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Topical Review

SNAP Assay Technology

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The most widely used immunoassay configuration is the enzyme-linked immunosorbent assay (ELISA) because the procedure produces highly sensitive and specific results and generally is easy to use. By definition, ELISAs are immunoassays used to detect a substance (typically an antigen or antibody) in which an enzyme is attached (conjugated) to one of the reactants and an enzymatic reaction is used to amplify the signal if the substance is present. Optimized ELISAs include several steps that are performed in sequence using a defined protocol that typically includes application of sample and an enzyme-conjugated antibody or antigen to an immobilized reagent, followed by wash and enzyme reaction steps. The SNAP assay is an in-clinic device that performs each of the ELISA steps in a timed sequential fashion with little consumer interface. The components and mechanical mechanism of the assay device are described. Detailed descriptions of features of the assay, which minimize nonspecific binding and enhance the ability to read results from weak-positive samples, are given. Basic principles used in assays with fundamentally different reaction mechanisms, namely, antigen-detection, antibody-detection, and competitive assays are given. Applications of ELISA technology, which led to the development of several multianalyte SNAP tests capable of testing for up to 6 analytes using a single-sample and a single-SNAP device are described.

Introduction

The enzyme-linked immunosorbent assay (ELISA) is one of the most sensitive and specific diagnostic technologies available. ELISA is the method of choice in reference laboratories worldwide because of its unparalleled accuracy and ease of use. The SNAP assay device contains reagents needed to conduct the steps associated with the ELISA in-clinic with minimal hands-on time. The SNAP assay was developed by scientists at IDEXX Laboratories to overcome limitations of other clinic-based assays, which included first generation ELISA-, colloidal gold-, and latex particle–based tests. The SNAP product combines the simplicity and immediate results of an in-office test with the accuracy of a reference laboratory-format ELISA.

The SNAP assay is by far the market leader in veterinary office diagnostics—approximately 19 million SNAP devices are run by veterinarians each year.[1,2] Since the introduction of the heartworm antigen SNAP assay in the early 1990s, the number of assays using SNAP technology has expanded and currently includes over 20 different assays across a variety of assay formats for use in widely different applications using diverse sample types (whole blood, serum, plasma, feces, and milk). These include assays for detection of antigens and antibodies typically associated with the diagnosis of infectious diseases, the quantification of metabolic and cardiac hormone levels, and tests for antibiotic drug-residue levels in milk. In-clinic SNAP assays designed for use as point-of-care tests in veterinary clinics are described in this article.

Features of the SNAP Assay

ELISA Format

The SNAP assay is an example of an ELISA. By convention, ELISAs are immunoassays, which contain an enzyme-labeled antibody or antigen (generally referred to as a conjugate) and typically include an antigen- or antibody-binding step, a wash step, and a color-generating step that involves enzymatic reaction to produce a colored reaction product. Each of the basic steps in the ELISA procedure has been incorporated in the SNAP assay. The SNAP assay was developed to provide timed, automatic, and sequential flow of sample, conjugate, and wash, substrate reagents in a simple easy-to-use device that can be run in clinic.[1]

Basic SNAP Assay Procedure

The SNAP assay device is shown in Fig 1. Procedurally, an enzyme-labeled conjugate is mixed with serum, plasma, or whole blood in a tube and added to the sample well of the SNAP device. The sample-conjugate mixture flows through the matrix, interacts with test and control spots deposited on the matrix, and reaches the activation circle in approximately 30-60 seconds. The device is then activated (by depressing or "snapping" the activator), which results in the release of wash buffer and substrate solution from reagent reservoirs contained within the device. Positive results are visualized by the formation of colored reaction products; the assay is complete in 6-10 minutes depending on the test. The development of color in the positive control indicates that the assay reagents are functional.[1]

Detailed SNAP Assay Mechanism

The SNAP device architecture and its functional mechanisms are shown in Fig 2. The basic steps in the ELISA procedure are fully described.

Initial Antigen or Antibody Conjugate Reaction

The conjugate is an antibody or antigen covalently attached to the enzyme horseradish peroxidase (HRPO). The conjugate

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Fig. 1. The SNAP assay device.[1]

reagent is mixed with the sample in a sample tube before application to the SNAP device (Fig 2.1). The conjugate reagent reacts specifically with the target analyte in the sample (if present) and forms an HRPO-labeled antigen-antibody complex.

Flow Matrix and Sample Flow

The flow matrix is a brilliant white porous polyethylene material, which is the centerpiece of the SNAP device. Operationally, sample, conjugate reagent, wash buffer, and substrate solution all flow through the polyethylene matrix. Immobilized reagents are applied to the matrix in separate areas as test and control spots in a pattern that allows reactions to occur independently in a reagent-specific fashion (Fig 2.2).

The sample-conjugate reagent mixture applied to the sample well flows through the matrix and reaches the test spots (Fig 2A). HRPO-labeled antigen-antibody complexes (if present) bind to the antigen or antibody reagents deposited in these spots to form immune complexes (Fig 2.3).

Device Activation and Bidirectional Flow

The SNAP device is manually activated (or automatically with a SNAP Pro Mobile Device) when the flow of the sample-conjugate mixture reaches the activation circle. Activation of the SNAP device initiates additional steps in the ELISA procedure as described below:

(1) Separate wash buffer and substrate solution reservoirs are punctured; wicks are forced into contact with the matrix and direct the flow of wash buffer and substrate solution to the matrix.
(2) The sample well end of the matrix is forced into contact with an absorbent block that absorbs liquid and reverses the flow of liquid through the matrix.

Activation of the SNAP device initiates the reverse flow process —the flow of wash buffer (Fig 2B) and substrate solution (Fig 2C) from the reagent reservoirs through the matrix to the absorbent block in the opposite direction of the initial flow of sample and conjugate reagent.

Wash Step

The wash buffer is a solution containing detergent, nonspecific protein, and preservatives. The wash buffer removes unbound debris and unreacted conjugate reagent from the matrix and produces a clean, untarnished background so that results can be easily interpreted (Fig 2.5).

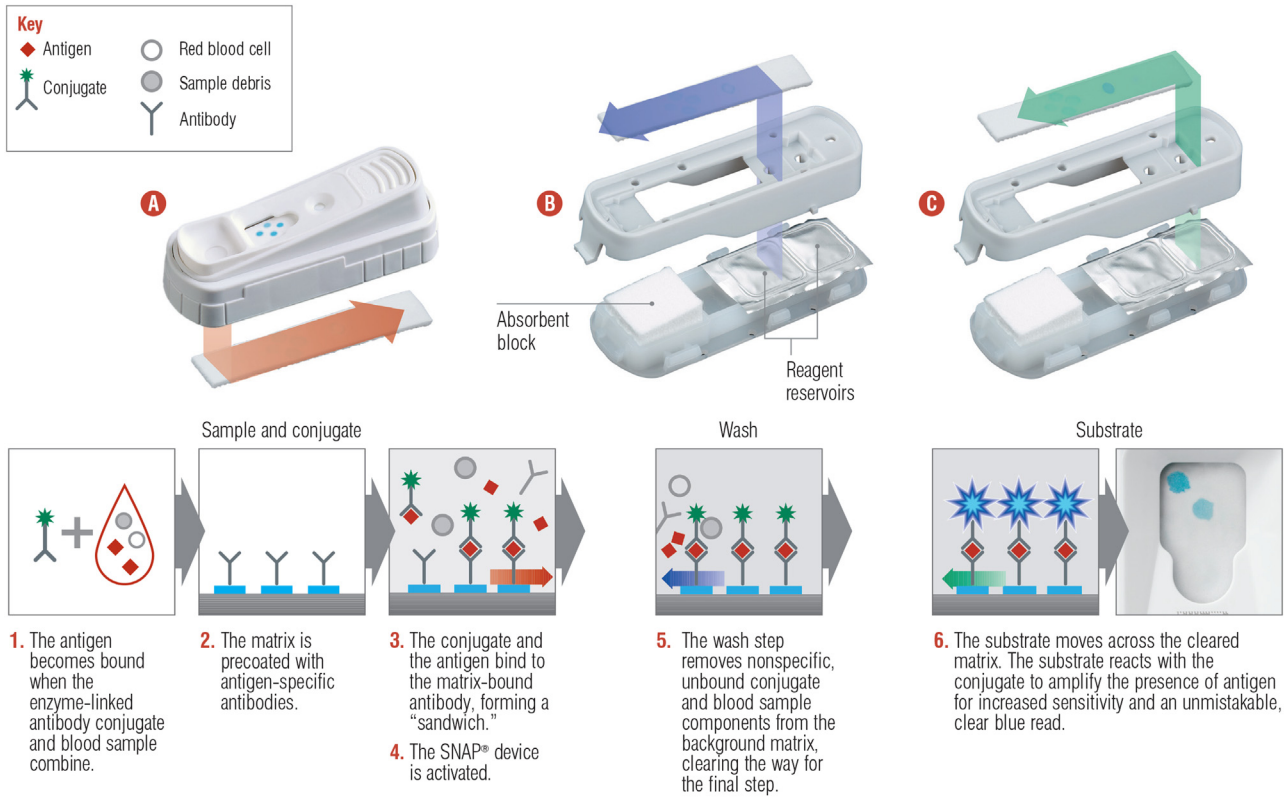


Fig. 2. Detailed mechanism of the SNAP device. Reaction mechanism is shown for an antigen-detection assay using immobilized antibody and an antibody-HRPO conjugate. An antibody-detection assay would be similar but would use immobilized antigen and an antigen-HRPO conjugate.

Substrate Step

The substrate solution used in the SNAP assay contains TMB (3,3', 5,5'-tetramethylbenzidine). This is a precipitating substrate solution—upon reaction mediated by the enzyme HRPO, the substrate turns distinct blue in color and becomes insoluble. This signifies and marks the location of positive results (Fig. 2.6).

Advantages of Bidirectional Flow of Wash and Substrate

A schematic representation of the reverse flow process showing spot placement and reagent flow is given in Fig 3. Sample-conjugate reagent mixture is applied to one end of the flow matrix and flows in the forward direction, whereas wash buffer and substrate solution are applied to the opposite end (when the device is activated) and flow in the reverse direction. The flow of wash buffer and substrate solution in the reverse orientation is very efficient and has several advantages:

(1) Wash buffer interacts directly with the test spots in the read window and does not pass through unreacted conjugate and sample debris in the vicinity of the sample well; this reduces the amount of unbound material that needs to be washed off the matrix and minimizes background coloration.
(2) The time needed to wash the matrix is reduced.
(3) The sample-conjugate reagent mixture is exposed to the solid phase reagents in both forward and reverse directions, which increases contact time and offers a second opportunity to bind with the test spots.
(4) The wash buffer and substrate solution are contained in separate reservoirs and flow through the matrix in a tandem fashion. The wash buffer and substrate solution do not mix, a frontal boundary forms between the reagents as they migrate through the matrix—nonspecifically bound components and unreacted conjugate reagent are removed by washing before exposing the matrix to substrate. This reduces nonspecific background and enhances the ability to read results.

The wash step, and particularly its reverse flow orientation, is a key element of SNAP assay technology. Notwithstanding the efficiency of the wash reagent itself, the reverse flow orientation and the sequential flow of wash and substrate are largely responsible for the almost pristine white background observed in the SNAP assay.

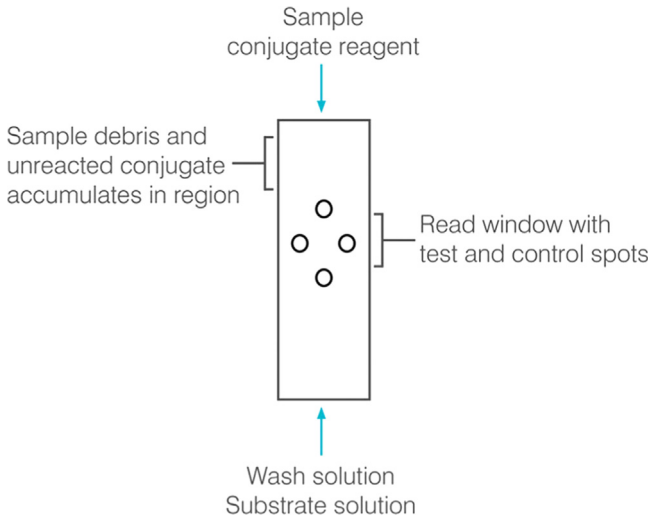


Fig. 3. Schematic representation of the SNAP flow matrix showing the flow of the sample-conjugate reagent in the forward direction and the reverse flow orientation of the wash buffer and substrate solution.

Comparison of the SNAP Assay to Other In-Clinic Technologies

Several lateral-flow immunoassays have been developed for use in veterinary clinics that use colloidal gold or colored latex particles attached to diagnostic reagents in place of the enzyme conjugates used in ELISA. The accumulation of gold or colored latex particles on the diagnostic test spot or line signifies a positive result.

The SNAP ELISA has several advantages over the colloidal gold- and latex particle–based lateral-flow assays. As described previously, SNAP ELISA uses reverse directional flow, a wash step to eliminate nonspecific binding, an enzymatic reaction step to amplify signal development, and a distinct blue-colored reaction product that is easy to observe against the background of the white-colored matrix. A practical example that illustrates the advantages of the wash and enzyme reaction steps is found when using hemolyzed samples. Results for hemolyzed samples can be difficult to interpret using colloidal gold-format assays because of red background coloration on the flow matrix, which makes it difficult to observe color development on the test line. In the SNAP ELISA, there is little or no background color, and blue-colored positive results are easily interpreted because of the high color contrast between spot and sample color. Fig 4 shows results of a colloidal gold-format assay and SNAP ELISA using a hemolyzed weak heartworm antigen-positive sample. In this case, the result is readily observable in the SNAP ELISA but very difficult to detect in the colloidal gold-format assay. Differences in the SNAP assay and lateral-flow assay are summarized in Table 1. Studies have shown sensitivity limitations for several colloidal gold-format lateral-flow assays.[3] The SNAP-format ELISA was shown to be more sensitive than colloidal gold lateral-flow assays for feline leukemia virus antigen[4] and detection of antibody to tick-borne agents (*Anaplasma* spp., *Ehrlichia* spp., and *Borrelia burgdorferi*).[5,6]

Diversity of SNAP Assay Applications

Antigen and Antibody-Detection Assays

Assays with fundamentally different reaction mechanisms, namely antigen- and antibody-detection, can be performed using the SNAP device. Antibodies and antigens can be immobilized on the matrix or conjugated to HRPO. An antibody pair (immobilized antibody and conjugated antibody) is used in antigen-detection assays and an antigen pair (immobilized antigen and conjugated antigen) is used in antibody-detection assays. Fig 5 is a representation of the immune

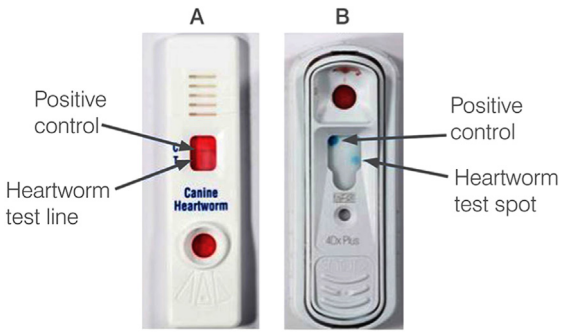


Fig. 4. Assay devices obtained following testing of a hemolyzed known heartworm antigen-positive sample using a commercially available colloidal gold-format lateral-flow assay (A) and the SNAP 4Dx Plus ELISA (B).

Table 1.
Comparison of SNAP Assay to Colloidal Gold Lateral-Flow Assay

Assay Step	Colloidal Gold-Based Lateral-Flow Assays	SNAP Assay
Wash step	No wash step	Wash step removes unbound sample components and unreacted conjugate before addition of substrate.
Flow orientation	Unidirectional flow	Bidirectional flow of sample and wash or substrate provides a second chance of binding and eliminates nonspecific color development.
Mechanism of signal generation	Accumulation of gold particles	Enzymatic signal amplification
Color of result	Read result may be difficult to interpret especially with whole blood samples	Distinct blue dot enhances ability to read result

complexes formed on the matrix following antigen- and antibody-detection assays. These assays take place independently of each other and can be carried out on the same SNAP assay device.

Multianalyte Combination Assays

Several SNAP-format combination assays have been developed, which test for several analytes in a single-assay step. The reagents used in these combination assays are highly optimized and are analyte-specific—reagents used in one assay do not cross-react with the other assays on the same device. One or more antigen- or antibody-detection assay can be performed simultaneously using the same assay device from a single-sample aliquot. This versatility has led to a number of combination assays that are widely used in veterinary medicine to test for several analytes simultaneously and have revolutionized in-office diagnostic testing.

The wash step and bidirectional flow of sample and reagents are key features of the SNAP assay needed to produce clean backgrounds in multianalyte assays. Traditional combination assays require the use of multiple conjugates that increases the conjugate load and can result in nonspecific coloration of the matrix. This can make it difficult to distinguish nonspecific background from color produced by analyte-specific reactions. In the SNAP combination assays, sample debris and unreacted conjugate are efficiently removed from the matrix, which results in a clean white background so that false-positive results due to nonspecific reactions are almost nonexistent and weak-positive results can be easily detected.

The initial SNAP-format assay was a single-analyte assay for heartworm (*Dirofilaria immitis*) antigen, which was introduced in 1992. Since that time, additional analytes have been added to the original test in an incremental fashion—starting with the combination assay for heartworm antigen and *Ehrlichia canis* antibody and leading to the SNAP 4Dx Plus test (2012) that added assays for antibodies to 4 additional tick-borne agents. Fig 6 gives brief details on the IDEXX SNAP point-of-care tests; additional information is available on the IDEXX Website.[7]

Semiquantitative SNAP Assays

Several SNAP-format semiquantitative assays have been developed, which enable quantitative measurement of analyte levels in patient's samples. The assays contain a reference spot and a sample spot, which binds antigen—results are determined by comparing the intensity of color in the patient sample spot to the reference spot. Relative color intensity can be assessed visually or with an automated SNAP assay reader that electronically measures and compares the optical differences of the 2 spots.[8]

Pancreatic Lipase SNAP Test

There are 2 semiquantitative SNAP-format assays (SNAP cPL and SNAP fPL) that have been designed to provide a measure of the immunoreactive pancreatic-specific lipase in canine and feline serum samples. The tests use monoclonal antibodies that react with different epitopes of the canine and feline pancreatic lipases as conjugate and matrix reagents. The pancreatic lipase present in the sample binds to the conjugate antibody and the capture antibody. The SNAP result shows a blue-colored reaction product that is proportional to the amount of pancreatic lipase in the sample.[9]

SNAP Feline proBNP Test

The SNAP Feline proBNP test provides a semiquantitative measurement of the concentration of a breakdown product of BNP (brain natriuetic peptide) referred to as NT-proBNP. The test uses antibodies as conjugate and matrix reagents. The sample is mixed with the antibody conjugate that binds NT-proBNP and

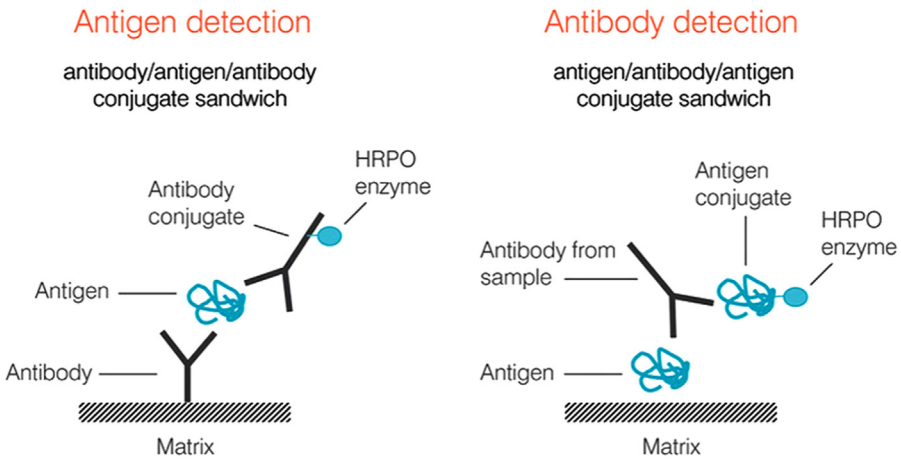
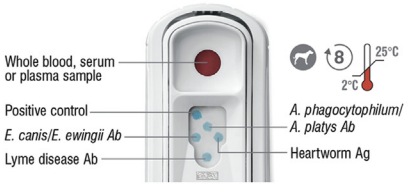


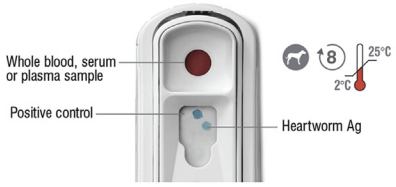
Fig. 5. Representation of the immune complexes formed on the SNAP flow matrix following antigen- and antibody-detection assays.

Interpreting results

Infectious Disease Tests



SNAP® 4Dx® Plus—Screen dogs annually for six vector-borne diseases.

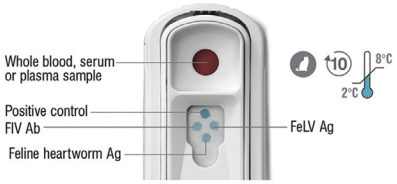


SNAP® Heartworm RT—Screen annually for canine heartworm.

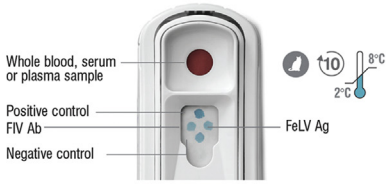
KEY

- Species
- 8 Development time in minutes.
- Store at room temperature or in refrigerator.
- Store in refrigerator.

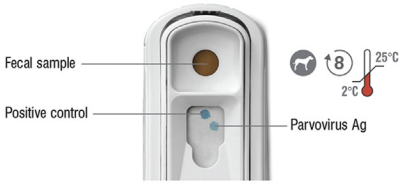
ALL components MUST be at room temperature before running the test.



SNAP® Feline Triple®—Test all new to household, sick and at-risk cats and cats with respiratory or vomiting symptoms.



SNAP® FIV/FeLV Combo—Test all new to household, at-risk and symptomatic cats.



SNAP® Parvo—Test dogs that present with sudden onset of lethargy, vomiting, fever and diarrhea.

SNAP® *Giardia*—Test dogs and cats that present with diarrhea.

SNAP® Lepto—Test dogs when leptospirosis is suspected.

Pancreatic Tests

Abnormal
Sample spot is the same color or darker than reference spot.

Normal
Sample spot is lighter than reference spot.

SNAP® cPL™—Test dogs that present with vomiting, anorexia and/or abdominal pain.
SNAP® fPL™—Test cats that present with lethargy, decreased appetite, dehydration, weight loss, vomiting and/or diarrhea.

Cardiac Tests

Abnormal
Sample spot is the same color or darker than reference spot.

Normal
Sample spot is lighter than reference spot.

SNAP® Feline proBNP—Test cats at risk for cardiac disease (murmur, breed, age).

Fig. 6. IDEXX SNAP point-of-care tests.

then reacts with antibody immobilized on the matrix. Upon completion of the SNAP assay, a blue-colored reaction product is formed that is proportional to the concentration of NT-proBNP in the sample.[8]

SNAP Device Automation

IDEXX Laboratories has produced several instruments that aid in running and recording SNAP assay results.

SNAPshot Dx Analyzer

The SNAPshot Dx Analyzer (Fig 7) is an advanced optical system that allows the user to load certain SNAP devices immediately following the activation step. The analyzer quickly and efficiently reads the SNAP test result at the appropriate time, interprets SNAP test results consistently and accurately, and integrates the results into the patient record and invoice. The SNAPshot Dx Analyzer connects to the IDEXX VetLab Station, which provides a comprehensive picture of the patient's health.

SNAP Pro Mobile Device

The SNAP Pro Mobile Device (Fig 8) automatically activates the SNAP device at the appropriate time following sample application, properly times the run and captures an image of the final SNAP result. The final result can be stored electronically and displayed on the SNAP Pro Mobile screen. The SNAP Pro Mobile Device connects wirelessly to the IDEXX VetLab Station and ensures that all SNAP test results are recorded in the patient record and entered into the customer record for billing.

SNAP Device Studies

The accuracy of SNAP-format assays has been commonly accepted; the SNAP assay is the most frequently used in-clinic diagnostic platform worldwide and has become the standard of comparison for veterinary in-clinic assays.[1] The results of a large number of studies have been published to validate individual SNAP assays and to report infection rates in small regional and large national studies.

Fig. 7. IDEXX SNAPshot Dx Analyzer.

Fig. 8. IDEXX SNAP Pro Mobile Device.

The following is a list of recent publications that describe the use of SNAP assays: SNAP 4Dx Plus,[10-13] SNAP 4Dx,[14-16] SNAP 3Dx,[17-19] SNAP Heartworm RT,[20,21] SNAP Feline Triple,[22] SNAP FIV/FeLV Combo,[23-25] SNAP FIV/FeLV Combo Plus,[26] SNAP Parvo,[27-29] SNAP *Leishmania*,[30-32] SNAP *Giardia*,[33] SNAP Lepto,[34,35] SNAP cPL,[9] SNAP fPL,[9] and SNAP Feline Pro BNP,[8].

References

1. OConnor T, Lawrence J, Andersen P, Leathers V, Workman E. Immunoassay applications in veterinary diagnostics. In: Wild D, editor. *The Immunoassay Handbook*. 4th ed. Great Britan: Elsevier; 2013. p. 623
2. IDEXX Laboratories Inc Trust your results with ELISA technology 2015 [cited 16.10.15. Available at: https://www.youtube.com/watch?v=pscgOZerxhg&list=PLzewGG_yZcVkHxkdf9XmzjosVgSwPvYQ6&index=2.
3. Thatcher B, Beall M, Liu J, Goldstein R, Chandrashekar R. Performance of the Anigen Rapid Caniv-4 Test Kit using characterized canine samples. *J Vet Intern Med* **29:**1209, 2015
4. Thatcher B, Liu J, Bewsey H, Beall M, OConnor T, Chandrashekar R. Evaluation of three in-clinic serological tests for specific detection of FeLV antigen in cats. 58th American Association of Veterinary Laboratory Diagnosticians; Oct 22, 2015; Providence, RI.
5. Thatcher B, Liu J, Andrews B, Breitschwerdt E, Beall M, Chandrashekar R. Comparative evaluation of two rapid in-clinic serological assays for detection of antibodies to canine monocytic and granulocytic ehrlichiosis. 27th Meeting of the American Society for Rickettsiology; June 20, 2015; Lake Tahoe, CA.
6. Thatcher B, Liu J, Bewsey H, Beall M, Chandrashekar R. Comparative evaluation of in-clinic tests for antibodies to Anaplasma and Ehrlichia species in dogs. 58th American Association of Veterinary Laboratory Diagnosticians; October 22, 2015; Providence, RI.
7. IDEXX Laboratories Inc. IDEXX Products and Services 2015. Available at: https://www.idexx.com/corporate/products-and-services/products-and-services.html.
8. Machen MC, Oyama MA, Gordon SG, et al. Multi-centered investigation of a point-of-care NT-proBNP ELISA assay to detect moderate to severe occult (pre-clinical) feline heart disease in cats referred for cardiac evaluation. *J Vet Cardiol* **16:**245–255, 2014. http://dx.doi.org/10.1016/j.jvc.2014.09.002 [PubMed PMID: 25456274]

9. Xenoulis PG, Steiner JM. Canine and feline pancreatic lipase immunoreactivity. *Vet Clin Pathol* **41:**312–324, 2012. http://dx.doi.org/10.1111/j.1939-165X.2012.00458.x [PubMed PMID: 22861648]
10. Little SE, Beall MJ, Bowman DD, Chandrashekar R, Stamaris J. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in the United States, 2010-2012. *Parasit Vectors* **7:**257, 2014. http://dx.doi.org/10.1186/1756-3305-7-257 [PubMed PMID: 24886589; PubMed Central PMCID: PMCPMC4057565]
11. Goldstein RE, Eberts MD, Beall MJ, Thatcher B, Chandrashekar R, Alleman AR. Performance comparison of SNAP® 4Dx® plus and AccuPlex®4 for the detection of antibodies to *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. *Int J Appl Res Vet Med* **12:**141–147, 2014
12. Starkey LA, Barrett AW, Chandrashekar R, et al. Development of antibodies to and PCR detection of *Ehrlichia* spp. in dogs following natural tick exposure. *Vet Microbiol* **173:**379–384, 2014. http://dx.doi.org/10.1016/j.vetmic.2014.08.006 [PubMed PMID: 25213230]
13. Stillman BA, Monn M, Liu J, et al. Performance of a commercially available in-clinic ELISA for detection of antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* and *Dirofilaria immitis* antigen in dogs. *J Am Vet Med Assoc* **245:**80–86, 2014. http://dx.doi.org/10.2460/javma.245.1.80 [PubMed PMID: 24941391]
14. Chandrashekar R, Mainville CA, Beall MJ, et al. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* and *Dirofilaria immitis* antigen in dogs. *Am J Vet Res* **71:**1443–1450, 2010. http://dx.doi.org/10.2460/ajvr.71.12.1443 [PubMed PMID: 21117995]
15. Murdock JH, Yabsley MJ, Little SE, et al. Distribution of antibodies reactive to *Borrelia lonestari* and *Borrelia burgdorferi* in white-tailed deer (*Odocoileus virginianus*) populations in the Eastern United States. *Vector Borne Zoonotic Dis* **9:**729–736, 2009. http://dx.doi.org/10.1089/vbz.2008.0144
16. Little SE, O'Connor TP, Hempstead J, et al. *Ehrlichia ewingii* infection and exposure rates in dogs from the southcentral United States. *Vet Parasitol* **172:**355–360, 2010. http://dx.doi.org/10.1016/j.vetpar.2010.05.006 [PubMed PMID: 20541322]
17. O'Connor TP, Hanscom JL, Hegarty BC, Groat RG, Breitschwerdt EB. Comparison of an indirect immunofluorescence assay, western blot analysis, and a commercially available ELISA for detection of *Ehrlichia canis* antibodies in canine sera. *Am J Vet Res* **67:**206–210, 2006. http://dx.doi.org/10.2460/ajvr.67.2.206 [Epub 2006/02/04. PubMed PMID: 16454622]
18. O'Connor T, Esty KJ, MacHenry P, Hanscom JL, Bartol BA, Lawton T. Performance evaluation of *Ehrlichia canis* and *Borrelia burgdorferi* peptides in a new *Dirofilaria immitis* combination assay. In: Seward RL, editor. *Recent Advances in Heartworm Disease: Symposium '01*. Batavia, IL: American Heartworm Society; 2001. p. 77–84
19. Levy S, O'Connor TP, Hanscom JL, Shields P. Utility of an in-office C6 ELISA test kit for determination of infection status of dogs naturally exposed to Borrelia burgdorferi. Vet Ther **3**:308-315, 2002. [Epub 2002/11/26. PubMed PMID: 12447839].
20. Lee AC, Bowman DD, Lucio-Forster A, Beall MJ, Liotta JL, Dillon R. Evaluation of a new in-clinic method for the detection of canine heartworm antigen. *Vet Parasitol* **177:**387–391, 2011. http://dx.doi.org/10.1016/j.vetpar.2010.11.050 [PubMed PMID: 21211910]
21. Atkins CE. Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. *J Am Vet Med Assoc* **222:**1221–1223, 2003 [PubMed PMID: 12725308]
22. Ortega-Pacheco A, Aguilar-Caballero AJ, Colin-Flores RF, Acosta-Viana KY, Guzman-Marin E, Jimenez-Coello M. Seroprevalence of feline leukemia virus, feline immunodeficiency virus and heartworm infection among owned cats in tropical Mexico. *J Feline Med Surg* **16:**460–464, 2014. http://dx.doi.org/10.1177/1098612X13509995
23. Levy JK, Scott HM, Lachtara JL, Crawford PC. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. *J Am Vet Med Assoc* **228:**371–376, 2006. http://dx.doi.org/10.2460/javma.228.3.371 [Epub 2006/02/02. PubMed PMID: 16448357]
24. Little S, Sears W, Lachtara J, Bienzle D. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in Canada. *Can Vet J* **50:**644–648, 2009[PubMed PMID: 19721785; PubMed Central PMCID: PMCPMC2684053]
25. Lee IT, Levy JK, Gorman SP, Crawford PC, Slater MR. Prevalence of feline leukemia virus infection and serum antibodies against feline immunodeficiency virus in unowned free-roaming cats. *J Am Vet Med Assoc* **220:**620–622, 2002 [PubMed PMID: 12418520]
26. Hartmann K, Griessmayr P, Schulz B, et al. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. *J Feline Med Surg* **9:**439–445, 2007. http://dx.doi.org/10.1016/j.jfms.2007.04.003 [pii: S1098-612X(07)00093-9; Epub 03.07.07; PubMed PMID: 17604205]
27. Decaro N, Desario C, Beall MJ, et al. Detection of canine parvovirus type 2c by a commercially available in-house rapid test. *Vet J* **184:**373–375, 2010. http://dx.doi.org/10.1016/j.tvjl.2009.04.006 [PubMed PMID: 19410488]
28. Abd-Eldaim M, Beall MJ, Kennedy MA. Detection of feline panleukopenia virus using a commercial ELISA for canine parvovirus. *Vet Ther* **10:**E1–E6, 2009 [PubMed PMID: 20425728]
29. Neuerer FF, Horlacher K, Truyen U, Hartmann K. Comparison of different in-house test systems to detect parvovirus in faeces of cats. *J Feline Med Surg* **10:**247–251, 2008. http://dx.doi.org/10.1016/j.jfms.2007.12.001 [PubMed PMID: 18243743]
30. Ferroglio E, Centaro E, Mignone W, Trisciuoglio A. Evaluation of an ELISA rapid device for the serological diagnosis of *Leishmania infantum* infection in dog as compared with immunofluorescence assay and Western blot. *Vet Parasitol* **144:**162–166, 2007. http://dx.doi.org/10.1016/j.vetpar.2006.09.017 [pii: S0304-4017(06)00534-6; Epub 2006/10/19; PubMed PMID: 17046162]
31. Couto CG, Lorentzen L, Beall MJ, et al. Serological study of selected vector-borne diseases in shelter dogs in central spain using point-of-care assays. *Vector Borne Zoonotic Dis* **10:**885–888, 2010. http://dx.doi.org/10.1089/vbz.2009.0063
32. Marcondes M, Biondo AW, Gomes AA, et al. Validation of a *Leishmania infantum* ELISA rapid test for serological diagnosis of *Leishmania chagasi* in dogs. *Vet Parasitol* **175:**15–19, 2011. http://dx.doi.org/10.1016/j.vetpar.2010.09.036 [pii: S0304-4017(10)00547-9; Epub 30.10.10; PubMed PMID: 21030153]
33. Carlin EP, Bowman DD, Scarlett JM, Garrett J, Lorentzen L. Prevalence of *Giardia* in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP *Giardia* test. *Vet Ther* **7:**199–206, 2006. [PubMed PMID: 17039442]
34. Curtis K, Foster P, Smith P, et al. Performance of a recombinant Lip32 based rapid in-clinic ELISA (SNAP Lepto) for the detection ot antibodies against leptospira in dogs. *Int J Appl Res Vet Med* **13:**182, 2015
35. Winzelberg S, Tasse S, Goldstein R, et al. Evaluation of SNAP Lepto in the diagnosis of leptospirosis infections in dogs: twenty two clinic cases. *Int J Appl Res Vet Med* **13:**194, 2015