Zomedica® TRUFORMA®

The first and only in-clinic ACTH assay

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Previously, Dr. Wood was a lead scientist at Swift Biosciences and developed next-generation sequencing assays. She served as a research assistant professor and completed a postdoctoral fellowship (awarded by the American Cancer Society) in the Cell & Molecular Biology department at Northwestern's Feinberg School of Medicine. She earned a PhD in molecular biology from Johns Hopkins University.

THE PROBLEM

Adrenal dysfunction can result in significant health issues for dogs, and understanding the cause of Cushing's disease and Addison's disease is important to provide the pet owner with an accurate prognosis and to establish an appropriate treatment plan. Accurately diagnosing and monitoring these conditions can be challenging in practice, because testing methodologies are complex. Endogenous adrenocorticotropic hormone (eACTH) testing is the most reliable and straightforward method to differentiate pituitary and adrenal-dependent Cushing's disease, as well as primary and secondary Addison's disease.

Before Zomedica developed the TRUFORMA platform, eACTH was available only at reference laboratories. The molecule is extremely unstable and samples that are not handled properly after collection can undergo rapid enzymatic degradation, compromising the test results. To preserve the sample's integrity, plasma must be kept cold, and frozen if stored longer than 12 hours. These requirements cause significant challenges during shipping to reference laboratories, and lapses in cold storage frequently result in unusable samples. Most general practitioners don't want the hassle of eACTH testing, so mostly specialists who have immediate access to reference laboratory instruments use this methodology.

ZOMEDICA TRUFORMA

THE SOLUTION

The more quickly a sample can be processed, the less likely the sample will be degraded and the more accurate and reliable the test result. Ideally, veterinarians should be able to use a point-of-care diagnostic eACTH test to ensure the sample is not compromised, eliminating the need to ship or store samples for an extended period. An in-house test would provide faster results and allow veterinarians to start treating the patient sooner. However, creating an appropriate device is challenging because eACTH testing requires sensitive and precise technology to ensure results are reliable for accurate clinical decision making.

Current point-of-care diagnostic devices use fluorescence or colorimetric detection methods that cannot adequately achieve the

sensitivity and precision required for a reliable eACTH test.

THE INNOVATION

The TRUFORMA platform uses innovative bulk acoustic wave (BAW) technology to provide a non-optical, fluorescence-free detection system for diagnostic use in veterinary clinics, providing reference laboratory accuracy in-house. When we first conceived the TRUFORMA device, our goal was to develop a point-of-care device that provided reference lab–quality data. Existing commercially available point-of-care diagnostic devices use optical detection methods that limit assay sensitivity.

When looking to eliminate these shortcomings, we identified BAW sensors. Industries such as telecommunications and aerospace have used BAW technology for decades. BAW sensors have also proven effective in cell phones, and their ability to withstand movement made them perfect for our aspirations for an eACTH point-of-care device. In addition, BAW sensors are not highly sensitive to environmental variations and do not require substantial maintenance. By partnering with Qorvo Biotechnologies, we were able to bring BAW technology to veterinary medicine.

A BAW biosensor is comprised of multiple resonators, which are composed of piezoelectric material subjected to an electrical field. Detection reagents, such as antibodies and nucleic acids, can be used to coat the resonators for immunoassay and molecular testing. This technology provides much more sensitive results, because current enzyme-based immunoassays rely on optical sensors to detect luminescence or color change, while BAW biosensors measure binding events and the insoluble product that the enzymes generate, which accumulates on the sensor surface. Steps include:

- **Coating the sensor** — The BAW sensor is coated with a monoclonal capture antibody.
- **Antigen binding** — Antigen in the sample binds to a polyclonal detection antibody in the solution, and the capture antibody on the sensor surface recognises the complex.
- **Substrate conversion** — After several wash steps, an enzyme substrate is added and bound enzyme converts the substrate to an insoluble product that accumulates on the BAW biosensor surface. The BAW biosensor measures this as a shift in frequency that is proportional to the amount of analyte in the sample.

Endocrine testing, including cortisol testing for low dose dexamethasone suppression tests, ACTH stim testing, and eACTH testing, can be performed on the TRUFORMA platform. The device is easy to run and requires minimal training and no previous technical experience. The process involves:

- Using a provided disposable pipette to load the serum or plasma into the cartridge.
- Using the touchscreen to enter the sample information.
- Loading the cartridge into the device and starting the run.

The assays, which take less than 20 minutes to run and produce results, are far more efficient than sending samples to a referral lab.

Using BAW technology, we were able to develop the TRUFORMA device to provide a sensitive, accurate and reliable eACTH assay that can be run at the point-of-care, greatly facilitating clinical decision making. The TRUFORMA eACTH assay's analytical sensitivity is below 5 pg/ml, which is the lower end of the gold-standard reference lab eACTH assay. The TRUFORMA assay will allow general practitioners to improve diagnostics and treatment when caring for animals with Cushing's and Addison's disease.

THE DIFFERENCE MAKER

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