



# Use of serology in canine and feline dermatology



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**S**erology is based on the use of antibodies to detect circulating antibodies (immunoglobulin) or antigens. Largely used in the diagnosis of infectious diseases, it is now often replaced by DNA or RNA detection of infectious agents by molecular biology techniques such as polymerase chain reaction (PCR, rt-PCR). However, antibody detection can have some advantages on PCR techniques and numerous diagnostic evaluations use serological techniques especially for the diagnosis of immune-mediated diseases.

## ■ Principle of serological techniques

Numerous techniques are used (*Table 1, Figure 1*). The main home tests are based on enzyme-linked immunosorbent assay (ELISA) and immunochromatography (IC) (*Figure 2*) and laboratory techniques on indirect immunofluorescence assay (IFA), ELISA and agglutination (latex or RBC) (*Figure 3*).

Different immunoglobulin isotypes can be detected depending on the technique or antisera used. IgM are usually detected in the IFA technique using an anti-IgM-FITC and in all cases of latex agglutination. IgG are detected with most techniques. For IgE detection, the most sensitive techniques are used with ELISA or the avidin-biotin system. Radioimmunosorbent assay (RIA) and bioluminescence are not used in veterinary practice for serology, but mainly for hormone measurements.



**Figure 1.** Numerous kits are commercialized for serological diagnosis of infectious diseases for in-clinic or laboratory use.

**Table 1.**  
Serological techniques

Technique	Advantages	Limitations	Example
Indirect immunofluorescence (IFA)	Simple, numerous antigens, adaptability to different species	Interlaboratory variations, not adaptable to large scale work	Leishmaniasis, ANA*, toxoplasmosis, ehrlichiosis, anaplasmosis, neosporosis, borreliosis
Enzyme-linked immunosorbent assay	Can be developed as a commercial or in-clinic kit	Limited to a few antigens	FeLV, FIV, leishmaniasis, ehrlichiosis, dirofilariasis, <i>Sarcoptes scabiei</i>
Immunochromatography	Simple	No positive control, not adapted to multiple samples	FeLV, FIV, leishmaniasis, ehrlichiosis, dirofilariasis
Latex agglutination	Simple	Reading sometimes difficult	Cryptococcosis, rheumatoid factors, brucellosis
Electrosyneresis	Specificity	Specialized laboratory	Aspergillosis

\*Antinuclear antibody

### ■ Interpretation

The presence of circulating antibodies indicates previous exposure to an antigen. It is not systematically a marker of the disease. Immunization can be observed in healthy dogs or cats and serology cannot have a good diagnostic value if there is not a clear difference between the healthy population and the sick population (*i.e.*: toxoplasmosis, borreliosis, neosporosis). When there is an overlap between both populations, it is necessary to define a threshold of positivity which is a compromise between sensitivity and specificity (*i.e.* leishmaniasis, allergen specific IgE). If there is a clear difference between both populations, serology is a very effective diagnostic tool (*i.e.* cryptococcosis, aspergillosis, antinuclear antibodies, sarcoptic mange). False negative results can be observed after long term corticosteroid therapy.



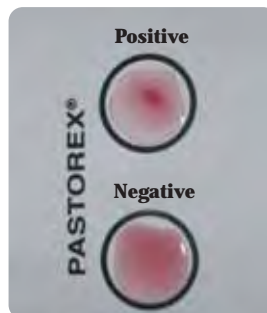
**Figure 2.** Positive test using the immuno-chromatography technique (leishmaniasis).

### ■ PCR, culture or serology?

Currently, there are three ways to diagnose an infectious disease:

- Direct isolation: cytology, histology, culture
- DNA or RNA isolation by PCR or rt-PCR techniques
- Specific antibodies isolation: serology

When direct isolation is easy (fungi, bacteria, most parasites) indirect techniques like serology or PCR are not useful. If isolation is difficult they are mandatory. PCR techniques are very sensitive if the right probe is used. However, this very high sensitivity can limit interpretation when the presence of an infectious agent is not pathologic (*i.e.* leishmaniasis in endemic area). PCR needs a specialized laboratory and its cost is higher than most serological tests. This is why serology is one of the major techniques to diagnose numerous diseases in canine and feline dermatology (**Table 2**).



**Figure 3.** Positive cryptococcus latex agglutination.

**Table 2.**  
Indications of PCR and serology in canine and feline dermatology

	Direct examination or culture	PCR	Serology
Ectoparasites	+	-	- *
Fungi	+	+	- **
Bacteria	+	-	-
Mycobacteria	+	+	-
Rickettsia	+/-	+	+
Protozoa	+	+	+
Virus	-	+	-

\* Except for *sarcoptic mange*

\*\* Except for *cryptococcosis*

## ■ Infectious and fungal diseases

### Viral

Numerous in-clinic and laboratory kits have been developed for the diagnosis of retroviral infection in the cat. They are based on ELISA or immunochromatography techniques for the detection of circulating antibodies (FIV) and antigens (p27 antigen for FeLV). Their sensitivity and specificity are very high. They can be used to diagnose retroviral infection associated with an infectious dermatosis. However, when a viral dermatosis is suspected, PCR techniques applied to skin biopsies are more accurate for retroviral and for other viral infections including feline herpes and calici, papillomavirus, and canine distemper and parvovirus.

### *Ehrlichia*, *Anaplasma*

Serology for rickettsia infection is rarely used in Europe except in cases of suspected acquired immunodeficiency associated with such diseases (e.g.: generalized bacterial cellulitis, adult generalized demodicosis). IFA techniques are the more accurate techniques developed to diagnose chronic forms of the disease. ELISA kits developed for the serology of *Ehrlichia canis* are sensitive enough when used in endemic areas, where reinfestation is frequently associated with a high antibody response.

### *Bartonella*, *Borellia*

Even if serological techniques are developed for the diagnosis of *Bartonella sp.* and *Borellia burgdorferi* infection (IFA) they have no diagnostic value in dermatology. When cutaneous manifestation of these diseases is suspected, PCR must be used.

### *Malassezia*

*Malassezia* specific IgG or IgE have been studied in dogs with atopic dermatitis or recurrent *Malassezia dermatitis*. These dogs present higher levels of malassezian antibodies compared to a healthy canine population. Different ELISA techniques were developed using allergenic extracts of *Malassezia pachydermatis*. However, the serological techniques do not seem to be reliable enough compared to intradermal testing (1). Furthermore, indications for this serological assay are not clear since *Malassezia* immunotherapy, based on such tests, has not been studied. Skin tests with crude extracts could be a more simple and reliable approach to identify dogs with a strong allergic reaction to these yeasts.

### Dermatophytosis

Even if a few serological techniques were developed for the diagnosis of dermatophyte infection, such an approach is neither efficient (lack of sensitivity, persistent seropositivity), nor recommended (2,3). A definitive diagnosis of dermatophytosis is achieved by mycological culture, which is the only way to identify the offending dermatophytes.

### Deep and subcutaneous mycosis

Different serological tests (e.g. antigen detection for *Cryptococcus sp.*) can be used to diagnose most subcutaneous or systemic mycosis (Table 3). However, the definitive diagnosis of these mycoses in dermatology is better achieved by cytology, histopathology and culture (specialized mycology laboratory) and in some cases by immunohistochemistry or PCR techniques. In the case of systemic cryptococcosis or aspergillosis, the semi-quantitative serologic techniques can help monitor treated animals (e.g. decreasing titers (Figure 4)).

## ■ Protozoa

### *Leishmaniasis*

Leishmaniasis is a frequent cause of skin disease in the Mediterranean area and serology is the most widely used technique to obtain a definitive diagnosis. Most laboratories use IFA or ELISA techniques giving semi-quantitative results. In-clinic kits are based on ELISA and IC and detect high titers of antibodies. A negative result with these in-clinic tests in a dog showing clinical signs compatible with leishmaniasis must always be verified by a laboratory technique.

**Table 3.**  
Serological tests used in the diagnosis of mycosis

Mycosis	Serological test	Sensitivity	Specificity	Best diagnostic methods
Aspergillosis	Electrosyneresis (serum)	low	high	CT-scan
Cryptococcosis	Latex agglutination (serum, CSF, urine)	high	high	Cytology, histopathology, culture (PCR for <i>C. neoformans</i> )
Sporotrichosis	-			Cytology, histopathology, culture
Blastomycosis	ELISA (Ag or Ab)	high	low	Cytology, histopathology, immunohistochemistry, culture
Histoplasmosis	Latex agglutination	low	low	Cytology, histopathology, immunohistochemistry, culture, PCR
Coccidioidomycosis	Immunoprecipitation	low	low	Cytology, histopathology, culture
Pythiosis	ELISA	high	high	Cytology, histopathology, culture, PCR
Lagenidiosis	ELISA	low	low	Cytology, histopathology, culture, PCR



**Figure 4.** Positive electrosyneresis of an *Aspergillus fumigatus* serology. Note the multiple arcs.

Clinical signs of leishmaniasis are variable and serology can be positive in dogs in endemic area without clinical leishmaniasis. This is why, if clinical signs are highly suggestive, serology can be performed to confirm the diagnosis; in other cases skin biopsies can be performed to help minimize the differential diagnosis (**Figures 5 and 6**).

The high sensitivity of PCR is interesting for epidemiological studies but is too high to be used as a first step in the diagnosis of clinical

leishmaniasis. False positive results are rare with serology since there are no cross reactions with other organisms. Negative serology can be observed when organisms are easily isolated, such as in the papular or nodular form of the disease.

**Neosporosis**

Neosporosis serology in the dog can be achieved by IFA techniques (VMRD) or adapted ELISA techniques (from bovine kits). These techniques are highly sensitive and specific. However, interpretation cannot be made out of clinical context since numerous healthy dogs can be seropositive (10 to 20% in most European countries). The cutaneous form of neosporosis is diagnosed after isolation of tachyzoites in skin biopsies or fine needle aspiration. Differentiation with *Toxoplasma gondii* tachyzoites can be achieved by immunohistochemistry or PCR on skin biopsies.

**Toxoplasmosis**

Cutaneous signs of toxoplasmosis can be directly due to infection of the skin (nodular dermatitis) or secondary (ulcers due to vasculitis, partial seizures mimicking pruritus). Serology is useful since a negative result has a high negative predictive value. However, high IgG levels have no diagnostic value. The diagnosis of toxoplasmosis can be achieved with positive IgM serology and/or isolation of the organism (cytology, histopathology, PCR).



**Figure 5.** Leishmaniasis in a senior Yorkshire Terrier: differential diagnosis include at least epitheliotropic lymphoma, dermatophytosis, leishmaniasis and demodicosis.

## ■ Ectoparasites

### *Sarcoptic mange*

Sarcoptic mange is one of the main causes of violent pruritus and sometimes of chronic or mild pruritus. As the isolation of the organism is often difficult, serology can offer a very useful diagnostic alternative. Serodiagnostic techniques for sarcoptic mange have been developed for swine in Sweden where the disease is endemic.

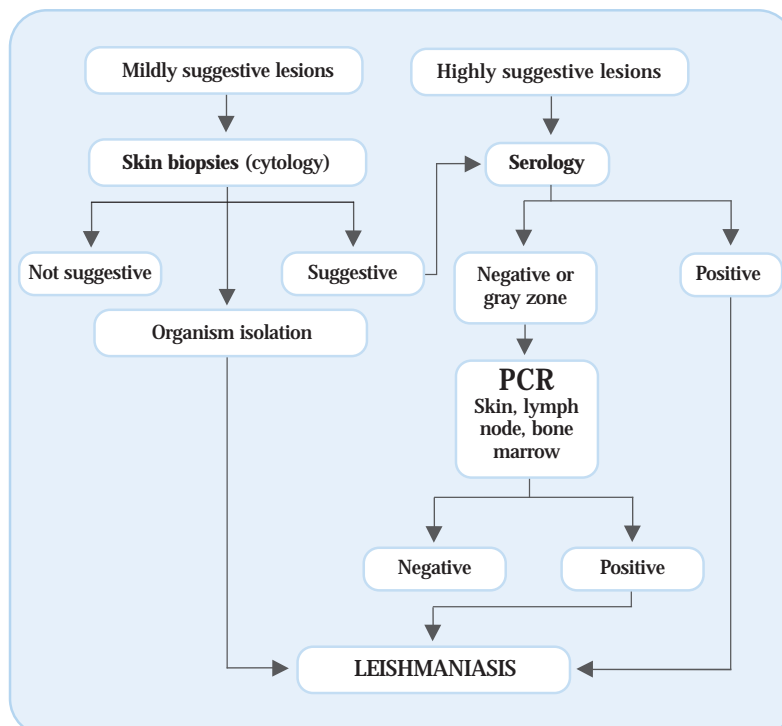
Adaptation of this serology test to the dog led to the commercialization of an ELISA kit. Its sensitivity (85%) and specificity (90%) are high when comparing healthy dogs to dogs with sarcoptic mange (4). However, in practice this test is used in cases of chronic pruritus to exclude a diagnosis of sarcoptic mange (**Figure 7**). These dogs have often been treated with corticosteroids and various acaricidal drugs. In such dogs the efficacy of the technique is unknown and we often observe results in a gray zone. However, even after long term cortico therapy, serology is still positive when there are numerous parasites.

## ■ Immunologic diseases

### *Auto-immune skin diseases*

#### *Antinuclear antibodies*

Antinuclear antibodies are observed at high level in 97 to 100% cases of systemic lupus and this test is the corner stone of the disease (5). Higher titers are observed in most severe cases and decrease during treatment. These antibodies have different antigen specificities (**Table 4**) leading to various aspects of immunofluorescence. Anti-DNA are rare and anti-Sm and anti-type 1 are highly suggestive of systemic lupus.



**Figure 6.** The role of serology and PCR in the diagnosis of cutaneous leishmaniasis in the dog.



**Figure 7.** Sarcoptic mange. If no parasites are isolated, *Sarcoptes scabiei* serology can help define a diagnosis of sarcoptic mange.

The main serologic technique used is IFA using rat liver or Hep 2 cells as substrate (**Figure 8**). Titers over 1/160 are generally considered as high. As antinuclear antibodies can be observed in other diseases with polyclonal stimulation of the immune system (*i.e.* leishmaniasis, dirofilariasis) or in a few healthy dogs (German Shepherd), the diagnosis of systemic lupus relies on the observation of at least 4 diagnostic criteria (**Table 5**).

### ■ Anti-desmoglein antibodies

Auto-antibodies involved in the development of pemphigus foliaceus (anti-desmoglein 1) and pemphigus vulgaris (anti-desmoglein 3) could be used as a diagnostic tool. Different techniques have been developed from IFA on skin or esophagus to highly specific ELISA. However, these techniques are devoted to fundamental laboratory research and the sensitivity of this approach is poor at this time.

### *Auto-immune endocrinopathy*

Anti-thyroxin or thyroglobulin antibodies detection can be interesting in the diagnosis of auto-immune thyroiditis, since it is the main cause of hypothyroidism in the dog. Most techniques are based on ELISA or hemagglutination and are specific for the dog. These tests can help classify hypothyroidism, but are not useful in clinical practice. They cannot be considered for the early diagnosis of hypothyroidism. The main indication of such a serology is the observation of large discrepancies between thyroxinemia and clinical signs: high free T4 level with symptoms of hypothyroidism. Such discrepancies can be due to anti-T4 antibodies when a competitive technique is used. The anti-T4 antibodies of the patient block the labeled antibodies leading to a result showing high FT4 level. In these cases FT4 dosage must be controlled with an equilibrium dialysis technique.

### *Allergic skin diseases*

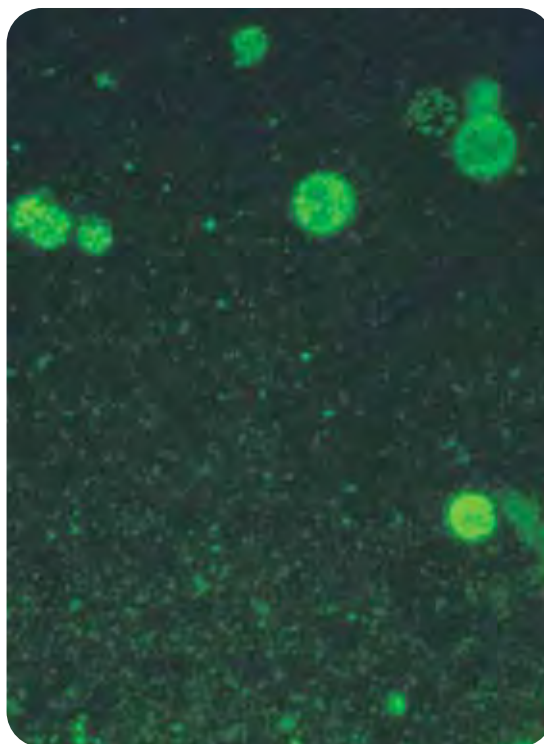
Allergen specific IgE dosage, commonly named *in vitro* allergy testing is widely used. However, its real indications and limits are often unknown and commercial laboratories often promise non-realistic diagnostic performance.

The only indication of allergy testing in dermatology is to choose allergens for aeroallergen-specific immunotherapy in the dog (6). It has no diagnostic value for other allergens (food (7,8)), it

**Table 4.**  
Antinuclear antibodies specificity observed in canine systemic lupus (5)

Total antinuclear antibodies	100%
Anti-DNA	< 3%
Anti-histones	66%
Anti-ENA*	40%
Anti-Sm	16%
Anti-RNP	8%
Anti-type 1	20%
Anti-type 2	9%
Anti-SSA	4%
Anti-SSB	0%
Other antinuclear antibodies	
Anti-HMG 1	6%
Anti-HMG 2	18%

\*ENA : extractible nuclear antigens



**Figure 8.** Positive IFA for antinuclear antibodies on rat liver (dog with systemic lupus erythematosus).

does not allow the diagnosis of allergic disease and it has no known diagnostic value in the cat and horse. Feline IgE levels are in fact identical in healthy cats and cats with allergic skin diseases for all the techniques used (polyclonal anti-IgE, monoclonal anti-IgE or FcεR1) (9,10).

**Table 5.**  
ARA\* criteria adapted to the diagnosis of canine lupus (5)

Criteria	Definition
Erythema	Localized to light exposed skin (face)
Cutaneous lupus	Depigmentation, erythema, ulcers (face: nose, lips eyelids)
Photosensitivity	Worsening of lesions after solar exposure
Oral ulcers	Mouth or pharynx
Arthritis	2 or more articulations with no deformation
Serous inflammation	Pleural or pericardial sterile effusion
Renal signs	<b>a)</b> Proteinuria > 0.5 g/L or <b>b)</b> Cylindruria or microscopic hematuria or hemoglobinuria
Central nervous system signs	<b>a)</b> Seizures or <b>b)</b> Behavioral alterations
Hematological signs	<b>a)</b> Hemolytic anemia with reticulocytosis or <b>b)</b> Leucopenia (< 3 000 /mm <sup>3</sup> ) or <b>c)</b> Lymphopenia (< 1 000 /mm <sup>3</sup> ) or <b>d)</b> Thrombocytopenia (< 100 000 /mm <sup>3</sup> )
Immunological signs	<b>a)</b> Anti-histone antibodies or <b>b)</b> Anti-Sm or anti-type 1 antibodies or <b>c)</b> CD8 + lymphopenia
Antinuclear antibodies	High IFA titers

\*American Rheumatoid Association

In practice the diagnosis of allergic skin diseases is based on epidemiological and clinical criteria and not on the results of allergy testing (**Table 6**) (11). For example, observation of dorsolumbar pruritus and lesions is more efficient to diagnose flea bite allergy than any allergy test!

### ■ Anti-canine IgE

Ig techniques are based on the same principle and utilizes a canine anti-IgE serum (**Figure 9**) with high sensitivity technique (*i.e.* avidin-biotin), since IgE concentration is very low compared to other isotypes. The specificity of the anti-IgE is often discussed, but this is not the main quality criterion of an IgE dosage. In fact, the analytical sensitivity and specificity of the technique does not correlate with diagnostic sensitivity and specificity. For example, monoclonal anti-IgE or human recombinant Fc $\epsilon$ RI (h $\alpha$ Fc $\epsilon$ RI) are reactive with low affinity (12). Since liaison with IgE is poor, it is necessary to use a longer incubation time or less diluted serum. This can increase the risk of unspecific binding and minor

specificity of the technique. Therefore a polyclonal can be as effective as a monoclonal or Fc $\epsilon$ RI reactive in practice. The quality of the technique depends on the way these reactives are used.

### ■ Technical limits

The main difficulties in allergen specific IgE measurement are: the quality of allergenic extracts, the need for controls for each allergen, and the definition of a positive threshold for each allergen. In fact, most laboratories use a negative control (pool of healthy or atopic dog serum with negative skin tests) and a positive control for up to 3 allergens. However, IgE fixation is highly variable depending on each allergen. This is why interpretation is possible for common aeroallergens for which positive control is used, but not for the others.

Aeroallergen specific IgE levels are higher in atopic dogs but there is a large overlap between the healthy and the atopic population. If the threshold of positivity is too low, the technique

**Table 6.**  
Major diagnostic criteria for the diagnosis of atopic dermatitis

1. Age of onset between 6 months and 3 years
2. Steroid sensitive pruritus
3. Bilateral otitis externa
4. Perioral erythema or cheilitis
5. Anterior bilateral pododermatitis

is too sensitive and the positive predictive value of the result is poor. If the threshold is high enough to limit false positive results, the positive predictive value of the result is correct, but in most cases sensitivity is low (70 to 40% depending on the allergens).

### ■ Conclusion

Like any laboratory test, a serological test must be used when a specific disease is suspected. The interpretation will always be difficult when



**Figure 9.** Anti-IgE reagent used in *in vitro* techniques are not the key stone of the quality of the dosage.

serology is used as a screening test evaluated outside of the context of clinical and epidemiological data. Serology must not be abandoned for PCR techniques, since the two are complementary.

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