# SNAP 4Dx Plus Test: Expanded capability, same great performance

## Introduction

Vectors and the diseases they transmit have become increasingly prevalent throughout Europe. A recent publication summarizing over 224.000 SNAP* 4Dx* Plus Test results from 2016–2020 found that dogs are commonly exposed to vector-borne pathogens, such as the tick-borne disease agents *Anaplasma* spp. and *Ehrlichia* spp.[1] The geographic distribution of both arthropod vectors and the pathogens they transmit continues to expand. Selected countries (> n = 1.000) are projected on a map with the proportion positives for *Anaplasma* spp. and *Ehrlichia* spp. (figure 1).

The SNAP 4Dx Plus Test from IDEXX can be used to detect the antigen of *Dirofilaria immitis* and antibodies against *Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Ehrlichia canis* and *Ehrlichia ewingii* in a single whole blood, plasma or serum sample.[2,3] To help veterinarians routinely screen for and more accurately diagnose these vector-borne diseases, IDEXX has improved the SNAP 4Dx Plus Test by adding three new peptides to the existing spots used: species-specific peptides for *A. phagocytophilum* and *A. platys* to the *Anaplasma* spp. spot and an *E. canis* peptide to the *Ehrlichia* spp. spot. These peptides have been previously evaluated in canine experimental infections as well as patient samples using different test platforms.[4,5]

This improved test is thus expected to better meet the needs of practicing veterinarians and their patients and enables them to confidently screen for these tick-borne pathogens. Equally important, the SNAP 4Dx Plus Test helps uncover evidence that dogs have been exposed to multiple infectious organisms either through bites from multiple tick vectors or coinfections carried by the same vector. This helps in diagnosis, treatment and awareness of tick-borne diseases. Further, the test continues to deliver consistent, accurate detection for heartworm antigen and antibodies to the $C_6$ peptide of *B. burgdorferi*, the causative agent for Lyme disease.

Fig. 1

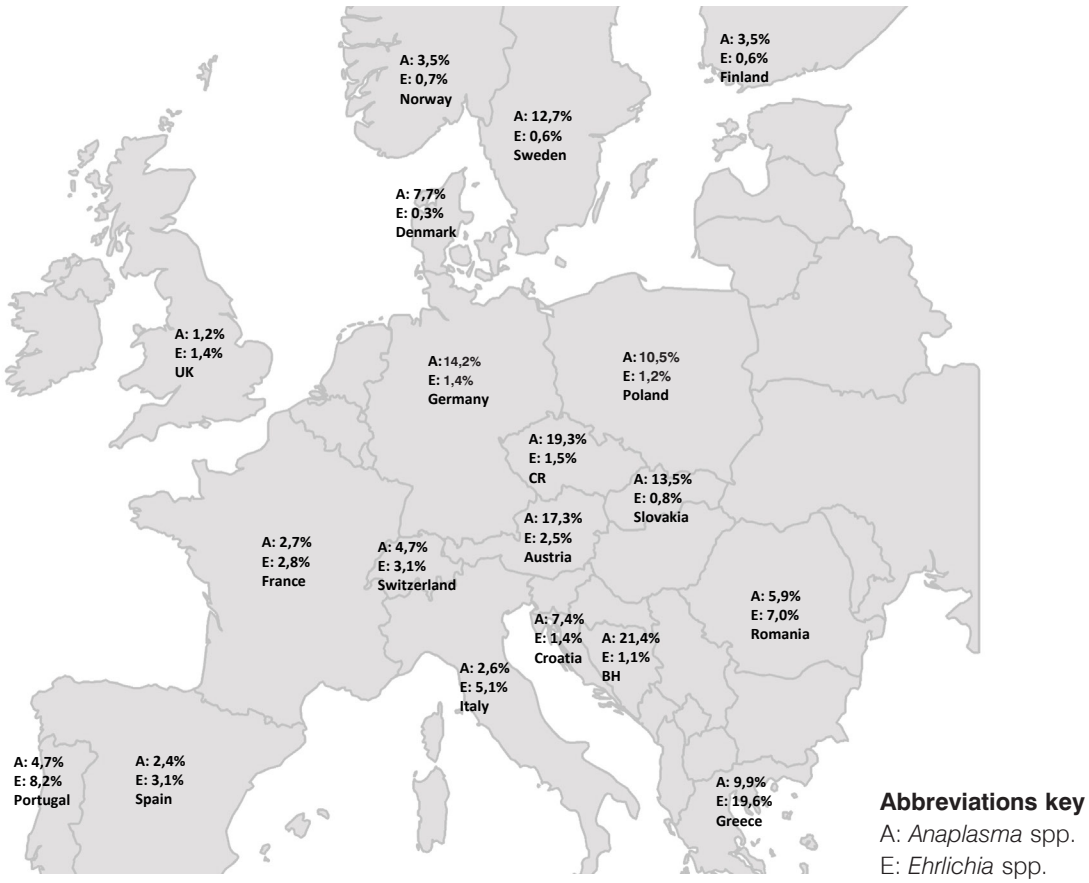


**Figure 1:** *Anaplasma spp*. and *Ehrlichia spp*. seropositivity (%) by country.[1] A: antibodies to *Anaplasma spp*., E: antibodies to *Ehrlichia spp*.; total numbers of tested dogs: Austria (n = 4.572), BH / Bosnia and Herzegovina (n = 3.671), CR / Czech Republic (n = 6.238), Croatia (n = 2.417), Denmark (n = 7.784), Finland (n = 6.084), Germany (n = 20.582), Greece (n = 6.488), France (n = 18.070), Italy (n = 64.879), Norway (n = 3.051), Poland (n = 3.812), Romania (n = 13.995), Slovakia (n = 1.584), Switzerland (n = 1.006), Spain (n = 39.526), Sweden (n = 10.047), Portugal (n = 1.285), UK (n = 2.631).

## Same great performance with enhanced detection of *Anaplasma* spp.

The SNAP* 4Dx* Plus Test continues to exhibit sensitivity and specificity consistent with the performance shown in numerous peer-reviewed publications.[2,3] The additional markers improve the sensitivity and specificity for *Anaplasma* spp. while improving the specificity for *Ehrlichia* spp. detection (see table 1). When the enhancements to *Ehrlichia* spp. and *Anaplasma* spp. detection were evaluated compared to the previous antibody markers on the same sample set (see table 1), the additional antibody markers enabled detection of more positive samples for both *Anaplasma* spp. and *Ehrlichia canis* than previously. In 510 samples evaluated, 21 more antibody-positive samples were detected for *Anaplasma* spp. and 4 more antibody-positive samples were found for *Ehrlichia* spp.

| Analyte | Reference standard | SNAP 4Dx Plus Test result | | Total | Sensitivity (95% CL) |
|---|---|---|---|---|---|
| | | + | – | | Specificity (95% CL) |
| *Dirofilaria immitis*[a] | + | 48 | 1 | 49 | 98.0% (89.1%–99.9%) |
| | – | 0 | 461 | 461 | 100.0% (99.2%–100%) |
| *Anaplasma* spp.[b] | + | 80 | 5 | 85 | 94.1% (86.8%–98.1%) |
| | – | 7 | 418 | 425 | 98.4% (96.6%–99.3%) |
| *Ehrlichia* spp.[c] | + | 99 | 7 | 106 | 93.4% (86.9%–97.3%) |
| | – | 13 | 391 | 404 | 96.8% (94.6%–98.3%) |
| *Borrelia burgdorferi*[d] | + | 21 | 1 | 22 | 95.5% (77.2%–99.9%) |
| | – | 3 | 485 | 488 | 99.4% (98.2%–99.9%) |

**Table 1.** Improved SNAP 4Dx Plus Test versus reference methods.[6]

**Reference methods**

a. Necropsy or PetChek* Heartworm ELISA positive and PetChek* Heartworm ELISA negative

b. *A. phagocytophilum* IFA and *Anaplasma* spp. ELISA

c. *E. canis* IFA and *E. ewingii* ELISA

d. Lyme immunoblot and $C_6$ ELISA

The increased sensitivity of the SNAP 4Dx Plus Test for detection of *Anaplasma* spp. antibodies is also evident in endemic European areas. The overall serum test set (n = 1.604) was obtained from dogs located in UK, France and Spain (convenience samples sent to reference laboratory for biochemistry analysis) as well as Germany and Italy (canine vector-borne disease [CVBD] profiles or hunting dogs, respectively).[6] In these 1.604 European samples, the proportion positives for *Anaplasma* spp. antibodies with the improved version of the test was 18,6% as compared to data recently generated by the SNAP 4Dx Plus Test that ranged from 1,2% in UK to 14,2% for Germany (see figure 1).[1]

In these endemic regions, the increased sensitivity allows veterinarians to uncover dogs that may have vague or no clinical signs at the time of testing, giving them the opportunity to further evaluate for evidence of anaplasmosis. Accurate diagnosis enables timely treatment in clinically affected dogs and helps support discussions with pet owners on tick control and preventative recommendations.

Furthermore, earlier detection of pathogens is important because, with some tick-borne pathogens, acute disease might occur soon after tick attachment. For example, in most dogs, the clinical signs of canine anaplasmosis are nonspecific and confined to the acute phase of the infection.[7] Thrombocytopenia was evident in dogs experimentally infected with *A. platys* or *A. phagocytophilum* within 10 days post-infection.[8,9] Thus, anaplasmosis poses a diagnostic challenge, and early detection is relevant.

## Earlier detection of *Anaplasma phagocytophilum*

Experimental tick infection of 8 young-adult beagles with *A. phagocytophilum* was performed according to Chandrashekar et al.[4] Seroconversion using the improved SNAP* 4Dx* Plus Test preceded antibody detection with the current test in 7/8 dogs by 3–14 days (figure 2).[6]

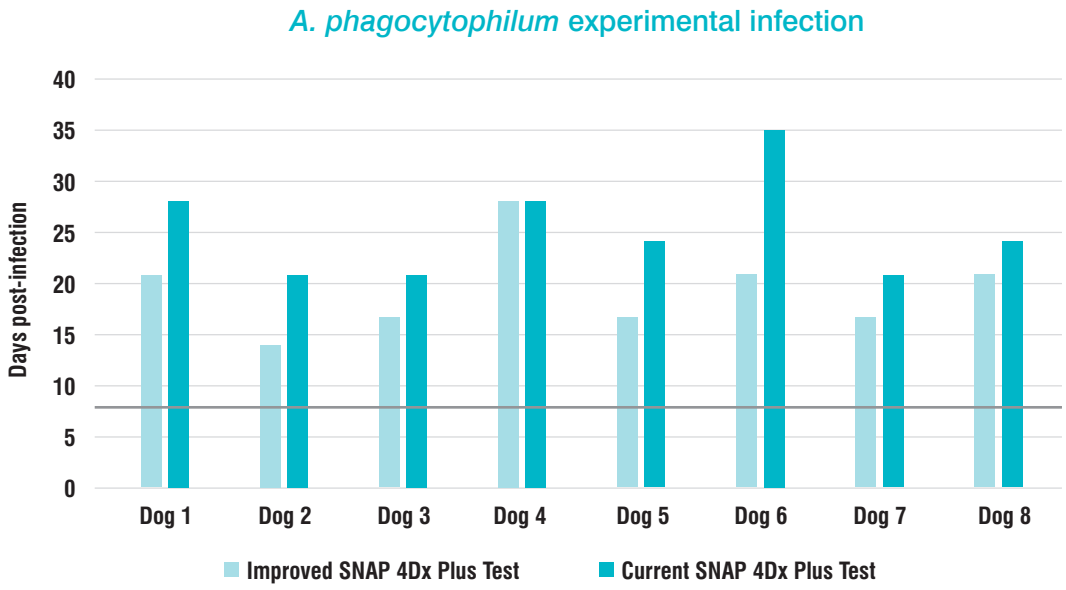


**Figure 2.** *A. phagocytophilum* experimental infection – positive result days post-infection.[6] Horizontal line represents when the dogs were first PCR positive (day 7); difference in seroconversion between improved and current version of the SNAP 4Dx Plus Test is shown using the paired bars on the graph.

## Earlier detection of *Anaplasma platys*

Earlier detection could be shown also for *A. platys* by experimental infection of 6 six-month old, female hound-type dogs that were inoculated intravenously with *A. platys* according to Gaunt et al.[10] Four dogs seroconverted 4–22 days earlier than the current test (figure 3).[6]

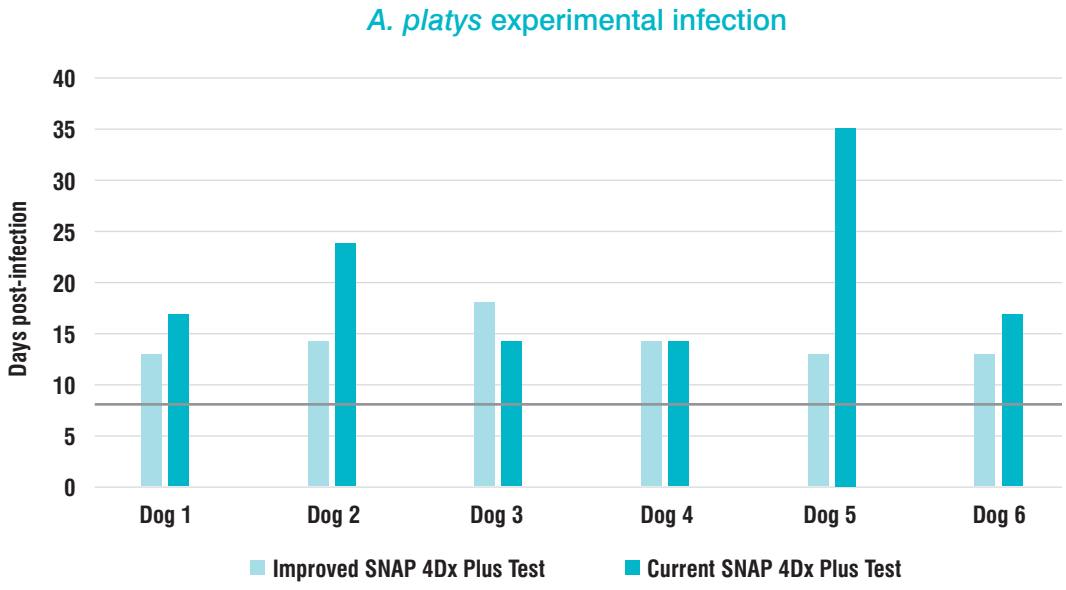


**Figure 3.** *A. platys* experimental infection – positive result days post-infection.[6] Horizontal line represents when the dogs were first PCR positive (day 7 as a median value for the group); difference in seroconversion between improved and current version of the SNAP 4Dx Plus Test is shown using the paired bars on the graph.

## Earlier detection of *Ehrlichia canis*

Experimental infection data has suggested improved alignment with the SNAP* 4Dx* Plus Test and PCR-positive dogs at the onset of clinical symptoms for post-infection detection of *E. canis*. Of 6 dogs experimentally infected with *E.* canis (tick infestation; *Rhipicephalus sanguineus*), 3 dogs had detectable antibodies for *E.* canis that correlated with the onset of observable clinical signs and were PCR positive.[6] The remaining 3 dogs were antibody positive within 6 days following onset of clinical signs and PCR-positive results (figure 4). This gives veterinarians the potential for an earlier diagnosis in acutely ill dogs.

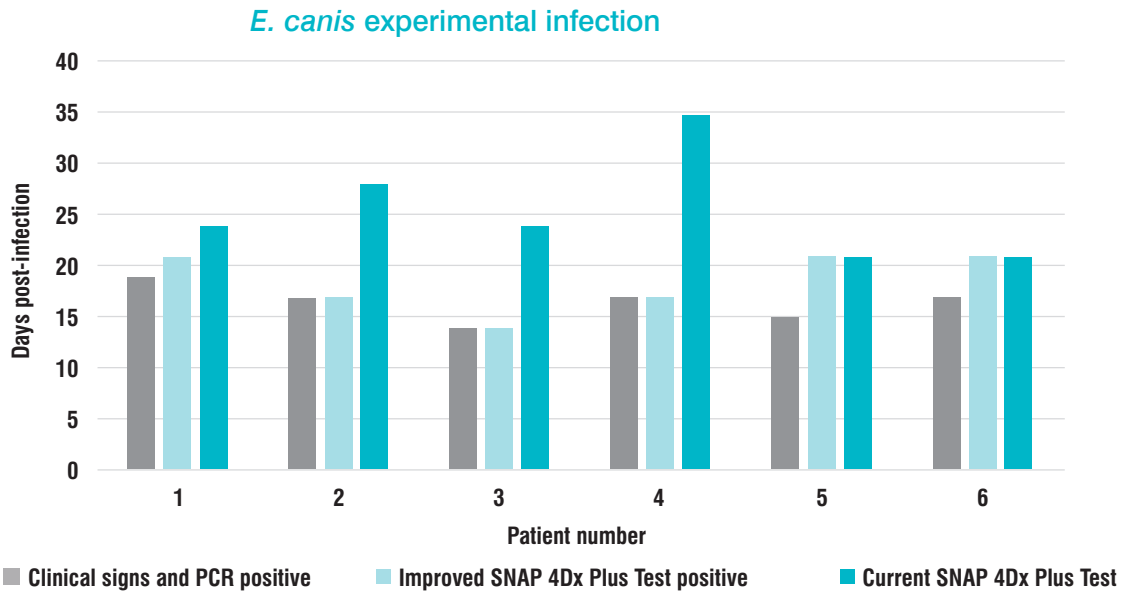


**Figure 4.** *E. canis* experimental infection – positive result days post-infection.[6]

### *Anaplasma platys:* challenges and issues in different geographical regions

*A. platys* was first identified in 1978 in dogs from Florida, and thereafter reported from several regions around the world. In Australia and the United States, it can be a subclinical or asymptomatic disease but in other areas, e.g., South America or Southern Europe (Croatia, France, Greece, Cyprus, Italy, Portugal, Romania and Spain), Northern Africa, Israel and Asia, this agent can cause a severe disease.[11] Moreover, a high proportion of dogs (approximately 62%) were considered as nonresponders to treatment.[11] The reasons for this different clinical presentation are not completely understood and could include different genetic strains, coinfections or other individual factors as concurrent diseases, genetic factors, immune status, physical condition, etc. As for *A. phagocytophilum*, a favorable response to treatment in dogs is accepted.[12] Therefore, special attention should be given to compare these two *Anaplasma* species as they are both relevant to Europe but differ in several aspects.

### *Anaplasma* species and coinfections

In respect to its distribution within Europe, *A. platys* is prevalent in the Mediterranean area, where its tick vector *R. sanguineus* is common, and *A. phagocytophilum* is prevalent across the continent with high endemic areas where favorable conditions for its vector *Ixodes ricinus* are present (see figure 5). Co-infections with either *B. burgdorferi* (with *A. phagocytophilum*) or *E. canis* (with *A. platys*) are reflecting the exposure to these two ticks and have important clinical and laboratory implications.[13,14] *Anaplasma* spp. and *B. burgdorferi* showed highest prevalence and co-positivity proportion in Northern Europe (figure 5a). A co-exposure as seen on the SNAP 4Dx Test (figure 5b) in this region most likely reflects exposure to *A. phagocytophilum* based on their common tick vector *Ixodes ricinus*. Antibodies to *Ehrlichia* spp. were most common for Southern Europe followed by antibodies to *Anaplasma* spp. (figure 5a). The co-positivity to *Anaplasma* spp. and *Ehrlichia* spp. (figure 5b) were encountered most frequently in this region, and it most probably represents co-exposure to *E. canis* and *A. platys* considering that they use the same tick vector (*R. sanguineus*), which is the most common tick in this region (figure 5c).


**Figure 5.** a) A total of 108.803 canine samples were tested by veterinarians with SNAP* 4Dx* Test in Europe during 2011–2015 (Northern Europe, 15 countries, n = 51.357) versus Southern Europe, 13 countries, n = 57.446);[15] b) 2 SNAP 4Dx tests as examples of co-exposure with either *B. burgdorferi* and *Anaplasma* spp. (left) or *Ehrlichia* spp. and *Anaplasma* spp. (right); c) approximate European distribution of the brown dog tick *R. sanguineus* (darker green color),[16] images of *Ixodes ricinus* (top) and *Rhipicephalus sanguineus* (bottom) are projected within their preferred climatic zones on the map.

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