![Gold Standard Diagnostics logo]

Canine INgezim PLEX Environmental Sensitization kit
R.15.CEA.K.61

Canine INgezim® PLEX Environmental Sensitization kit is an ELISA-microarray immunoassay designed to simultaneously measure the semi-quantitative level of 30 environmental allergen-specific IgE in canine serum. In addition, the microarray features CCD (Carbohydrate Cross Reactivity Determinants) duplicated spots for detecting CCD-specific IgE antibodies, that do not have any clinically relevance. An optional serum pre-adsorption incubation with the INgezim PLEX anti-CCD blocker allows to identify only IgE antibodies specific to pollen peptides epitopes. The kit is intended to offer veterinarian clinicians a support for designing an allergen-specific immunotherapy (ASIT) along with the patient clinical history.
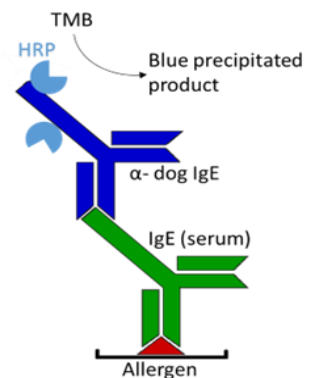
KIT FEATURES

APPLICATION
The kit is intended for the determination of allergen-specific IgE levels that may have clinical significance in dogs previously diagnosed with canine topical dermatitis. It is exclusively valid for sera from dogs suffering this pathology.

TECHNICAL BASE
The Canine INgezim® PLEX Environmental Sensitization tests are solid-phase immunoassays in which allergen extracts are deposited in duplicate spots forming a microarray onto microplate wells. The immunoassay is performed in an indirect ELISA-microarray format, in which the allergen-specific IgEs present in a sensitized dog serum will bind to the corresponding allergen spots.

Only twenty microlitres of serum, diluted at a 1:5 ratio are incubated in the ELISA-microarray well containing the allergen microarray. After incubation, non-specific IgE is washed off. The specific allergen-IgE binding is detected by the HRP-conjugated anti-dog IgE antibody. After a second washing step, the binding is developed by a colorimetric peroxidase substrate (TMB) which is converted to an insoluble, coloured product that precipitates specifically onto the allergen-specific IgE reactive spots. The enzyme-substate reaction is stopped by washing with ddH$_2$O to remove TMB substrate. The amount of precipitate is proportional to the level of allergen-specific IgE in the patient sample.

The assayed microarrays can be analyzed with our Hailstorm analyzer or by any commercial colorimetric reader compatible with 96-well microplates.

ALLERGEN PANEL
The microarray panel comprises 29 extracts (6 mites, pollens from 6 grass, 8 weeds and 5 trees and 4 moulds) and one major allergen component deposited in duplicated spots on each microplate well. The microarray also features control spots to check the assay (dog IgE) and the serum sample (anti-dog IgE mAb spots) functionality and five positional marker spots for the Hailstorm's software image analysis.

Mites	Grass	Weeds	
D. farinae	Perennial ryegrass	Saltwort	Nettle
D. pteronyssinus	Red fescue	English plantain	Goosefoots
A. siro	Cock´s-foot	Ragweed	Sticky weed
L. destructor	Timothy-grass	Mugweed	Sheep's shorrrel
T. putrescentiae	Kentucky bluegrass		
B. tropicalis	Bermuda grass		

Trees	Moulds	Others
Birch tree	*A. alternata*	rCup a 1 Cupressus natural major allergen
Ash	*P. notatum*	CCD (Carbohydrate Cross Reactivity Determinants)
Olive	*C. herbarum*	Dog IgE (positional markers)
London plane	*A. fumigatus*	Dog IgE (conjugate control spots)
Black poplar		α-dog IgE mAb (canine serum control)
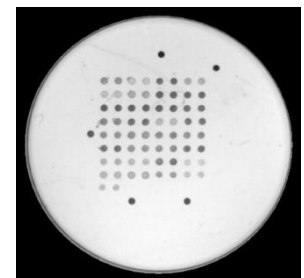
RESULT INTERPRETATION

Allergen-specific IgE reactivity is reported as signal intensity (SI) value in arbitrary units (AU). Spot intensity is measured as the grey signal intensity median of the spot pixels minus the grey signal intensity median of the background spots. Allergen-specific IgE reactivity (SI) is calculated as the average of the signal intensity of the spot duplicates.

The test determines reactive and non-reactive specific IgE to each of the 30 allergens included in the panel, based on a cutoff point established to discriminate between allergen-specific levels with or without potential clinical relevance. The microarray also includes both duplicate spots for evaluating the assay performance and the functionality of the serum.

The microarray features a duplicated target that specifically detects CCD-specific IgE antibodies. In addition, an optional pre-incubation step with the INgezim PLEX anti-CCD blocker (Prod. Ref: R.15.BLOK61; not provided in the kit) suppresses the IgE binding to clinically

irrelevant CCD, ensuring a reliable detection of pollen-specific IgE antibodies against peptide epitopes in a single test session. The CCD spots in the microarray serve as a positive control during the blocking step verifying the effectiveness of the serum pre-adsorption with the anti-CCD blocker.

As there is no evidence that the level of allergen-specific IgE correlates with the severity of clinical disease, the veterinary practitioner shall prescribe an allergen-specific immunotherapy (ASIT) based on the positive or negative results, the most reactive and prevalent allergens of patient residence area as well as the patient´s clinical history.

ASSAY VALIDATION

VALIDATION DATA

The results obtained of prevalence from a collection of sera from dogs diagnosed with CAD (n=236) coincide with those previously published in Spain (Pérez-Aranda, M. et al., 2016), with the mite group sensitization (n=147; 62%) being more prevalent than that of pollens (n=70; 30%) and fungi (n=26; 11%), and among those, *D.farinae* being the most prevalent sensitization (n=56; 24% and 38% regarding total sera and mite sensitized sera, respectively).

Utility of INgezim PLEX Environmental for immunotherapy design. Since there is no gold standard test and it has been reported that there is poor correlation between commercial assays, INgezim PLEX Environmental performance was evaluated by assaying the efficacy of the vaccines designed based on the results of our test and the dogs' clinical history. The vaccines were manufactured as depot preparations by Immunal or Roxall Medizine. The symptoms and the frequency and quantity of both flares and accompanying treatments were reduced to different extents in 16 of the 20 vaccinated dogs (80% efficacy), so that vaccine administrations were prolonged for up to 2 years. This level of efficacy is in range with those of competitor vaccines (70 - 85%).

Accuracy in inter-batch qualitative results (positive / negative): 97%

CV intra-batch: 2% - 23%

CV inter-batch: 8% - 25

HAILSTORM ANALYZER AND STORM 3.0 SOFTWARE DESCRIPTIONS

HAILSTORM ANALYZER

The Hailstorm (GSD US) is an automatic device of the bolt family which supports various technologies (ELISA, CLIA and ELISA microarray) and test formats (96 well MTP and Mono Test Strips).

The Hailstorm is a standalone two-plate device that can processes INgezim® PLEX microarray kits, providing analysis and interpretation of the results as specified in the kit instructions for use. The samples and reagents are processed in wells on a 96 well microtiter plate according to the assay protocol defined in the Hailstorm Test File. In this format, samples and reagent bottles are loaded into a reagent rack on the instrument. Hailstorm calculates the required volume to perform the assay depending on the sample number.

The Hailstorm allows to select the number and the position of the samples in the 96-well microplate. Ninety-six sera sample (100 µl serum per assay) analysis takes approximately 6 hours. The assay procedure is followed by an automatic scanning, greyscale image acquisition and analysis and data processing. Allergen-specific IgE reactivity is expressed as signal intensity (SI) values in arbitrary units (AU). In addition, performs and analyzes the microarray immunoassay providing a report of the patient's sensitization profile.

The relevant system components of the Hailstorm for microarray processing are:

- A high-resolution camera/lens to capture microarrays images on the microplate wells. It provides 10 bit gray scale images (1024 gray levels) which are 1.3 MegaPixels and 5.3 µm of pixel size.
- A LED illumination unit consisting of a circular array of 14 white LEDS, which can be moved to any microplate well position.
- A removable Diffusion Plate to create a uniformly illuminated background.

STORM 3.0 SOFTWARE

The Storm 3.0 software captures and analyzes microarray images from Ingezim PLEX microarray kits:

- Execute Microarray test files and display and report results as well as subsets of results.
- Store microarray images and data as well as backup and purge microarray data.

KIT COMPOSITION
- Allergen microarray microplate
- Bottle with serum dilution buffer
- Bottle with HRP-Conjugate
- Bottle with Washing Solution
- Bottle with stop solution
- Bottle with substrate for HRP (TMB)

IMQ CERTIFIED

MANAGEMENT SYSTEM
QUALITY · ISO 9001
ENVIRONMENTAL · ISO 14001

9191.INGE
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