Development of Diagnostic In-Situ Hybridization for a Commercial Platform

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Technology description

Executive Summary

In situ hybridization (ISH) uses complementary DNA or RNA probes to localize a specific DNA or RNA sequence in in tissue sections. Because ISH targets nucleic acid sequences, as opposed to proteins, ISH can detect both expressed and unexpressed genes, making it a versatile and less costly diagnostic tool. As with other detection techniques, ISH requires that the sequence of the target be known so that complementary probe sequence can be synthesized and tagged, with visualization aids, for use as hybridization probes.

Canine herpesvirus 1 is a leading cause of sudden death in puppies between 1 - 4 weeks of age. The virus is generally transmitted as litters pass through the birth canal, with a near 100% mortality rate. Current diagnoses of the mother include a combination of assays that are limited because they rely on antibody recognition, leading to frequent misdiagnoses. Using in-situ hybridization (ISH) to diagnose canine herpesvirus increases the accuracy of proper diagnoses compared to current methods by detecting in the genome.

Equine warts are the most common skin tumors in horses between 1 -3 years of age, affecting a wide range of cutaneous sites. These warts can result in health problems because of physical location, and also detract from the aesthetic appeal of the animal. However, further studies of showed that warts caused by equine papillomavirus type 2 (EcPV-2) might promote skin cancer in horses. ISH is able to more accurately detect EcPV-2 than current methods. Accurate detection of equine papillomavirus is crucial to prevent individual horses from developing malignant skin cancers from common warts.

Zoonotic visceral leishmanisis (VL), or black fever, is the second largest parasitic killer in the world after malaria. The two most culpable Leishmania species, L. infantum and L. donovani affect an estimated 500,000 each year, and result in more than 50,000 deaths worldwide. The current control strategies for VL rely on reservoir and vector control, with dogs acting as the main vector of L. infantum. An accurate diagnosis of infection in dogs is fundamental for the control of zoonotic VL. While histopathology and immunohistochemistry are frequently used to diagnose the presence of L. infantum in dogs, they show limited accuracy, specifically in detecting and discriminating L. infantum from other leishmanial species found in dogs. In-situ hybridization can detect L. infantum with significantly increased sensitivity and specificity in histological samples from infected dogs.

Description of Technology

This invention from Michigan State University is a set of oligonucleotide probes, current protocols, and troubleshooting services for the ISH-based diagnostics of four veterinary maladies: canine and feline herpes, equine papilloma, and canine Leishmania. The following are included:
In situ hybridization protocols using 5’ -digoxygenin labeled oligoprobes
HERPES-dog oligoprobes and corresponding control block
HERPES-cat oligoprobes and corresponding control block
Equine papilloma oligoprobes and corresponding control block
Leishmania oligoprobes and corresponding control block.

Application area

Veterinary diagnostic tool/methodology
Transmission control of zoonotic VL

Advantages

Accurate technique: demonstrated to be more sensitive and selective than PCR and IHC
Higher exclusivity: detects target with high specificity
Increased proper diagnosis: does not rely on gene expression (e.g., proteins, antibodies) for detection

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