

## Validation of a novel epicutaneous delivery system for patch testing of house dust mite-hypersensitive dogs

Thierry Olivry\*†, Keith E. Lindert†‡, Judy S. Paps\*, Petra Bizikova\*, Stan Dunston\*, Nathalie Donne§ and Lucie Mondoulet§

\*Department of Clinical Sciences, College of Veterinary Medicine, †Center for Comparative Medicine and Translational Research and

‡Department of Population Health and Pathobiology, North Carolina State University, Raleigh, NC, USA

§DBV Technologies, 80/84 rue des Meuniers, 92220 Bagneux, France

Correspondence: Thierry Olivry, Department of Clinical Science, North Carolina State University, College of Veterinary Medicine, 1060 William Moore Drive, Raleigh, NC 27607, USA. E-mail: tolivry@ncsu.edu

**Background** –Patch tests with allergens are used for the evaluation of cellular hypersensitivity to food and environmental allergens in dogs and humans with atopic dermatitis. Viaskin is a novel allergen epicutaneous delivery system that enhances epidermal allergen capture by immune cells.

**Objectives** –To compare the use of Viaskin and Finn chamber patch tests in dogs hypersensitive to mite allergens.

**Methods** –Empty control or *Dermatophagoides farinae* house dust mite-containing Viaskin or Finn chamber patches were applied to the thoracic skin of six mite-hypersensitive Maltese-beagle crossbred atopic dogs. Lesions were graded 49 and 72 h after patch test application, and skin biopsies were collected after 72 h. Overall microscopic inflammation, eosinophil and T-lymphocyte infiltrations were scored.

**Results** –Positive macroscopic patch test reactions developed at five of six Viaskin application sites and four of six Finn chamber application sites. Median microscopic epidermal and dermal inflammation, as well as eosinophil and CD3 T-lymphocyte dermal scores were always higher in biopsies collected at Viaskin than at Finn chamber sites. Microscopic inflammation scores were significantly higher after mite allergen-containing Viaskin compared with empty patches, but this was not the case for mite-containing Finn chambers compared with control chambers. Scores obtained using Viaskin were not significantly different from those obtained using Finn chambers. Macroscopic and microscopic scores were significantly correlated.

**Conclusions and clinical importance** –In mite-allergic dogs, Viaskin epicutaneous delivery systems appear to induce stronger allergen-specific inflammation than currently used Finn chamber patch tests. Consequently, Viaskin patches might offer a better alternative for screening cellular hypersensitivity to food and environmental allergens.

### Introduction

To reproduce the natural lesions of canine atopic dermatitis experimentally<sup>1</sup> or to document the delayed type of reactivity to allergens in spontaneously allergic dogs,<sup>2</sup> epicutaneous allergen applications ('patch tests') can be used. In dogs, patch testing has been done previously using either aluminium cupules on an adhesive strip, the so-called Finn chambers,<sup>2</sup> or commercial adhesive bandages.<sup>1</sup> Which one of these two epicutaneous application devices yields the highest inflammation and lowest adhesive reaction has not yet been evaluated.

Viaskin epicutaneous delivery systems (EDSs; DBV Technologies, Paris, France) are composed of an 11-mm-diameter central translucent polyethylene membrane surrounded by an adhesive polyester crown that

maintains the chamber on the skin (Figure 1). Allergens (up to 500 µg per EDS) can be electrostatically deposited onto the underside of the membrane. After application, the newly created occlusive chamber generates moisture from sweat and transepidermal water loss, and it induces a barrier change. The moisture releases the allergens from the membrane, which permits their diffusion through the stratum corneum barrier and their capture by dendritic antigen-presenting cells localized in the very superficial layers of the epidermis.<sup>3</sup>

The objective of this study was to compare, in mite-sensitized atopic dogs, the macroscopic and microscopic inflammation induced by allergens using two patch tests, the novel EDS and the traditional Finn chambers (FCs; Smart Practice, Phoenix, AZ, USA).

### Materials and methods

The use of these dogs and all procedures were pre-approved by the North Carolina State University institutional animal care and use committee.

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**Conflict of Interest:** Nathalie Donne and Lucie Mondoulet are employed by DBV Technologies.

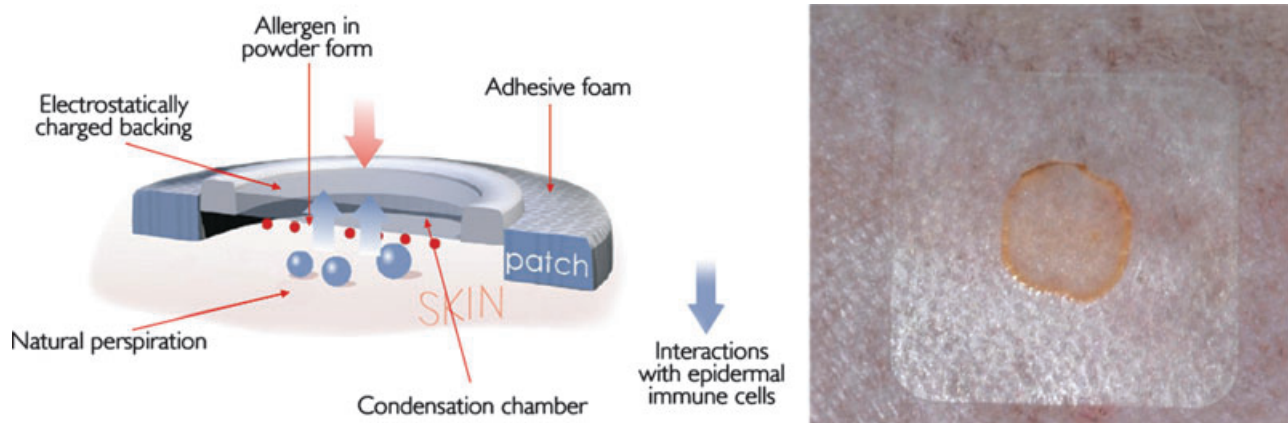


**Figure 1.** Diagram of Viaskin epicutaneous delivery system (left) and the application of a mite allergen-containing epicutaneous delivery system onto canine skin (right).

## Animals

We selected six Maltese-beagle atopic dogs previously sensitized to *Dermatophagoides farinae* (Df) and shown to have elevated Df-specific IgE serum levels and previous skin lesion development after epicutaneous Df allergen painting. There were three intact males and three females (two intact, one spayed); their ages ranged from 3.8 to 12.8 years (median 6.5 years).

## Patch tests

Hair was clipped from both sides of the thorax 24 h before patch testing. On the day of challenge, two FC patches were applied to the left thorax; the lowermost small FC contained a cellulose disk impregnated with 20 µL of phosphate-buffered saline, while a disk soaked with 500 µg of Df extract (Greer, Lenoir, NC, USA) was placed in a larger FC on the upper thorax. On the right side, two EDSs were applied. The lowermost EDS had an empty chamber, while the top one contained 500 µg of Df allergens (Greer). Patch test sites were covered with a bandage and a coat. Skin biopsies were collected 72 h after patch test application under sedation and local anaesthesia. Samples were bisected and snap frozen in liquid nitrogen or fixed in formalin.

## Assessment of macroscopic skin lesions

Patches were removed 48 h after application, and lesions were scored 1 and 24 h later, as done previously.<sup>1</sup> Grades at 49 and 72 h were added to yield a total lesional score.

## Assessment of microscopic inflammation

Five-micrometre-thick sections were obtained from each paraffin block and stained with haematoxylin and eosin, Luna stain for eosinophils or CD3, as done previously.<sup>1</sup> Microscopic evaluation was done blindly using randomly coded slides. Epidermal and dermal inflammation were scored using a five-point standard severity scale (from 'none, 0' to 'marked, 4'). The T lymphocytes and eosinophils were counted using a reticule, with the grid being placed at five regularly spaced intervals in the superficial dermis between infundibula.

## Statistical analyses

Statistical comparisons were made between scores obtained, for each patch type, between Df and control sites with one-tailed nonparametric paired *t*-tests (Wilcoxon signed rank tests) to account for the unidirectional hypothesis (Df > control values). In contrast, scores for Df patches were compared between the two devices using two-tailed Wilcoxon signed rank tests. Finally, we calculated the correlation between 72 h lesional scores and dermal inflammation, eosinophil and CD3 scores, or between the last two values using Spearman's rank correlation.

## Results

### Assessment of macroscopic skin lesions

Inflammation was seen at five of six and four of six Df-containing EDS and FC patch test sites, respectively (Figure 2). One dog did not react to any of the patches. Total lesional scores were significantly higher at Df than empty EDSs, but not for Df compared with control FC patches (Table 1). When comparing EDS versus FC patches, the total lesional scores were not significantly different. After 49 and 72 h, the presence of mild reactive erythema at the site of adhesive contact was seen in eight and six of 12 EDSs and six and zero of 12 FCs, respectively.

### Assessment of microscopic inflammation

All parameters evaluated were significantly higher in Df than empty EDS patches, but not in Df-containing compared with control FCs (Table 1). While microscopic scores were almost always equal or higher for Df-containing

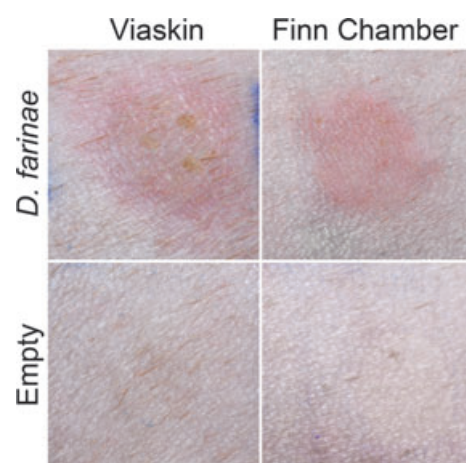


**Figure 2.** Photo composite of patch test reactions of the same dog at 72 h, illustrating grade 3 reactions to *Dermatophagoides farinae* antigen after both Viaskin epicutaneous delivery system (erythema, oedema, induration and vesiculation) and Finn chamber application (erythema, oedema and induration). There were no visible reactions after testing with empty or control devices.

**Table 1.** Clinical and microscopic scores

|                                   | <i>Dermatophagoides farinae</i> | Empty            | <i>Dermatophagoides farinae</i> versus control |
|-----------------------------------|---------------------------------|------------------|------------------------------------------------|
| Viaskin epidermal delivery system |                                 |                  |                                                |
| Total lesional score (49 + 72 h)  | 2.0 (0.2–4.8)                   | 0.0 (0.0–0.0)    | $P < 0.05$                                     |
| Dermal inflammation score         | 4.0 (1.6–5.0)                   | 1.5 (0.1–2.6)    | $P < 0.05$                                     |
| Epidermal inflammation score      | 2.5 (0.6–4.0)                   | 0.0 (0.0–1.2)    | $P < 0.05$                                     |
| Dermal eosinophil score           | 231.5 (15.6–468.4)              | 12.0 (0.0–26.5)  | $P < 0.05$                                     |
| Dermal CD3 score                  | 300.5 (119.6–474.7)             | 36.5 (1.2–115.4) | $P < 0.05$                                     |
| Finn chambers                     |                                 |                  |                                                |
| Total lesional score (49 + 72 h)  | 2.0 (0.2–3.5)                   | 0.0 (0.0–0.6)    | $P > 0.05$                                     |
| Dermal inflammation score         | 3.0 (1.6–4.0)                   | 1.0 (0.2–2.8)    | $P > 0.05$                                     |
| Epidermal inflammation score      | 1.0 (0.0–2.4)                   | 0.0 (0.0–0.6)    | $P > 0.05$                                     |
| Dermal eosinophil score           | 60.0 (1.2–150.4)                | 10.5 (0.0–35.5)  | $P > 0.05$                                     |
| Dermal CD3 score                  | 153.4 (46.9–352.1)              | 50.5 (0.0–294.0) | $P > 0.05$                                     |

Data are presented as medians (95% confidence intervals).

EDS than FC patches, the differences between these two groups were not significant (Table 1). Values of all microscopic parameters were significantly correlated to 72 h lesional scores from the same site ( $P < 0.01$ ); dermal eosinophil and CD3 scores were also correlated with each other ( $P < 0.0001$ ).

## Discussion

In this study, we sought to compare the allergen-specific allergic reactions induced by two different patch test devices, the novel Viaskin EDS and the traditionally used FC.<sup>2</sup> Our results suggest that both macroscopic and microscopic inflammation scores were, almost always, equal or higher at Df-containing EDS compared with FC patch test sites. However, differences in scores were not significantly different between the two devices. Of interest is the observation that all scores were significantly different between mite and control patches only in the EDS but not the FC samples. This observation suggests that the inflammation induced by the EDS is greater than that induced by traditional chambers. In this experiment, the macroscopic and microscopic reactions seen with both Df-containing EDSs and FCs were similar to those reported previously for patch testing with mite allergens in sensitized dogs.<sup>1</sup>

As the Viaskin EDS patches have proved to be useful for allergy diagnosis<sup>4</sup> and epicutaneous immunotherapy in children with spontaneous food allergies,<sup>5</sup> our results warrant further investigation of the benefit of this novel EDS as an aid for diagnosis or immunotherapy in dogs with atopic dermatitis.

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## Résumé

**Contexte** – Les patchs tests d'allergènes sont utilisés pour évaluer l'hypersensibilité cellulaire aux aliments et aux allergènes environnementaux chez l'homme et chez les chiens atteints de dermatite atopique. Viaskin est un nouveau système de distribution épicutanée d'allergène qui favorise la capture allergénique épidermique par les cellules immunitaires.

**Objectifs** – Comparer l'utilisation de Viaskin et de patchs tests Finn chamber chez les chiens sensibilisés aux allergènes d'acariens.

**Méthodes** – Des contrôles vides, des Viaskin ou des patchs Finn chamber contenant l'acarien de la poussière de maison *Dermatophagoides farinae* ont été appliqués sur la peau du thorax de six chiens atopiques croisés beagle-maltais allergiques aux acariens. Les lésions ont été notées 49 et 72 h après l'application des patchs et des biopsies cutanées ont été prélevées après 72 heures. L'inflammation microscopique globale, les infiltrations éosinophiliques et lymphocytaire T ont été notées.

**Résultats** – Les réactions positives macroscopiques des patchs se sont développées pour cinq des six sites d'application de Viaskin et pour quatre sur six des sites d'application des patchs Finn chamber. La moyenne microscopique de l'inflammation épidermique et dermique, les scores dermiques des éosinophiles et de lymphocytes T CD3 étaient toujours plus élevés pour les biopsies issues des sites de Viaskin que de Finn chamber. Les scores d'inflammation microscopique étaient significativement plus élevés

après l'application de Viaskin contenant des allergènes d'acariens qu'avec des patchs vides mais ce n'était pas le cas avec les patchs Finn chamber contenant les acariens comparé aux chambres contrôles. Les scores obtenus utilisant Viaskin n'étaient pas significativement différents de ceux obtenus par les patchs Finn chamber. Les scores macroscopiques et microscopiques étaient significativement corrélés.

**Conclusions et importance clinique** – Chez les chiens allergiques aux acariens, le système de diffusion épicutanée Viaskin semble induire une inflammation spécifique d'allergène plus forte que les patchs Finn chamber actuellement utilisés. Ainsi, les patchs Viaskin pourraient offrir une meilleure alternative pour le screening cellulaire de l'hypersensibilité aux aliments et aux allergènes environnementaux.

## Resumen

**Introducción** – las pruebas de parche con alergenitos se utilizan para la evaluación de la hipersensibilidad celular a los alimentos y alergenitos ambientales en perros y seres humanos con dermatitis atópica. Viaskin es un nuevo sistema de liberación de alergenitos por vía epicutánea que favorece la captura de alergenitos por las células del sistema inmunitario.

**Objetivos** – comparar el uso de Viaskin y las pruebas de parche de cámara de Finn en perros con hipersensibilidad a alergenitos de ácaros.

**Métodos** – se aplicaron controles vacíos o Viaskin o parches de cámaras de Finn conteniendo ácaros del hogar en la piel del tórax en seis perros cruzados atópicos Maltes-Beagle con hipersensibilidad a ácaros. Las lesiones se evaluaron a las 49 y 72 horas tras la aplicación del parche, y se tomaron biopsias de piel tras 72 h. Se valoraron el grado general de inflamación, y los infiltrados ricos en eosinófilos y linfocitos T.

**Resultados** – reacciones positivas macroscópicas a las pruebas de parche se desarrollaron en cinco de las seis localizaciones con la aplicación de Viaskin y en cuatro de los seis lugares con las cámaras de Finn. La media de los valores de inflamación microscópica en la epidermis y la dermis, así como los valores de infiltración con eosinófilos y linfocitos CD3 fueron siempre mayores en biopsias tomadas de los lugares aplicados con Viaskin comparados con los lugares con cámara de Finn. Los valores de inflamación microscópica fueron significativamente más altos tras la aplicación de Viaskin con alergenito de ácaros comparado con los parches vacíos, pero no fue así el caso de las cámaras de Finn con alergenito de ácaros comparado con las cámaras control. Los valores obtenidos con Viaskin no fueron significativamente diferentes de los valores obtenidos usando las cámaras de Finn. Los valores macroscópicos y microscópicos estuvieron significativamente correlacionados.

**Conclusiones e importancia clínica** – en perros con alergia a los ácaros, el sistema de aplicación epicutánea Viaskin parece inducir un mayor grado de inflamación específica de alergenitos que las actualmente utilizadas pruebas de parche de cámaras de Finn. Por consiguiente, los parches de Viaskin podrían ofrecer una mejor alternativa para investigar hipersensibilidad a alergenitos ambientales y alimentarios.

## Zusammenfassung

**Hintergrund** – Patch Tests mit Allergenen werden für die Evaluierung der zellulären Hypersensibilität auf Futter- und Umgebungsallergene bei Hunden und Menschen mit atopischer Dermatitis eingesetzt. Viaskin ist ein neues epikutanes Allergen-Transportsystem, welches das Abfangen der epidermalen Allergene durch Immunzellen verstärkt.

**Ziele** – Ein Vergleich von Viaskin und Finn Kammer Patch Tests bei Hunden mit einer Hypersensibilität auf Milbenallergene.

**Methoden** – Leere Kontrollen oder *Dermatophagoides farinae* Hausstaubmilben enthaltende Viaskin oder Finn Kammer Patches wurden an der Haut des Thorax von sechs Milben-hypersensiblen atopischen Malteser-Beagle Mischlingshunden aufgebracht. Die Veränderungen wurden nach 49 und 72h des Patch Tests beurteilt und Hautbiopsien wurden nach 72h entnommen. Es wurde die Entzündung, die eosinophile Infiltration sowie die Infiltration mit T Lymphozyten mikroskopisch bewertet.

**Ergebnisse** – An fünf von sechs Viaskin Applikationsstellen sowie an vier von sechs Finn Kammer Applikationsstellen entwickelten sich positive, makroskopisch erfassbare Patch Test Reaktionen. Der Medianwert für mikroskopisch erfassbare epidermale und dermale Entzündung, sowie eosinophile und CD3 T-Lymphozyten Werte waren immer in den Biopsien aus dem Viaskin höher als bei denen aus den Finn Kammern. Die mikroskopischen Entzündungswerte waren signifikant höher in den Viaskin im Vergleich zu leeren Patches, was aber bei Finn Kammern mit Milben im Vergleich zu den Kontrollkammern nicht der Fall war. Die Auswertung mittels Viaskin war nicht signifikant unterschiedlich im Vergleich zu jener der Finn Kammern. Die makroskopische und mikroskopische Auswertung korrelierte signifikant.

**Schlussfolgerungen und klinische Bedeutung** – Bei Milben-allergischen Hunden scheint das epikutane Viaskin Allergen-Transportsystem eine deutlichere entzündliche Reaktion auszulösen, wie der derzeitige verwendete Finn Kammer Patch Test. Folglich ist es möglich, dass Viaskin Patches eine bessere Alternative für ein Screening der zellulären Hypersensibilität auf Futter- und Umgebungsallergene darstellen.

## 要約

**背景** - アレルゲンのパッチテストは犬とヒトのアトピー性皮膚炎で、食事や環境アレルゲンの細胞性過敏反応の評価に使用されてきた。Viaskinは免疫細胞による表皮のアレルゲンの補足を増強する新規のアレルゲン経皮運搬システムである。

**目的** - ダニ抗原に対する過敏症を有する犬で、ViaskinとFinn chamberを用いたパッチテストを比較する。

**方法** - パッチに何も浸み込ませていない陰性対照群あるいは*Dermatophagoides farinae* ハウスダストマイト抗原を含むViaskinあるいはFinn chamber パッチを、6頭のダニ過敏症のマルチーズ・ビーグル交雑アトピー犬の胸部皮膚に貼付した。パッチテスト実施49時間と72時間後に病変をグレード評価し、72時間後に皮膚生検材料を採取した。総合的な顕微鏡的な炎症、好酸球とTリンパ球浸潤をスコア化した。

**結果** - 肉眼的に陽性なパッチテスト反応がViaskin適応部位の6か所中5か所で、Finn chamber適応部位の6か所中4か所で認められた。顕微鏡的な表皮と真皮の炎症および好酸球とCD3 Tリンパ球真皮スコアの中央値は、Finn chamberと比較してViaskinの部位から採取した生検材料で常に高かった。顕微鏡的な炎症スコアは陰性対照群と比較してダニアレルゲンを含むViaskin後で有意に高かったが、ダニ含有Finn chamber群と陰性対照群を比較した場合はそうではなかった。Viaskin群のスコアはFinn chamber群のスコアと有意な差はなかった。肉眼的、顕微鏡的なスコアは有意に相関していた。

**結論と臨床的な重要性** - ダニアレルギーの犬では、Viaskin の経皮運搬システムはこれまで使われているFinn chamber パッチテストより強いアレルゲン特異的炎症を誘発すると思われる。その結果として、Viaskin パッチは食事や環境に対する細胞性過敏症のスクリーニングによりよい代替法として提供できるかもしれない。

## 摘要

**背景** - 過敏原斑貼試験可用于评估犬和人异位性皮炎的食物和环境过敏原的细胞性过敏反应。Viaskin是一种新奇过敏原表皮传送系统，通过免疫细胞增强捕获表皮过敏原。

**目的** - 比较在螨虫过敏原过敏的犬上使用Viaskin和Finn 室斑贴试验。

**方法** - 空白对照或含美洲尘螨（粉尘螨）的Viaskin或Finn室贴片，贴于6只螨虫过敏的异位性马尔济斯-比格杂交犬的胸部皮肤。斑贴试验49和72小时后对病变评分，72小时后采集皮肤活检样本。对全部显微镜下炎症、嗜酸性粒细胞和T-淋巴细胞浸润评分。

**结果** - Viaskin 法6个中的5个贴片部位，和Finn室法6个中的4个贴片部位，均出现了肉眼可见的斑贴试验反应阳性。Viaskin活检中，显微镜下表皮和真皮炎症中间值，以及嗜酸性粒细胞和CD3 T-淋巴细胞皮肤评分均高于Finn室。含螨虫抗原的Viaskin贴片后显微镜下炎症评分显著高于空白贴片，但含螨虫的Finn贴片与对照贴片相比并非如此。使用Viaskin法与Finn室得出的评分没有显著差异。肉眼和显微镜下观察评分显著相关。

**结论和临床价值** - 在螨虫过敏的犬上，Viaskin经表皮传送系统诱导的特异性过敏原炎症似乎强于当前的Finn室斑贴试验。因此，Viaskin斑贴可能提供更好的替代方法，用于筛选食物和环境过敏原的细胞过敏。