Reproducibility of serum testing for environmental allergen-specific IgE in dogs in Europe

Katja N. Baumann* †, Natalie K.Y. Gedon†, Teresa M.S.A. Boehm* ‡, Laura Udraite-Vovk* and Ralf S. Mueller* ‡

*Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Veterinärstraße 13, 80539 Munich, Germany
†Tierklinik Oberhaching, Bajuwaren Ring, 82041 Oberhaching, Germany
Correspondence: Katja Baumann, Centre for Clinical Veterinary Medicine, Veterinärstraße 13, 80539 Munich, Germany. E-mail: katja.bmn@gmail.com

Background – Serum testing for allergen-specific immunoglobulin (Ig)E is commonly employed to identify allergens used for allergen-specific immunotherapy in dogs, yet the reliability of results has been a matter of debate.

Objective – The aim of this study was to evaluate the reproducibility of serum tests for environmental allergen-specific IgE in three European laboratories.

Animals/Methods – Serum was obtained from 33 client-owned dogs diagnosed with atopic dermatitis, divided into three aliquots and sent to the laboratories under different names. Two aliquots were sent simultaneously to one of the laboratories on the first day; the third sample was then sent to the same laboratory on the subsequent day. The laboratory for each patient was chosen according to a predetermined randomization list. The agreement between different samples from the same dog for each of the laboratories was calculated with a Cohen’s Kappa test. Spearman’s rank coefficients (r_s) as well as the coefficients of variation (CV) additionally were calculated.

Results – The intra- and interassay agreements for laboratories A, B and C were 0.79 and 0.75, 0.92 and 0.90, and 0.90 and 0.85, respectively. The CVs were 18.92% and 22.95%, 14.43% and 18.79%, and 15.38% and 18.75% (respectively) and the r_sp 0.73 and 0.68, 0.95 and 0.92, and 0.82 and 0.74 (respectively).

Conclusion and clinical relevance – The differences in reproducibility between laboratories complicate test interpretation and underline the importance of interpreting results of serum testing for allergen-specific IgE in the context of the patient’s clinical history.

Introduction

Canine atopic dermatitis (cAD) is an inflammatory and pruritic skin disease based on a genetic predisposition. The dogs show pruritus and cutaneous inflammation.1,2 CAD is associated, in the majority of cases, with immunoglobulin (Ig)E antibodies, mostly directed against environmental allergens.3

Apart from symptomatic therapy with anti-inflammatory drugs, there is currently only one form of specific treatment for environmental allergy, namely allergen-specific immunotherapy (AIT). Allergens for AIT are determined individually by correlating the results of either an intradermal test (IDT)4 and/or a serum allergen test (SAT) for environmental allergen-specific IgE antibodies with the clinical history of the dog.5 SATs are used by general practitioners and veterinary dermatologists as they are simple to perform and readily available. However, owing to the frequent occurrence of environmental allergen-specific IgE antibodies in clinically nonaffected as well as atopic dogs, allergen tests are unsuitable for the diagnosis of allergy,6,7 and are used only to identify possible allergens for an AIT in patients already diagnosed with cAD.

Allergen-specific IgE-assays share a common methodology. The most commonly used SATs are enzyme-linked immunosorbent assays (ELISAs) for which the patient’s serum is combined with an individual allergen extract bound mostly to a solid phase. After washing away unbound antibodies, the allergen-bound IgE antibodies are detected using an IgE-specific reagent linked to an enzyme to allow the photometric measurement of IgE-specific reagents. The signal strength is proportional to the amount of bound allergen-specific IgE.2,5 SATs have several advantages over IDTs: blood samples can be obtained quickly and easily without the risk of possible anaphylactic reaction to injected allergens as in the IDT, and SATs can be used in patients with severely inflamed skin, where IDT cannot.

Accepted 3 November 2020

Source of Funding: The study was funded by the German Society of Veterinary Dermatology.

Conflicts of Interest: None of the laboratories was notified about the study, or had any influence on the study design, the data evaluation or the preparation of the manuscript. None of the authors are associated with any of the laboratories. In the past Ralf Mueller worked as a consultant for the following companies or received support for studies or lectures: Artu Biologicals, Bayer Animal Health, Boehringer, Decha, Elanco Animal Health, Greer Laboratories, Idexx Laboratories, Hill’s, LCDA, Merial, MSD, Novartis, Royal Canin, Selectavet, Synlab, Virbac and Zoe- tis. There is no conflict of interest for any of the other authors.

This study was presented as an abstract at the North American Veterinary Dermatology Forum 2019 in Texas, USA as well as at the German Society of Veterinary Dermatology Meeting 2019 in Augsburg, Germany.

© 2021 The Authors. Veterinary Dermatology published by John Wiley & Sons Ltd on behalf of the ESVD and ACVD, 32, 251–e67. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
In considering the role of these data in choosing allergens for AIT, AIT based on an IgE serum test is reported to be as effective as AIT based on IDT results. Unfortunately, the reported reproducibility of SATs has been highly variable.8–13 IgE-specific serum tests are neither 100% specific nor sensitive,14–17 although studies need to be interpreted with caution as presently there is no definite gold standard test for allergic reactions to environmental allergens in dogs or cats.

In order to improve the sensitivity, interassay reproducibility and intermethod agreement, the total serum IgE tests of numerous laboratories in human medicine are tested regularly. Performance monitoring is overseen by the College of the American Pathologists commissioned by the Clinical Laboratory Improvement Advisory Committee (CLIAAC). The results of the tested challenge sera are collected, the interassay variation calculated, and sent back to both of the participating laboratories and the accrediting companies.18,19 If a laboratory’s results differ by more than three times the standard deviation (SD) from the mean value of the peer group, this laboratory runs the risk of losing its approval for the respective allergen test.18 Currently, in veterinary medicine, there is no comparable system.

A number of studies have evaluated the reproducibility of allergen-specific IgE-testing in various laboratories, yet in Europe many laboratories have not been evaluated.9,11,13 The aim of this study was to determine the intra- (“same-day-”) and interassay (“different-day-”) variability of three European veterinary laboratories.

Methods and materials

Dogs
Thirty-three privately kept dogs with atopic dermatitis (AD) were included in this prospective, single-blinded study. All owners gave informed consent for blood sampling and multiple serum allergen testing. As all dogs underwent sampling to evaluate which allergens were to be selected for immunotherapy as part of normal clinical practice, an approval by the regulatory governmental agency in Bavaria was not necessary.

Diagnosis of cAD based on history, clinical signs (Favrot’s criteria)20 and exclusion of appropriate differential diagnoses such as adverse food reaction, ectoparasites, infections, endocrine or autoimmune diseases.

Laboratories
All three selected laboratories used the monoclonal antibody cocktail (mac)ELISA method for the determination of environmental allergen-specific IgE antibodies. Laboratories A and C used oligoclonal antibodies (OLYGO.3mAb), which are a mixture of three monoclonal antibodies derived from recombinant dog-IgE targeting different IgE epitopes, whereas Laboratory B used a mixture of three monoclonal antibodies specifically binding to the Fc region of canine IgE.

Study design
From each dog, 15–20 mL of blood were obtained and centrifuged within 2 h. A volume of 1.5–2 mL of harvested serum was placed into each of three vials, each vial being labelled with a different name. The samples were stored at 4°C until dispatch. The first and second samples were sent simultaneously to one of the laboratories on the first day. The third sample was sent on the subsequent day to the same laboratory (if this day was a Friday, the sample was sent the following Monday). The sample shipping protocol is described schematically in Figure 1. The order of the laboratories to which the samples were sent was determined before the start of the study using a computer randomization tool provided by GraphPad (www.graphpad.com/quickcalcs/randomize2; last accessed on Jan 11, 2020). All three laboratories were blinded to the duplicate natures of the samples.

Statistics
The agreement of the categorical values of the individual laboratories was based on the laboratory’s reference range. The intra- and interassay agreement was determined with Cohen’s Kappa test; the assessment can be seen in Table 1. In addition to categorizing the results as either positive or negative, two laboratories added an intermediate category termed “questionable”. For these laboratories, three different Cohen’s Kappa values were calculated in which the questionable category was considered as a positive result, a negative result or not included in the calculations. Spearman’s rank-order correlation coefficients (rs) for both intra- and interassay agreement were calculated for all laboratories. The coefficients of variation (CVs) of the intra- and interassay variability were calculated for each sample by dividing the SD of the two given values by their mean and multiplying it by 100. The mean value of all CVs then was calculated. Overall, the interassay variability was based on the values of the samples sent to the same laboratory on the same day. For the interassay variability, the values of the samples sent to the same laboratory on two consecutive days were used. Statistical analysis was performed using Excel (Microsoft Corporation; Redmond, WA, USA) and Prism 6g (GraphPad; San Diego, CA, USA).

Results
A total of 33 dogs were included in the study; within this number, eight of 18 males and five of 15 females were neutered. The age of the dogs ranged from one year and one month to nine years (mean age 4.3 years). Cross-bred dogs were most common (seven dogs), followed by four Rhodesian ridgebacks and two German shepherd dogs. The remaining dog breeds were each represented once: pug, Parson Russell terrier, miniature bull terrier, Magyar Vizsla, French bulldog, bearded collie, Coton de Tuléar, Labrador retriever, Swiss mountain dog, Yorkshire terrier, American bulldog, Biewer terrier, small Münsterländer, Siberian husky, West Highland white terrier, Berger blanc Suisse, silken windsprite and Bavarian mountain dog.

From the 33 dogs, there were two dogs missing tree pollen results owing to insufficient sample volume, and
two dogs in which a serum sample was lost in transit to the laboratory. The last three complete test results from Laboratory C were not included in the calculations as the authors were suspicious that the blinding was no longer in place. Accordingly, only 31 sera could be used for the intraassay variabilities and only 28 sera for the interassay variabilities. By deducting the missing 12 results of the tree pollen allergens, the 30 and 29 results as a consequence of the lost test sera, and the three sets of 30 results from Laboratory C, a total of 2,457 usable test results and 1,817 value pairs were obtained.

Table 2 presents the agreement coefficients obtained with different statistical methods. A different and generally high agreement of the test results was achieved. When considering the Cohen’s Kappa coefficients based on the evaluation score in Table 1, it is noticeable that for intra- and intercorrelation, respectively, Laboratory A showed a high agreement (0.61 and 0.79), while laboratories B (0.92) and C (0.86 and 0.90) showed an almost complete agreement.

The CVs of the agreement of allergen pairs were lowest in Laboratory A (18.92% and 22.95%), and although laboratories B and C showed a higher agreement, they were not significantly different to one another (B: 14.43% and 18.79%; C, 15.38% and 18.75%) (respectively). Spearman’s rank-order coefficients showed a similar pattern with Laboratory B’s agreement being the highest (0.95 and 0.92), followed by Laboratory C (0.82 and 0.74) and then Laboratory A (0.73 and 0.68) (respectively).

For more detailed study results, see the Supporting information, Tables S1–S3.

Discussion

The evaluation of intra- and interassay variabilities of serum allergen tests for environmental allergen-specific IgE in three different laboratories, showed a variation in agreement between substantial and almost perfect agreement. However, several differences would have led to a different formulation of the allergen extract used for immunotherapy in some cases.

Possible variations in the results might have occurred as a consequence of the temperature variation during transport and the time taken to arrive at the laboratory. In this study, the samples were sent either directly on the day the blood was taken or, if that day was a Friday, on the following Monday to minimize transport times. However, the time from sampling to arrival at the laboratory and processing is likely to have varied slightly, leading to slight deviations of the IgE concentrations. Intraassay variabilities were approximately 4% greater than the interassay variabilities, which may be further evidence for this assumption. A very recent study showed an even greater increase in variability of approximately 7% when evaluating the same serum after 30 days.21 A decrease of IgE concentrations between 0% and 29% was observed after 25 freeze–thaw cycles, and 10 days storage at room temperature.22 Another study also showed 5.5% loss after 10 freeze–thaw cycles, and 2% loss of total IgE after six days storage at room temperature.23 However, both studies evaluated extreme conditions normally not encountered in practice. In most cases it can be assumed that serum IgE concentrations in the dog are not affected significantly by a delay in processing of 24 h. A previous study24 reported that test variations also may have occurred owing to both preanalytical and analytical factors. Possible reasons for analytical variation would be sample mix-ups or contamination, incorrectly calibrated or nonfunctional instruments or machines, expired reagents, insufficient time or temperature during processing or deviations from the assay protocol.11,24 These factors could not be evaluated in the context of the present study and cannot be influenced by the veterinarian in practice.

<table>
<thead>
<tr>
<th>Table 1. Assessment of Cohen’s Kappa coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohen’s Kappa coefficient</td>
</tr>
<tr>
<td>α &lt; 0.1</td>
</tr>
<tr>
<td>0.1 ≤ α &lt; 0.4</td>
</tr>
<tr>
<td>0.4 ≤ α &lt; 0.6</td>
</tr>
<tr>
<td>0.6 ≤ α &lt; 0.8</td>
</tr>
<tr>
<td>0.8 ≤ α &lt; 1.0</td>
</tr>
</tbody>
</table>

(Statsoft 2018)

<table>
<thead>
<tr>
<th>Table 2. Agreement coefficients of paired serum tests for allergen-specific immunoglobulin (Ig)E in three different European laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
</tr>
<tr>
<td>Intraassay</td>
</tr>
<tr>
<td>Questionable – positive</td>
</tr>
<tr>
<td>Questionable – negative</td>
</tr>
<tr>
<td>Questionable – not included in the calculations</td>
</tr>
<tr>
<td>Interassay</td>
</tr>
<tr>
<td>Spearman’s rank coefficient</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
</tr>
<tr>
<td>Intraassay</td>
</tr>
</tbody>
</table>

*For laboratories A and C three reference ranges existed: positive, negative and questionable, whilst for Laboratory B only two reference ranges (positive and negative) were differentiated.
In the present study, the cut-off values given by the individual laboratories were deliberately used as the basis for the evaluation to reflect private practice conditions. Veterinarians must select suitable allergens for AIT on the basis of the specified reference values in association with the individual patient’s history. The laboratories were not compared with each other, which made an adjustment of the reference ranges superfluous. The selection of other cut-off values might have led to different degrees of agreement.12 However, evaluating the reference ranges was not part of this study.

A comparison of the interassay variability between the individual laboratories was not carried out as there already is substantial information available about this topic.10–13 In addition, the three laboratories offered different allergen panels, which makes a direct comparison more difficult and would have required reducing allergens from an average of 31 to 15 allergens.

In the absence of a gold standard for determining the relevant importance of allergens in CAD, the sensitivity and specificity was not determined. The intradermal test, previously considered the “gold standard” of allergen detection, cannot distinguish between allergic and nonallergic dogs,4,7,25,26 and is therefore not suitable. Likewise, serum testing for allergen-specific IgE does not reliably differentiate normal from allergic dogs.7,14

The results pertaining to the mould allergens “Aspergillus,” “Penicillium” and “Cladosporium” from Laboratory C were particularly conspicuous during the evaluation. All test results of the respective allergen of the different dogs showed the same numerical values throughout – Aspergillus 74, Penicillium 25 and Cladosporium 35. Because different allergens and concentrations are important for each dog, different results would have been expected, as can be seen with the other allergens of the panel. The identical results are difficult to explain. However, all values were evaluated as negative, so these results did not influence the selection of allergens for immunotherapy.

Several studies have addressed the reproducibility of serum allergen tests. One tested a macELISA procedure from Greer Laboratories (Lenoir, NC, USA).1,11 Serum samples were sent two days apart, with the subsequent sample being stored at –70°C for the interim period. Greer Laboratories was aware of the study, and examined the samples on separate days without being informed about the history or origin of the samples. The results showed a much higher CV (90%) compared to the present study. Sixty-two percent of the positive results and 96% of the negative results were reproducible. Another study focused on the intra- and interassay variability and also, by contrast with the present study, on the interlaboratory reproducibility of five laboratories in comparison to Greer’s reference laboratory.12,13 However, the focus of the present study was on the intra- and interassay reproducibility of the same laboratory. The lower values of intra- (6–13%) and interassay (8–17%) variabilities in these two studies compared to the present study might be a consequence of the fact that Greer Laboratories provided the laboratories with all necessary materials such as serum samples, buffers and antigen-coated wells, reducing possible variation in the source and type of reagents. In comparison to the aforementioned studies, the current study was not funded by any of the tested laboratories, and the laboratories were blinded to the study. Therefore, it was possible to evaluate the results completely independently.

Another independent and single-blinded study tested the intralaboratory reproducibility of several samples sent to the same laboratory on the same day.3 The results showed an average intralaboratory variance of only 3.14%. In that study, a human FcεR1α receptor reagent was used to detect IgE, rather than the monoclonal antibodies used in our study. The FcεR1α reagent has been shown to have a high specificity for canine IgE in previous studies.16,27

There were limitations in the present study. In total, only a small number of dogs were enrolled (ten, eight and twelve dogs for laboratories A, B and C, respectively), and for each laboratory on average 31 allergens were tested. However, this corresponds to a large total number of 2,457 evaluated results and thus 1,817 pairs of values that were compared. Furthermore, we could not assume that all serum samples were tested as prescribed in the study protocol, as the laboratories were blinded. It was therefore possible that serum samples of a dog sent in on different days were examined in the same batch and thus the intra- and interobserver reliability distorted. In addition, although customers were not informed of any changes in testing procedures during the six month period of sample collection, unknown methodological changes could have been introduced in the individual laboratories during that time.

Currently, there is no external control centre in veterinary medicine which tests laboratories offering these tests and their results at regular intervals, and each laboratory is responsible for its own quality control.

Conclusion

In this independent, blinded study, none of the three tested European veterinary laboratories showed absolute reproducibility of the test results, although agreement was in general high. This suggests that, regardless of the serum test used, when selecting suitable allergens for ASIT, it is essential always to assess the results in light of the clinical history. Further work is needed to develop a laboratory monitoring system that compares test results with corresponding reference values on a regular basis.

Acknowledgements

The authors thank the German Society of Veterinary Dermatology (DGVd) for sponsoring the study and all dog owners who agreed to the inclusion of their dogs in this study. We also would like to thank Amelie von Voigts-Rhetz for her help in carrying out the study. Open access funding enabled and organized by ProjektDEAL.

References

SAT reproducibility in dogs


Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Individual results of allergen-specific immunoglobulin (IgE) testing in Laboratory A.

Table S2. Individual results of allergen-specific immunoglobulin (IgE) testing in Laboratory B.

Table S3. Individual results of allergen-specific immunoglobulin (IgE) testing in Laboratory C.
0.79 et 0.75, 0.92 et 0.90, et 0.90 et 0.85. Les CVs étaient respectivement 18.92% et 22.95%, 14.43% et 18.79%, et 15.38% et 18.75% et le rsp 0.73 et 0.68, 0.95 et 0.92, et 0.82 et 0.74 (respectivement).

Conclusion et importance clinique – Les différences de reproductibilité entre les laboratoires compliquent l’interprétation des tests et souligne l’importance de l’interprétation des résultats des tests sériques pour les IgE spécifiques d’allergènes dans le contexte clinique de chaque animal.

RESUMEN
Introducción – las pruebas séricas de inmunoglobulina (Ig)E específicas de alérgenos se emplean comúnmente para identificar los alérgenos utilizados para la inmunoterapia específica de alérgenos en perros, aunque la fiabilidad de los resultados ha sido un tema de debate.

Objetivo – el objetivo de este estudio fue evaluar la reproducibilidad de las pruebas séricas de IgE ambiental específica de alérgenos en tres laboratorios europeos.

Animales/Métodos – el suero se obtuvo de 33 perros de propietarios particulares diagnosticados con dermatitis atópica, se dividió en tres alícuotas y se envió a los laboratorios con diferentes nombres. Se enviaron dos alícuotas simultáneamente a uno de los laboratorios el primer día, la tercera muestra se envió al mismo laboratorio al día siguiente. El laboratorio de cada paciente se eligió de acuerdo con una lista de aleatorización predeterminada. La concordancia entre diferentes muestras del mismo perro para cada uno de los laboratorios se calculó con una prueba Kappa de Cohen. Además, se calcularon los coeficientes de rango de Spearman (rs) y los coeficientes de variación (CV).

Resultados – las concordancias intra e intersesay para los laboratorios A, B y C fueron 0.79 y 0.75, 0.92 y 0.90 y 0.85, respectivamente. Los CV fueron 18.92% y 22.95%, 14.43% y 18.79%, 15.38% y 18.75% (respectivamente) y el rs 0.73 y 0.68, 0.95 y 0.92 y 0.82 y 0.74 (respectivamente).

Conclusión y relevancia clínica – las diferencias de reproducibilidad entre laboratorios complican la interpretación de las pruebas y subrayan la importancia de interpretar los resultados de las pruebas séricas de IgE específica de alérgenos en el contexto de la historia clínica del paciente.

Zusammenfassung
Hintergrund – Serumtests für Allergen-spezifisches IgE werden häufig verwendet, um Allergene zu identifizieren, die zur Allergen-spezifischen Immuntherapie bei Hunden Verwendung finden, wobei die Verlässlichkeit der Ergebnisse ein Diskussionsthema darstellt.

Ziel – Das Ziel dieser Studie war eine Evaluierung der Reproduzierbarkeit von Serumtests für Umweltallergen-spezifisches IgE in drei europäischen Laboratorien.


Ergebnisse – Die Intra- und Interassay Übereinstimmungen für die Laboratorien A, B und C lagen bei 0.79 und 0.75 bzw. 0.92 und 0.90 bzw. 0.90 und 0.85. Die CVs lagen bei 18.92% und 22.95% bzw. 14.43% und 18.79% bzw. 15.38% und 18.75% und der rs 0.73 und 0.68 bzw. 0.95 und 0.92 bzw. 0.82 und 0.74.

Schlussfolgerungen und klinische Bedeutung – Die Unterschiede bei der Reproduzierbarkeit zwischen den Laboratorien machen die Interpretation der Tests kompliziert und unterstreichen die Wichtigkeit, die Serum-Testergebnisse für Allergen-spezifisches IgE im Zusammenhang mit der klinischen Patientenhistorie zu interpretieren.

要約
背景 – アレルゲン特異的免疫グロブリン (Ig)E血清検査は、犬のアレルゲン特異的免疫療法に使用されるアレルゲンを同定するために一般的に使用されているが、その結果の信頼性については議論の余地がある。

目的 – 本研究の目的は、ヨーロッパの3つの研究室における環境アレルゲン特異的IgE血清検査の再現性を評価することであった。

動物/方法 – アトピー性皮膚炎と診断されたオーナー所有犬33頭から血清を採取し、3分割し、異なる名前で検査室に送付した。初日に分断した2サンプルを同時に1つの検査室に送り、3つの目のサンプルを翌日に同じ検査室に送った。各患者の検査室は、あらかじめ決められたランダム化リストに従って選択された。各検査室について、同じ犬からの異なるサンプル間の一致度を、コーヘンのカッパ検定を用いて計算した。そしてアレルゲンの順位係数 (rs)および変動係数 (CV)を追加的に計算した。

結果 – 検査室A、BおよびCの検査室内および検査間の一致度はそれぞれ0.79および0.75、0.92および0.90および0.85であった。変動係数はそれぞれ18.92%および22.95%、14.43%および18.79%、15.38%および18.75%、rsはそれぞれ0.73および0.68、0.95および0.92、0.82および0.74であった。
結論と臨床的妥当性 - 検査室間の再現性の違いを検査の解釈を複雑にし、アレルゲン特異的IgE血清検査結果を患者の病歴と照らし合わせて解釈することの重要性を強調した。

摘　要
背景 - 適原特異性免疫球蛋白(IgE)の血清検査、通常には発症を起因にアレルギー性疾患の診断に用いられる。しかし、この検査法の信頼性はまだ議論されており、特に臨床的妥当性に関して多摩向きがある。

目的 - 本研究の目的は、適原特異性IgE血清検査の再現性を評価することである。

動物/方法 - 33匹の犬に適原特異性IgE血清検査の信頼性を評価するため、3等分に分け、それぞれ名前を付けた。同一の診断で、適原の種類別にサンプルを採取し、3等分に分ける。各等分は1週間ごとに測定を行ない、測定結果の相関を評価した。

結果 - 各等分の間での相関係数は0.73と0.68、0.95と0.92、0.82と0.74（それぞれ）であった。また、内観的信頼性（CV）は、18.92%、22.95%、14.43%、18.79%、15.38%、18.75%（それぞれ）であった。

結論 - これらの結果から、適原特異性IgE血清検査の再現性は高いことが示された。しかし、個別の説明的な要因を考慮に入れることが必要であることを示唆している。