunoCAP®100 with detailed patient histories and allergen specific skin prick tests (SPTs).

Materials and methods

Study population

Children and adolescents from 3 to 18 years of age who were evaluated at the pediatric allergy and immunology clinics in MacKay Memorial Hospital, Taipei, and National Cheng Kung University Hospital, Tainan, Taiwan, and who had blood drawn for routine management were eligible to participate in this study. The ethical committees in both medical centers have agreed to the study protocol. The patient history and physical examination, skin prick tests, and blood tests for allergen specific IgE levels were performed as part of standard clinical care. Written informed consent was obtained before enrollment. Patients with the following conditions were excluded from this study: those currently undergoing allergen immunotherapy, or had taken oral antihistamines within 5 days before performing skin prick test, and subjects with any other systemic diseases that were not suitable to be enrolled in the study. The sample size was determined to be 200 evaluable subjects to ensure recruiting at least 40 positive subjects and 40 negative subjects for each of the 9 target allergens, i.e. *Dermatophagoides pteronyssinus* (D1, category number of ImmunoCAP® InVitroSight™), *D. farinae* (D2), *Blomia tropicalis* (D201), German cockroach (I6), dog dander (E5), cat dander (E1), egg white (F1), milk (F2), codfish (F3). Allergy and related medical history of each study subjects were evaluated by allergic specialists

(S.D.S. and J.Y.W.). The standard procedure of skin prick test (SPT) was performed according to the recommendation by EAACI (27). Two hundred and thirteen subjects who met all eligible requirements with at lease one positive result of SPT for entry into the study were enrolled into this study for bio-sample collection and 212 of them were considered evaluable.

Serum samples

Sera from freshly drawn blood samples were aliquoted into 2 samples and stored at 4 ^{0}C. To prevent any bias between the assay procedures, the BioIC and ImmunoCAP100® testing were analyzed blindly and periodically on the same day by a licensed clinical laboratory. Random numbers instead of real subject identification were assigned to the vials.

Device description and assay procedural for microfluidic cartridge allergen test

The microfluidic cartridge for allergen screening consists of 5 reagent delivery channels that pump reagents from individual storage tanks into a common reaction zone where allergen extracts are immobilized and then, finally, into a waste tank containing all reaction by-products (Figure 1A). During the operation, 90 µl of undiluted plasma or serum, 450 µl of wash buffer, 120 µl of premixed substrate, and

120 µl of a 1:1000 dilution of HRP conjugate were added to the appropriate tanks within the cartridge prior to use using a micropipette. The cartridge was then inserted into the BioIC instrument (Fig. 1B) and an automated, chemiluminescent immunoassay was performed in less than 30 minutes. The instrument contains solenoid actuators to pump reagents within the cartridge to perform a 2-step ELISA with reaction times of 10 minutes each. The reaction temperature was controlled at 32°C. After the final substrate addition, the chemiluminescence signals are imaged using a low-resolution cooled CCD camera. Cartridges were discarded after use.

The screening panel is comprised of 20 allergen extracts (native proteins, 9 of which are the subject of this study) the human IgE serial dilution curve, plus negative controls which were immobilized in a single reaction zone. All targets are present in quadruplicate. Target intensities were calculated by removing the spot farthest from the mean, averaging the remaining 3 spots, and subtracting the negative control intensity (Fig. 1C). Semi-quantitative results were generated by linear interpolation of target intensities against the human IgE serial dilution curve to determine specific IgE response in arbitrary units (1-32 AU) as described in detail in our previous report (28). Class scores from 0-6 were determined by ranges: <1 AU, 1-2 AU, 2-4 AU, 4-8 AU, 8-16 AU, 16-32 AU, and >32 AU, respectively.

Criteria for evaluation

The primary endpoint of this study was to determine the sensitivity and specificity of BioIC and ImmunoCAP100 for detecting allergen-specific IgE antibodies (sIgE) of the selected primary allergens (i.e., target allergens) by using SPT plus medical history as gold standard. The secondary endpoints of this study were to find the total agreement rate between BioIC and SPT, and between ImmunoCAP100 and skin test for each of the target allergens, and one class agreement rate between BioIC and ImmunoCAP100 for each of the target allergens.

Statistical analysis

Demography and baseline characteristics were analyzed by descriptive statistics. Frequency tables were provided for categorical variables, while 95% confidence interval was calculated for continuous measurements. ANOVA test was used to compare the difference among groups. P-values < 0.05 were considered as significant. Kappa statistic was used to evaluate the agreement between the two specific allergen tests (29) as well as by the method suggested by Bland and Altman (30). The results of the BioIC and ImmunoCAP100 were also compared with allergen skin test used as a reference test (27). Receiver-operating characteristics (ROC) curves were plotted for each allergen separately and the area under the curve was computed. Furthermore, a

nonparametric test (31) was conducted to compare the area under the curve between ImmunoCAP (in kU/l) and BioIC (in AU) results for each allergen, assuming a null hypothesis without a difference between the two areas, both variables equally discriminating. SAS 8.02 (SAS Institute Inc., Cary, NC, USA) was used for the ROC analysis. P-values < 0.05 were considered as significant.

Results

Demographic and clinical characterization of the study population

The mean age of the evaluated 213 subjects was 8.1 $\pm$ 3.9 years old. There were 120 (56.6%) male and 92 (43.4%) female subjects. All subjects had experienced at least one allergic symptom when they were enrolled in the allergic clinic for routine evaluation (Table 1). Allergic rhinitis was the mostly reported (90.6%) in evaluable subjects' medical histories, followed by atopic dermatitis (73.1%), asthma (52.8%), urticaria (5.2%), and allergic conjunctivitis (4.7 %).

Correlation of sIgE test results between BioIC and ImmunoCAP

Figure 2 demonstrates a favorable comparison with the sIgE test results by BioIC and ImmunoCAP assays in a pilot study on 10 allergic asthmatic children and 10 age-matched controls without allergy history. Using three pooled, mite-sensitive sera

that contained high (> 500 IU/ml), medium (100-500 IU/ml), and low (< 100 IU/ml) concentrations of total IgE, the test results of sIgE of BioIC in AU and ImmunoCAP in kU /l showed good correlation between these two assays (r^2 = 0.9958, Fig. 2A). Serial dilution with mite-positive serum (11.1 kU/L by immunoCAP) was used for determining the limit of detection (Fig. 2B). The cutoff for the assay is 1.0 AU and this value has been estimated to be < 1 IU/ml from the dilution experiment. Using skin prick test as the reference assay, both ImmunoCAP (r^2 = 0.9845) and BioIC (r^2= 0.9445) showed favorable sensitivity and specificity in the diagnosis of Der p sensitization (Fig. 2C).

Comparison of test results among skin prick test (SPT), BioIC, and ImmunoCAP

All evaluable subjects took skin prick tests (SPT) for eight specific allergens, as listed in Table 2, when enrolled and were with at least one positive test result as required as an inclusion criterion. There are 200 (94.3%) and 168 (79.2%) evaluable subjects who tested positive (at least one allergen test positive) with BioIC and ImmunoCAP, respectively. We found there were similar positive rates of study population among these three tests, except in aeroallergen of cat dander (E1) and food allergen of codfish (F3) ($p < 0.05$, ANOVA). There were also similar positive rate of allergens tested between BioIC and ImmunoCAP, except in dog dander (E5) and Egg white (F1)

($p<0.05$, Student t test)

Comparative agreement in either two tests among SPT, BioIC, and ImmunoCAP

The total agreement between two tests was defined as the incidence of subjects whose 2 test results were identical as positive or as negative. The results were calculated using kappa statistics and expressed as percentage of agreement and 95% confidence interval (CI). Table 3 shows the percentage of agreement of all 8 allergens tested in either two assays were 64.9% (BioIC *vs.* SPT, 95% CI: 58.2-71.5%), 67.5% (ImmunoCAP *vs.* SPT, 95% CI: 60.6-73.8%), and 77.8% (BioIC vs ImmunoCAP, 95% CI: 71.6-83.3%), respectively. Considering individual allergens, the agreements of test results in Der p (D1), Der f (D2), German cockroach (I6), dog dander (E5), egg white (F1), and milk (F2) were similar to its corresponding agreement in either two tests of SPT, BioIC, and ImmunoCAP. While in cat dander (E1) and Codfish (F3) allergen tests, there was a very high degree of agreement, 93.9% and 96.7%, between BioIC and ImmunoCAP, respectively, but not with SPT (Table 3).

The sensitivity, specificity, and comparative ROC analysis of the BIoIC and ImmunoCAP using SPT as golden standard

Table 4 shows the sensitivity and specificity for BioIC and ImmunoCAP, respectively,

calculated by using SPT as gold standard. BioIC has similar sensitivities and specificities as ImmunoCAP in the diagnosis of all seven allergens except in dog dander ($p< 0.05$). To assess diagnostic performance, we performed ROC analysis by comparing the BioIC and ImmunoCAP results with the respect of sensitivity and specificity to SPT. As shown in Fig. 3, BioIC and ImmunoCAP performed at the same excellent level in the confirmation of skin prick test results with allergies to Der p, Der f, German cockroach, cat dander, and milk. This finding was reflected by the similar shapes of the ROC curves for these particular allergens (Fig. 3). Moreover, the area under the curve for the ROC plots did not differ in the case of these allergens. For the diagnosis of dog dander (D1) and egg white (F1), the BioIC analysis resulted in a slightly reduced specificity when compared with the ImmunoCAP (Fig, 3A and 3C), while these differences were not statistically different ($p= 0.065$ in dog dander and $p=0.055$ in egg white). The confirmation of allergies to codfish (F3) was less efficient when compared with the ImmunoCAP ($p < 0.05$, Fig. 3D), a problem originating from the low sensitivity of the BioIC in the detection of codfish-specific IgE (<10%), while still retaining a high level of specificity (98.1%) (Table 4).

Comparative agreement of the class results between BIoIC and ImmunoCAP

There is a common practice to adopt semi-quantitative results in the report of

allergen sensitivity tests. Results for both systems are expressible in continuous units, (kIU/L IgE and AU), as well as in terms of the traditional spectrum of 7 semi-quantitative classes, ranging from class 0 (all results < 0.35 kIU/L) up to class 6 (all results >100 kIU/L). The class results of each allergen as assayed by BioIC (AU) and ImmunoCAP (kIU/L) are provided in Table 5. The total agreement and 1-class agreement rates are also illustrated. The total agreement rate ranged from 56.1% to 96.7%. The 1-class agreement rate, defined as the incidence of subjects whose difference of BioIC and ImmunoCAP test results was no more than one class, ranged between 55.2% and 99.5%. Exceptionally high values of total agreement were seen in cat dander (E1) and codfish (F3), 93.9% and 96.7%, respectively and those of 1-class agreement were observed in German cockroach (I6; 89.6%), dog dander (E5; 90.1%), cat dander (E1; 97.6%), milk (F2; 88.2%), and codfish (F3; 99.5%).

Discussion

The main goal of this study was to compare the diagnostic accuracy of allergen-specific IgE values obtained by a new microfluidic immunoassay technology (BioIC), also know as lab-on-chips, to well-established in vivo (SPT) and laboratory methods (ImmunoCAP) for identifying sensitization to a representative spectrum of specific aeroallergens in allergic children on a daily basis of clinical practice. Our results have clearly demonstrated the good correlation of BioIC and ImmunoCAP, in the differential diagnosis of allergen-sensitivity by patients' clinical history (Fig 2). When in comparison with the test results of the sIgE against over-all allergens, the agreement in either two tests among the three assays performed showed that there was a good agreement percentage between BioIC and ImmunoCAP ((77.8%. 95% CI:72-83.3%), but not to SPT respectively (BioIC 64.9%, CI:58-72%, and ImmunoCAP 67.5%, CI:61-74%). This finding may result from the well known facts in clinical practice that many factors may influence SPT, and it is not uncommon to find that the results of SPT regarding food allergens are unreliable unless it is confirmed by clinical history (27, 32). In contrast, in vitro sIgE test to food allergens has shown a good correlation with patient history and double blind challenge tests (32, 33).

By using SPT as quasi standard, and ROC analysis to compare the sensitivity and specificity of each allergen tested, we found BioIC and ImmunoCAP have nearly the same performance of sensitivity and specificity in the confirmation of SPT results. Using semi-quantitative class system for the total and within one class agreements between BioIC and Immuno CAP, each allergen test result ranged between 55.2% and 99.5% with an overall average of 80.9% (Table 5). Overall, our results have demonstrated the performance of the microfluidic cartridge technology for the screening of allergen-specific IgE sensitivities in human serum has favorable comparison results with SPT and the diagnostic accuracy for representative allergens equivalent to the semi-automated ImmunoCAP technology.

Recent progress of nanotechnology has revealed wide application of microarrays not only for research but also for routine outpatient settings. Still there are several issues need to be addressed before the allergen-microarray can be incorporated into the routine diagnostic setting. Firstly, although it is claimed that more than 50 to 100 allergens can be simultaneously tested in one condition (34), generally speaking, the clinical relevance of each allergen needs to be validated separately prior to the implementation of multi-allergen panels, or it may only produce an over-load of information and cause more confusion to doctors and patients alike (7). Secondly,

since allergen extracts contain a very complex mixture of both allergenic and non-allergenic proteins, allergen-specific IgE assays typically rely on high surface areas for immobilizing such extracts (35). This is a major challenge for miniaturized assays as well as the stringent conditions needed to perform a protein-microarray assay, such as humidification, temperature, and non-specific signals (noise) on the solid surface of a glass slide. The microfluidic cartridge showed sensitivities similar to that of other microarrays employing allergen extracts (15-18), however, the microfluidics may take advantage of the capabilities to perform reactions under dynamic conditions rather than static in typical slide microarrays, and provide better elimination of non-specific bindings of non-allergenic proteins. Finally, in a medical center, the large volumes of tests are in need of automated operation and a centralized report system for test results. Protein-microarrays suffer from multiple steps, prolonged manual operation which can only be solved by expensive robotics and, in most cases, require 2-3 hours to perform a test. The BioIC microfluidic assay, however, provides an inexpensive multiplexed assay format with rapid, automated analysis for low-volume specific-allergen testing (28). Improvements in the instrumentation such as analyzing cartridges in a carousel format or simply in parallel (i.e. 10 cartridges could reasonably complete a run in 60-75 minutes), could provide higher throughput analysis.

Overall, the microfluidic cartridge immunoassay for sIgE assay is a promising option not only for research but also for routine outpatient settings, which has multiple advantages over allergen-microarray slides as an automated, easy-access system with an extended working range and with diagnostic accuracy similar to the presently used ImmnuoCAP assay. Moreover, this system has potential for de-centralized allergen screening and near-patient testing where short analysis times, ease of use, and low instrumentation cost are critical in the clinical laboratory.

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Disclosure

This was an investigator-initiated study that was supported insofar that the determination of specific IgE with BioIC in all patients and with ImmunoCAP in all patients from the two medical centers was performed in the laboratories of Agnitio Science & Technology, Hsinchu, Taiwan.

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Table 1. Number of subjects with allergic disease history – evaluable population

Allergic symptoms	BioIC® tested % (n =212)
Allergic rhinitis	90.6% (192)
Atopic eczema	73.1% (155)
Asthma	52.8% (112)
Urticaria	5.2% (11)
Allergic conjunctivitis	4.7% (10)

Table 2. The number and percentage of subjects with positive results of skin prick test (SPT), BioIC, and ImmunoCAP in evaluable population

Allergens	SPT	BioIC®	ImmunoCAP®
	n = 212 (%)	n = 212 (%)	n = 212 (%)
Overall	212 (100.0%)	200 (94.3%)	168 (79.2%)
D. Pteronyssinus (D1)	170 (80.2%)	171 (80.7%)	153 (72.2%)
D. farinae (D2)	132 (62.3%)	158 (74.5%)	147 (69.3%)
Blomia tropicalis(D201)	N/A	159 (75.0%)	116 (54.7%)
German cockroach (I6)	71 (33.5%)	36 (17.0%)	31 (14.6%)
Dog dander (E5)	88 (41.5%)	88 (41.5%)[a]	24 (11.3%)[a]
Cat dander (E1)*	103 (48.6%)[b,c]	8 (3.8%)[b]	9 (4.2%)[c]
Egg white (F1)	42 (19.8%)	55 (25.9%)[a]	29 (13.7%)[a]
Milk (F2)	41 (19.3%)	42 (19.8%)	27 (12.7%)
Codfish (F3)*	53 (25.0%)[b,c]	5 (2.4%)[b]	2 (0.9%)[c]

*$p < 0.05$, ANOVA test among SPT, BioIC, and ImmunoCAP.

[a] $p < 0.05$, Student t test between BioIC and ImmunoCAP.

[b] $p < 0.05$, Student t test between SPT and BioIC.

[c] $p < 0.05$, Student t test between SPT and ImmunoCAP.

Table 3. The percentage of agreement between either two tests of BioIC®, ImmunoCap®, and SPT.

Allergen	BioIC® & Skin Test	ImmunoCAP® & Skin Test	BioIC® & ImmunoCAP®
D. pteronyssinus (D1)	69.3% (62.6-75.5%)[a]	72.2% (65.6-78.1%)	73.6% (67.1-79.4%)
D. farinae (D2)	65.1% (58.2-71.5%)	67.5% (60.6-73.8%)	78.8% (72.6-84.1%)
German cockroach (I6)	64.6% (57.7-71.1%)	66.0% (59.2-72.4%)	75.0% (68.6-80.7%)
Dog dander (E5)	52.8% (45.8-59.8%)	56.6% (49.6-63.4%)	58.5% (51.5-65.2%)
Cat dander (E1)	51.4% (44.4-58.4%)	50.9% (44.0-57.9%)	93.9% (89.7-96.7%)
Egg white (F1)	70.3% (63.6-76.4%)	75.0% (68.6-80.7%)	72.6% (66.1-78.6%)
Milk (F2)	71.2% (64.6-77.3%)	76.4% (70.1-82.0%)	73.1% (66.6-79.0%)
Codfish (F3)	74.5% (68.1-80.3%)	75.0% (68.6-80.7%)	96.7% (93.3-98.7%)
Overall	64.9% (58.2-71.5%)	67.5% (60.6-73.8%)	77.8% (71.6-83.3%)

[a] (95% CI)

Table 4. Sensitivity and specificity of BioIC and ImmunoCAP as compared to skin prick tests

Allergens	BioIC® (n = 212)		ImmunoCAP® (n = 212)	
	Sensitivity	Specificity	Sensitivity	Specificity
D. pteronyssinus (D1)	81.2%(138/170)	21.4% (9/42)	77.6% (132/170)	50.0% (21/42)
D. farinae (D2)	81.8% (108/132)	37.5% (30/80)	79.5% (105/132)	47.5% (38/80)
German cockroach (I6)	22.5% (16/71)	85.8% (121/141)	21.1% (15/71)	88.7% (125/141)
Dog dander (E5)*	43.2% (38/88)	59.7% (74/124)	11.4% (10/88)	88.7% (110/124)
Cat dander (E1)	3.9% (4/103)	96.3% (105/109)	3.9% (4/103)	95.4% (104/109)
Egg white (F1)	40.5% (17/42)	77.6% (132/170)	21.4% (9/42)	88.2% (150/170)
Milk (F2)	26.8% (11/41)	81.9% (140/171)	22.0% (9/41)	89.5% (153/171)
Codfish (F3)	3.8% (2/53)	98.1% (156/159)	1.9% (1/53)	99.4% (158/159)

* p = 0.012

Table 5. Agreement between Classes of BioIC® and ImmunoCAP®

Class		BioIC®								Total Agreement	1 class Agreement
		0	1	2	3	4	5	6	Total		
D. Pteronyssinus (D1)											
ImmunoCAP®	0	22	21	14	2	0	0	0	59	73.6%	60.8%
	1	4	4	0	0	0	0	0	8		
	2	10	5	6	1	0	0	0	22		
	3	3	10	12	4	0	1	0	30		
	4	2	5	12	11	2	1	0	33		
	5	0	0	3	11	16	2	0	32		
	6	0	0	0	3	7	11	7	28		
	Total	41	45	47	32	25	15	7	212		
D. farinae (D2)											
ImmunoCAP®	0	37	16	10	2	0	0	0	65	78.8%	55.2%
	1	2	4	1	0	0	0	0	7		
	2	6	7	6	1	0	0	0	20		
	3	8	10	6	4	1	1	1	31		
	4	1	7	18	10	1	0	1	38		
	5	0	0	9	9	7	1	1	27		
	6	0	0	1	5	6	5	7	24		
	Total	54	44	51	31	15	7	10	212		
Blomia tropicalis (D201)											
ImmunoCAP®	0	28	36	23	7	1	1	0	96	56.1%	63.7%
	1	11	5	2	2	0	0	0	20		
	2	9	11	8	1	0	0	1	30		
	3	4	10	15	5	1	0	1	36		
	4	1	1	5	6	3	0	5	21		
	5	0	0	1	3	1	0	0	5		
	6	0	0	1	1	0	0	2	4		
	Total	53	63	55	25	6	1	9	212		
German cockroach (I6)											
ImmunoCAP®	0	152	15	12	2	0	0	0	181	75.0%	89.6%
	1	16	1	0	0	0	0	0	17		
	2	8	1	1	2	0	0	0	12		
	3	0	0	2	0	0	0	0	2		
	4	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	176	17	15	4	0	0	0	212		
Dog dander (E5)											
ImmunoCAP®	0	112	57	18	1	0	0	0	188	58.5%	90.1%
	1	10	3	2	0	0	0	0	15		
	2	2	4	2	0	0	0	0	8		
	3	0	0	0	0	0	0	0	0		
	4	0	0	0	0	0	0	1	1		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	124	64	22	2	0	0	0	212		

Class		BioIC®								Total Agreement	1 class Agreement
		0	1	2	3	4	5	6	Total		
Cat dander (E1)											
ImmunoCAP®	0	197	5	1	0	0	0	0	203	93.9%	97.6%
	1	4	0	0	0	0	0	0	4		
	2	3	0	0	0	0	0	0	3		
	3	0	1	0	1	0	0	0	2		
	4	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	204	6	1	1	0	0	0	212		
Egg white (F1)											
ImmunoCAP®	0	141	15	20	3	4	0	0	183	72.6%	83.0%
	1	9	1	3	1	0	0	0	14		
	2	7	5	2	0	0	0	0	14		
	3	0	1	0	0	0	0	0	1		
	4	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	157	22	25	4	4	0	0	212		
Milk (F2)											
ImmunoCAP®	0	149	23	8	4	1	0	0	185	73.1%	88.2%
	1	10	0	4	1	0	0	0	15		
	2	9	1	0	0	0	0	0	10		
	3	2	0	0	0	0	0	0	2		
	4	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	170	24	12	5	1	0	0	212		
Codfish (F3)											
ImmunoCAP®	0	205	4	1	0	0	0	0	210	96.7%	99.5%
	1	2	0	0	0	0	0	0	2		
	2	0	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0	0		
	4	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0		
	6	0	0	0	0	0	0	0	0		
	Total	207	4	1	0	0	0	0	212		

Figure legends:

Fig. 1 (A) Injection molded microfluidic cartridge for automated immunoassays. Top view photograph showing 5 reagent delivery channels and a single reaction zone. (B). Hardware setup for operation of the microfluidic cartridge. The entire immunoassay is automatically performed within the dark chamber after reagents are loaded onto the cartridge. (C). A low resolution cooled CCD camera for chemiluminescence detection, the computer image of the target intensities were calculated and reported can be stored and print.

Fig. 2 (A). The correlation of test results between BioIC and ImmunoCAP assay for three pooled sera samples. (B). The detection limits of mite (Der p)-specific sIgE of BioIC by serial dilution with mite-positive (11.1 kU/L by ImmunoCAP) serum. (C) The sensitivity and specificity of ImmunoCAP (r^2 = 0.9845) (open circles) and BioIC (r^2= 0.9445) (solid circles) in the diagnosis of Der p sensitization as judged by the Skin prick test results.

Fig.3. Using SPT as quasi standard, ROC curves were plots for the 8 study allergens by BioIC and ImmunoCAP. The sensitivity, specificity, and the percentage of area under curve of each allergen tested are also shown in its corresponding figure.

Fig. 1

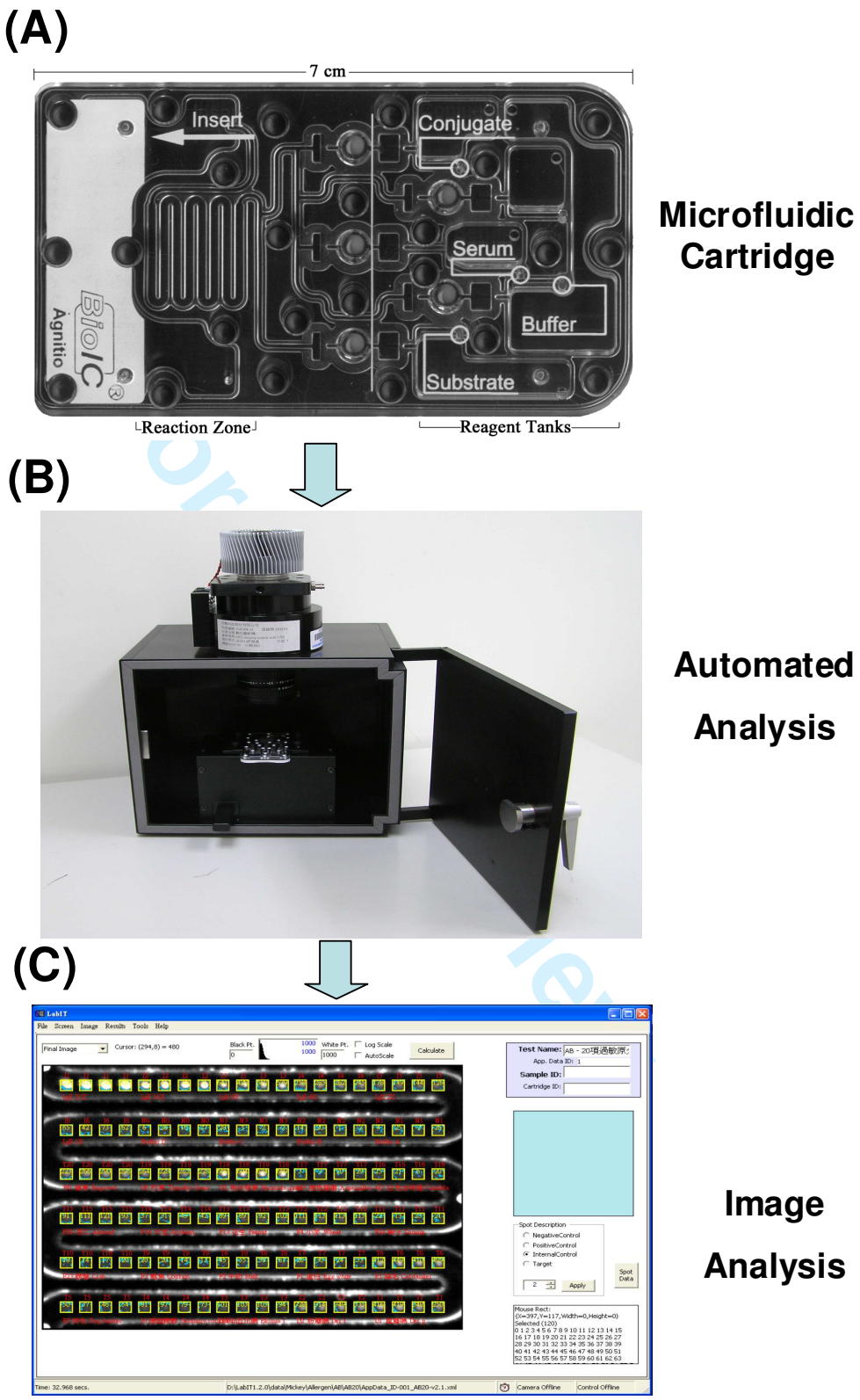

Fig. 2

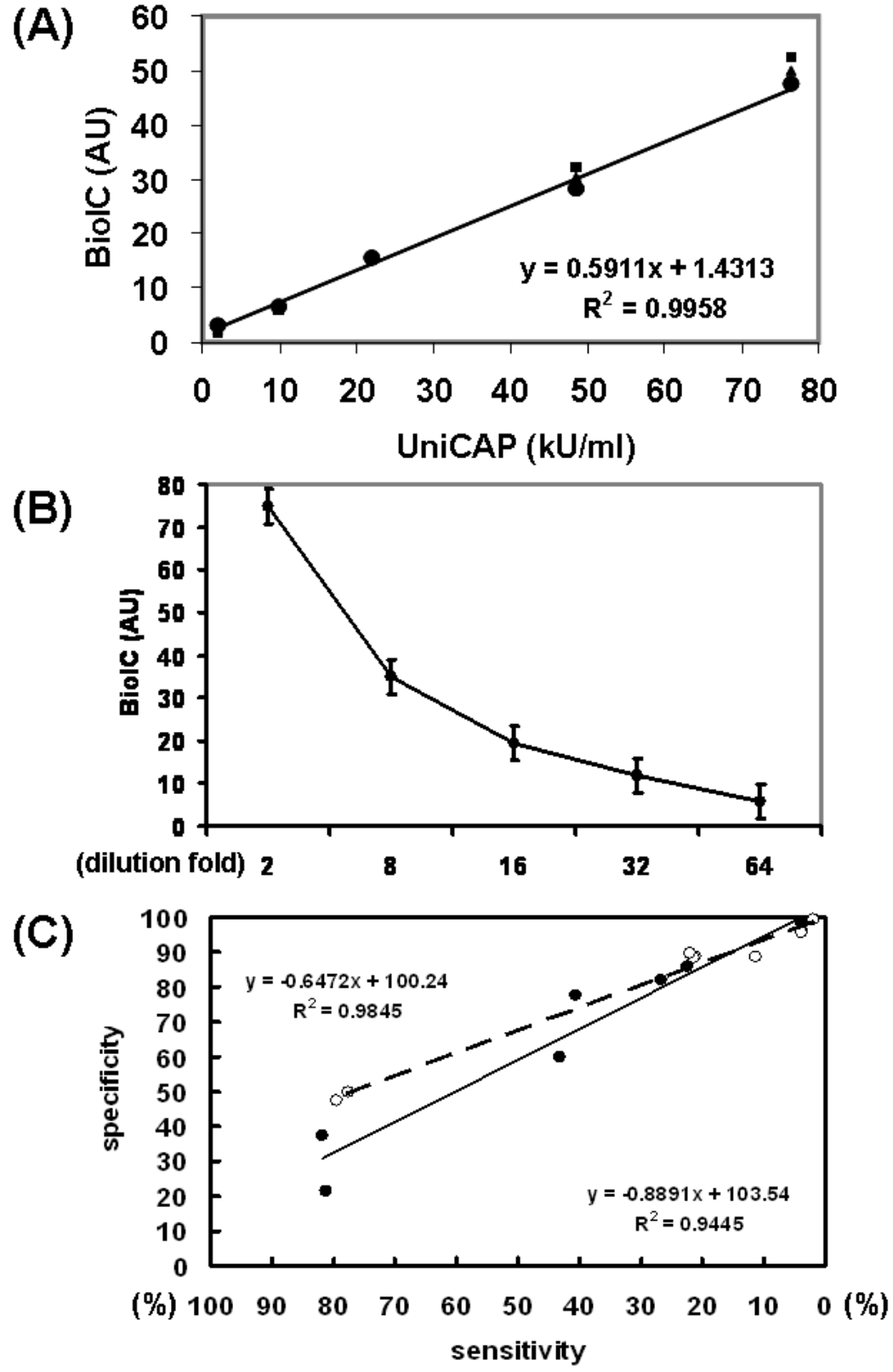

Fig. 3

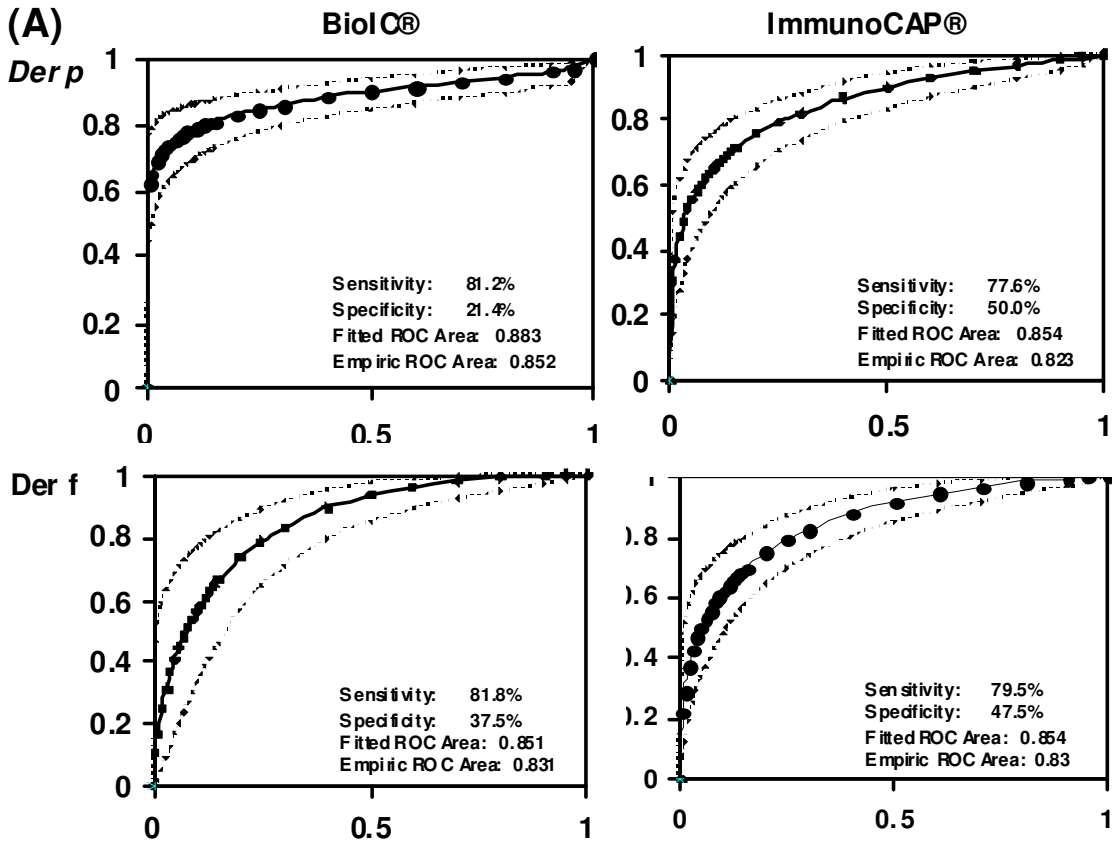
(A)
BioIC®
ImmunoCAP®
Der p
Sensitivity: 81.2%
Specificity: 21.4%
Fitted ROC Area: 0.883
Empiric ROC Area: 0.852
Sensitivity: 77.6%
Specificity: 50.0%
Fitted ROC Area: 0.854
Empiric ROC Area: 0.823
Der f
Sensitivity: 81.8%
Specificity: 37.5%
Fitted ROC Area: 0.851
Empiric ROC Area: 0.831
Sensitivity: 79.5%
Specificity: 47.5%
Fitted ROC Area: 0.854
Empiric ROC Area: 0.83

Fig. 3

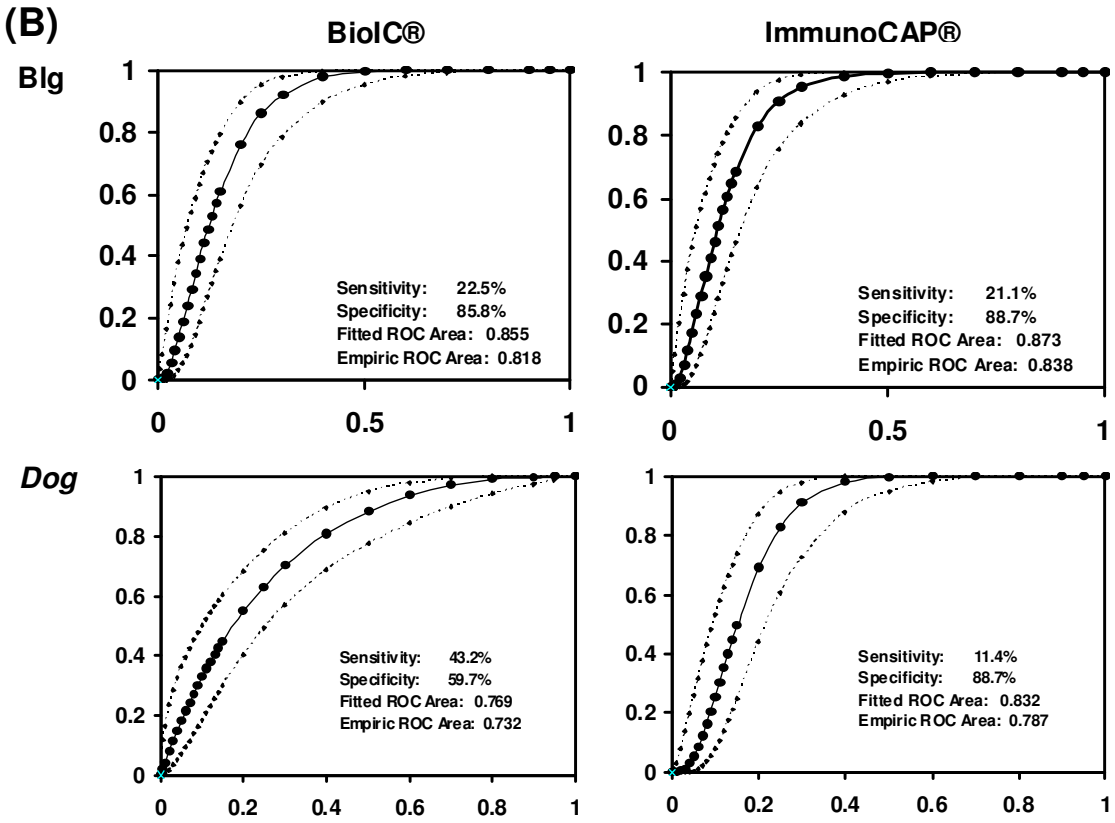

Fig. 3

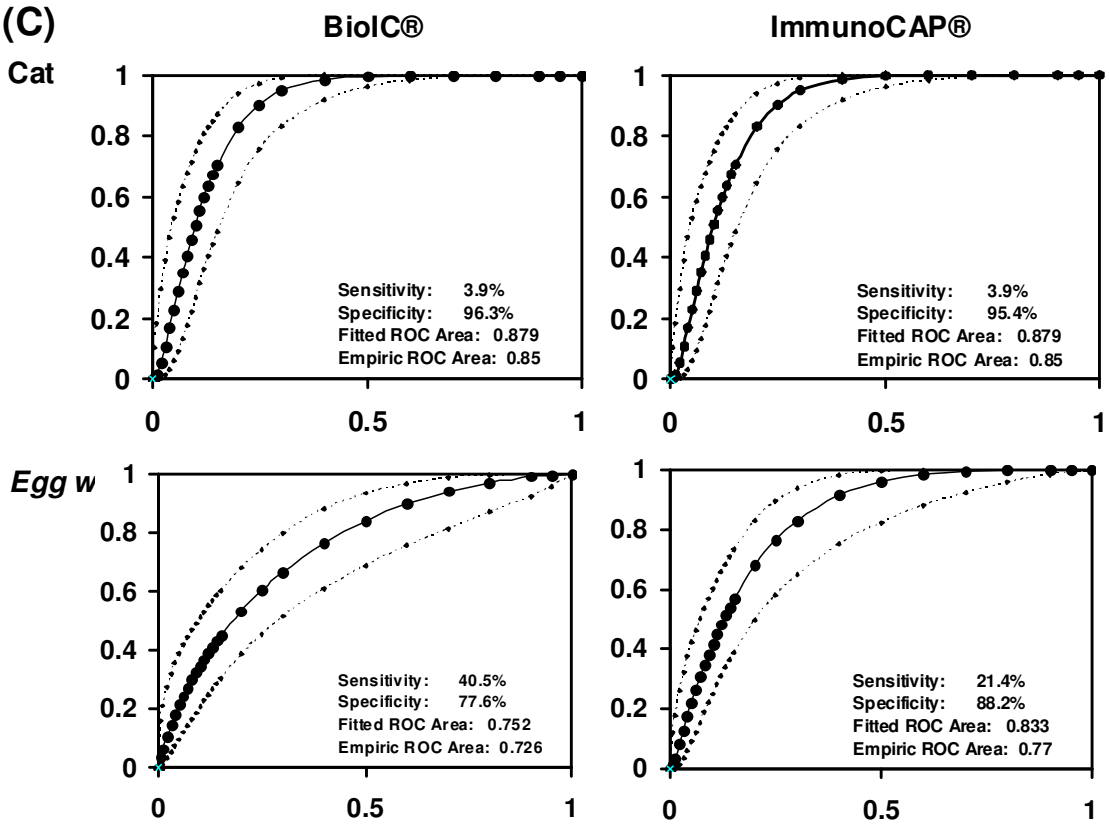

Fig. 3