Bulk Acoustic Wave Sensor Technology in Veterinary Medicine

Advancing Veterinary Medicine with The First Point-of-Care Veterinary Bulk Acoustic Wave Sensor Diagnostic Platform (TRUFORMA™)

Current State of Immunoassay Analyzers in Veterinary Medicine

Immunoassays are indispensable diagnostic tools commonly used in the practice of veterinary medicine. An immunoassay uses the inherent ability of an antibody to bind to specific molecular structure, thereby detecting biomarkers and aiding in the identification of disease. Biomarkers include proteins, hormones, nucleic acids, and other analytes found in biological samples, such as blood, urine, and feces.[1]

The binding of an antibody to the biomarker molecule to be measured is crucial to the process of immunoassay testing. Antibody binding must lead to the production of a signal that can be precisely measured, such as a change in color or luminescence, to quantify the biomarker of interest and allow reporting of a patient's result. Immunoassays are classified based on the method used to generate and detect the associated signal. Different methods for labeling and signal detection lead to variability in test performance, diagnostic accuracy, and test complexity.

Assay Performance is the Basis for Accurate Clinical Diagnosis

Diagnostic performance encompasses more than diagnostic specificity and sensitivity (i.e., the ability to correctly identify the presence or absence of disease). In fact, immunoassay analytical performance metrics, which ultimately determine diagnostic sensitivity and specificity, include analytical range, precision, accuracy, and the limits of detection and quantification. An immunoassay's analytical range refers to its ability to reliably quantify the concentration of an analyte over a range of concentrations with acceptable accuracy (correctness) and precision (repeatability). In other words, an analytical range represents the upper and lower limits of quantification where test results can be quantified with both confidence and reliability. At the lower end of an analytical range is a diagnostic's limit of detection, which is the lowest analyte concentration that can be distinguished from background signal noise but is not quantifiable. Accuracy over a wide range of analyte concentrations is necessary for acceptable diagnostic sensitivity and specificity. Improvements to immunoassay analytical performance metrics enhance diagnostic specificity and sensitivity, thus increasing an immunoassay's ability to differentiate healthy from diseased patients.

Overview of Conventional Technology in Immunoassay Testing

Immunoassays use specific methods to detect signals when antibodies—or sometimes antigens—bind to analytes. Antibody binding is reported by attaching substances referred to as "labels" or "reporters," which commonly include enzymes, fluorescent proteins, and radioactive isotopes. When the binding of antibody to analyte occurs, the label can be detected by color change, fluorescence, or radiation emission (see Figure below).[2] In general, gold standard immunoassays are predominately performed at reference labs and are divided into two groups: radioimmunoassays (RIAs) or chemiluminescent enzyme immunoassays (CEIAs).

Radioactive Isotope-Labeled Immunoassays

RIAs use radioactive isotope labels to signal antibody–analyte binding. RIAs typically use a scintillation counter to quantify bound radioisotopes. Although RIAs are regarded as highly accurate tests, the cumbersome workflows and stricter safety regulations required to use radioactive substances have greatly reduced the use of RIAs at reference laboratories.

Optical Detection-Based Immunoassays

Optical detection-based immunoassays depend on light generation to measure the quantity of a

biomarker present in a sample. Common optical-based immunoassays use fluorescent and enzyme-based reporters. Optical detection-based CEIAs involve a chemical reaction that emits light.

These optical detection-based immunoassay analyzers require photomultiplier tubes to amplify the light emitted and optical sensors to measure the amount of light produced in the chemical reaction. Measuring the quantity of light produced is an indirect measurement of the amount of biomarker present.

Point-of-Care (POC) Immunoassays

Current commercially available POC immunoassays use one of two technologies – either lateral flow tests, which require no instrumentation or homogeneous enzyme immunoassays (EIA) which are run on analytical chemistry instruments. Both lateral flow and EIA tests use single-step methods that couple reactive-enzyme labelled antibodies and antigens to optically detect the presence of select biomarkers. These two test modalities may not have adequate wash and incubation time leading to lower diagnostic performance and reduced clinical accuracy. Current POC tests are substandard for the diagnosis of most diseases, most notably thyroid and adrenal conditions.[3] The development of a diagnostic platform that can provide POC immunoassay testing without sacrificing gold standard performance and clinical accuracy is critical for advancing veterinary diagnostic testing and improving testing methods for complex conditions.

Barriers to Gold Standard Accuracy with Point-of-Care Convenience

Gold standard CEIA and RIA methods are predominately performed at reference laboratories. These methods are not suitable for POC testing. CEIAs and RIAs produce weak chemiluminescent and radioactive signals that must be amplified by specialized photomultiplier tubes for the reaction to be measured and reported. High instrument costs, the need for skilled technicians to run the tests, and the large footprints associated with the immunoanalyzers have made use of CEIA and RIA at POC non-viable. Smaller, cheaper, and easier-to-use POC optical detection instruments are available but deliver significantly lower performance, limiting both accuracy and the number of tests that can be run on these instruments. A new approach to immunoassay testing is needed to bring the power of gold standard tests—and their associated high performance and accuracy—to veterinary clinics.

Zomedica Product Development Processes Guided by Core Principles

Zomedica’s mission is advancing the health, effectiveness, and financial well-being of veterinarians by creating products for clinical use. These guiding principles are what shape our product development process.

Customer focused
Zomedica engages general-practice veterinarians, veterinary healthcare teams, and veterinary specialists to develop products for clinical use based on clinical needs. Board-certified veterinary internists with disease-state expertise guide development of rigorous target specifications for performance of our assays to ensure that our analytical ranges, accuracy, and precision targets will enable first-in-class quantitative measurements for canine and feline populations with a diverse array of medical needs. The company continues to engage general practitioners and their teams to help improve POC testing workflow and results reporting.

Identifies a growing clinical need
After extensive review of current POC performance metrics and unmet needs across a number of diseases and technologies, Zomedica identified a significant need for improved diagnosis of disease through gold standard POC thyroid and adrenal immunoassay testing in dogs and cats.

Meets rigorous standards
Zomedica seeks to produce diagnostic testing platforms that meet the most rigorous standards. The target specifications for all assays reflect aggressive performance metrics that aim to surpass those of corresponding gold standards. TRUFORMA's™ assay performance requirements were specifically chosen to meet or exceed reference laboratory performance, thus providing unparalleled service at the POC.

Leads to clinical confidence
Zomedica's goal is to bring gold standard performance to POC testing to improve diagnosis of disease. Improved test performance at the POC provides veterinary healthcare teams with control over their workday and confidence in their ability to diagnose diseases sooner. Improving immunoassay performance—whether through analytical range, accuracy, precision, or other key metrics—at the POC could lead to more accurate and convenient methods for diagnosing complex endocrine and metabolic disorders.

With Focus on the Value of Point-of-Care Testing
POC testing allows veterinarians to make faster clinical decisions and treat patients sooner. When a diagnosis is made at the clinic during or soon after a patient visit, the client is more likely to be engaged and interested in learning about test results and available treatment options. When clinic staff spend less time trying to reach the client, more time is spent on teaching and client education. Additionally, POC testing allows for faster identification of errors or problems in sample collection, processing, or transport, which can be corrected before the patient leaves the clinic. Clinical decisions are more likely to be made in the clinic before the day ends. However, accuracy cannot be sacrificed for POC testing speed and ease.

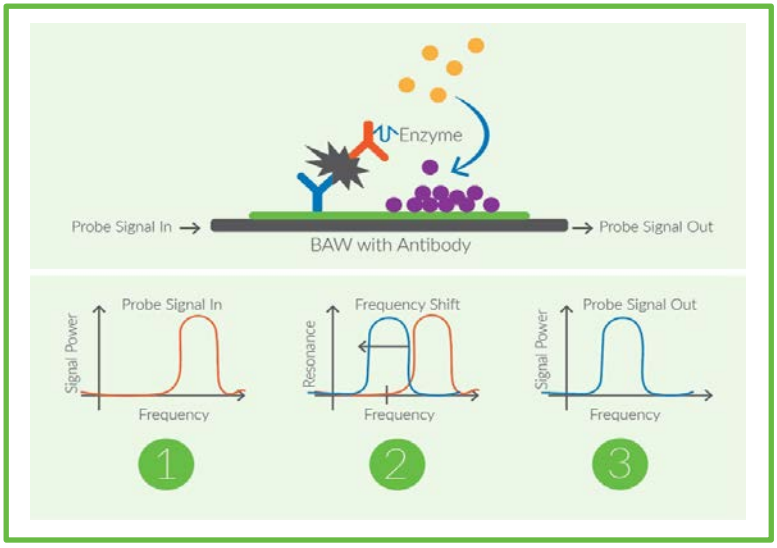
Advancing Point-of-Care Immunoassays in Veterinary Medicine

Transforming Point-of-Care Immunoassays with Resonant Frequency Detection
As previously discussed, gold standard performance at the POC is impossible to achieve with current detection technologies. Therefore, a new class of biosensors is needed. The leading manufacturer of bulk acoustic wave (BAW) filters, Qorvo Biotechnologies, LLC. (Qorvo), has developed a solution. BAW filters—one of the most common types of radio frequency (RF) filters—are deployed in millions of mobile devices worldwide. BAW RF filters are intricate electrical components that allow a mobile device to receive a desired wireless signal while

filtering out undesired signals. In a new application of this technology, Qorvo has spent years validating that these BAW filters could be functionalized (e.g., coated with biologic reagents) to enable the detection of biomarkers, such as proteins and nucleic acids. Such functionalized BAW filters are being integrated into cartridge and instrument systems for biomarker detection in liquid samples.

In 2018, Zomedica entered into a development and supply agreement with Qorvo Biotechnologies, LLC (Qorvo), a wholly owned subsidiary of Qorvo, Inc. focused on bringing its piezo-electric Bulk Acoustic Wave (BAW) sensor to the veterinary health sector. Under the terms of this agreement, Zomedica has exclusive, global rights to develop and market Qorvo's investigational point-of-care diagnostic platform for veterinary use.

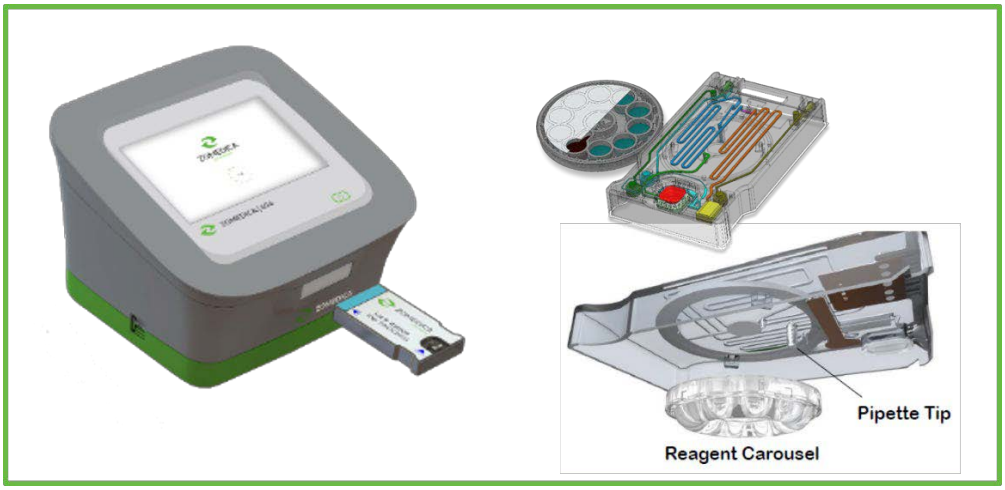
Qorvo's functionalized BAW biosensors consist of multiple resonators, each comprised of a piezoelectric material subjected to an electrical field. The resonators are coated with detection reagents, such as antibodies and nucleic acids. In the context of immunoassays, conventional enzyme-based immunoassay formats can be used with BAW biosensors. Whereas current enzyme-based immunoassays use optical sensors to detect the generation of luminescence or color change, BAW biosensors measure binding events and the insoluble product that is generated by the enzyme reactions that accumulate on the sensor surface, creating a frequency shift in resonance proportional to the mass accumulated on the sensor (Display Item 1). BAW biosensors use at least two resonators as part of an immunoassay test; one reference resonator coated with inert control antibodies and one test resonator coated with the capture antibody. The reference resonator helps to eliminate any minor signal interference resulting from various factors, such as temperature and viscosity changes by measuring and subtracting this interference from the test frequency shift results via the instrument's software.



Display item 1

Immunoassay testing using BAW biosensor detection is accomplished using a small bench-top instrument equipped with disposable testing cartridges. (Display item 2) The disposable cartridge is comprised of two pieces that are packaged as one pre-assembled piece: a main cartridge body and a random-access reagent carousel. The liquid reagents required for a test are stored in sealed wells on the reagent carousel. The main cartridge body contains the BAW biosensor accessed by a microfluidic channel, an automated pipette feature for accessing reagents, and a sample port where the sample is added. At the start of the test, the instrument

transfers the sample to an empty well on the carousel. The instrument rotates the carousel to access each of the wells and moves the carousel up and down to allow the reagents to be aspirated into the cartridge and across the sensor surface through the pipette feature on the cartridge body.



Display item 2

With the innovation of the BAW biosensor, there is finally clear potential for POC testing that is equivalent or even superior to gold standard reference laboratory immunoassays without the expense, labor, and space required for traditional optical sensor-based and RIA testing.

TRUFORMA™: A Complete Platform for Point-of-Care Thyroid & Adrenal Immunoassays

Thyroid and adrenal diseases are complex endocrine disorders with stringent performance requirements for diagnostic testing. As pets continue to live longer, age-related adrenal and thyroid disorders, are likely to be more commonly diagnosed. Unfortunately, no current POC immunoassays provide the performance required to deliver accurate, reliable data when testing for these disorders. Samples must be submitted to reference laboratories to ensure an accurate diagnosis. Although animals with adrenal and thyroid disease rarely present with urgent, life-threatening clinical signs, diagnostic challenges could be managed more effectively if POC immunoassays meeting the rigorous performance metrics required for accurate diagnosis were made available. TRUFORMA™ offers both the convenience of complete adrenal and thyroid testing at the point of care and the confidence of gold standard performance.

Diagnosing Thyroid Disease

Diagnosis of feline and canine thyroid disease may be straightforward with measurement of a single total thyroxine (T4) or it may be complex, requiring measurement of multiple thyroid hormones, including total T4, free T4, and thyroid stimulating hormone (TSH). Currently, there are no adequate POC immunoassays that measure total T4, free T4, or TSH, which leads to an inconvenient dilemma for veterinary practitioners. Veterinarians must either submit blood samples to run multiple tests at a reference laboratory, incurring unnecessary costs associated with potentially redundant testing or basing diagnostic decisions on subpar total T4 POC testing. Thus, pet owners are hassled by additional trips to the clinic and veterinary clinicians are inconvenienced by resampling and retesting of total T4 levels. TRUFORMA™ provides

complete thyroid testing at the POC, including accurate measurement of total T4, free T4, and TSH validated for dogs and total T4 and TSH validated for cats. In addition to bringing accurate thyroid testing to point of care, TRUFORMA™ will also bring to veterinarians the first ever TSH assay developed for and validated in cats. TSH validated for cats will help veterinarians more readily differentiate between hyperthyroidism and euthyroid sick syndrome and diagnose tough cases. TRUFORMA™ offers clinicians complete control over the testing process for diagnosing thyroid disease in dogs and cats.

Diagnosing Adrenal Disease

Diagnosis of adrenal disease (hyperadrenocorticism or Cushing's disease and hypoadrenocorticism or Addison's disease) in dogs currently relies on adrenal function testing. Hyperadrenocorticism is diagnosed and monitored by measuring the response of the adrenal glands to synthetic adrenocorticotropic hormone (ACTH) stimulation and/or to low-dose dexamethasone suppression. Hypoadrenocorticism is diagnosed by measuring the response—or lack thereof—of the adrenal glands to ACTH stimulation. Blood is obtained before and after administration of synthetic ACTH or dexamethasone and submitted to the reference laboratory for cortisol measurement.

In addition to testing serum cortisol in response to functional testing, the scientific literature suggests the measurement of endogenous ACTH could reduce reliance on functional testing for the diagnosis of hypoadrenocorticism and differentiation between pituitary dependent hyperadrenocorticism and adrenal dependent hyperadrenocorticism. Unfortunately, the true clinical utility of evaluating endogenous ACTH (eACTH) in testing patients for adrenal disease has not been fully explored due to the molecule's instability and difficulties associated with its transport to a reference laboratory.

Furthermore, there are currently no accurate POC tests for obtaining baseline cortisol measurements for screening and treatment monitoring purposes. TRUFORMA™ will provide cortisol- and eACTH-testing at POC, allowing for the use of new diagnostic testing protocols, such as cortisol-to-eACTH ratio for the diagnosis of hypoadrenocorticism, as well as reliable screening and monitoring protocols.

Future State and Platform Capabilities

Future potential capabilities of the TRUFROMA™ platform include multiplexing and molecular testing. First-in-class multiplex and molecular testing at the POC are development program goals, with the ultimate aim being development of a platform where these tests can be conducted on the same cartridge-based system. These capabilities add tremendous value, as they would drastically reduce turnaround time compared with both single biomarker POC assays and reference laboratory testing. Cartridge-based molecular testing requires no technical training or analysis. Additionally, all new assays developed in the future could be run on the same instrument with no additional investment or loss of laboratory bench space.

Easy to Use

The TRUFORMA™ operating system provides a user-friendly interface that requires minimal staff training. In brief, a sample (approximately 150 µL for serum, blood and plasma) is loaded into a disposable cartridge for a desired test. The cartridge is then inserted into the system. From there, the system reads a radio-frequency identification tag integrated into the cartridge to determine which test is to be performed (e.g., test protocol, calibration data, expiration date, quality control/batch traceability). The system then performs automated testing to provide quantitative, gold standard results to the user.

Increases Productivity
TRUFORMA™ eliminates the time required to send samples to a reference laboratory for testing, the high cost associated with reference laboratory testing, the chance for lost or misplaced samples at the reference laboratory, the time required for the veterinary healthcare team to follow up with clients, and the time for re-testing due to inadequate sample volume. TRUFORMA™ eliminates the delays that occur with reference laboratory testing and brings the focus back to where it belongs—the patient. By simplifying and streamlining the testing process, veterinarians can focus on providing effective, timely, and quality patient care.

TRUFORMA™ will transform POC testing in veterinary medicine and is set to create a new standard in the industry for immunoassay testing. The TRUFORMA™ platform offers a simplified and streamlined testing process that facilitates real-time diagnosis and implementation of appropriate treatment strategies. Diagnosis at POC puts control back into the hands of veterinarians and provides the opportunity for better patient outcomes and practice operation.

References

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