Shah, J. S.; Mao, M.; Ward, S.; Mariano, P.; Stricker, R.; Harris, N. S., 2002: PCR assay for direct detection of Babesia-WA1 in whole blood. Abstracts Of The Interscience Conference On Antimicrobial Agents & Chemotherapy. 42: 421 Poster # 1514

Revised Abstract

Babesiosis is a tick-borne disease caused by an intraerythrocytic parasite. At present, four species of Babesia are known to infect man. They are *Babesia microti*, *Babesia divergens*, *Babesia MO1* and *Babesia-WA1*. *B. microti* was thought to be present in NE Coastal regions of the US, but recently cases have been reported from Switzerland too. *B. divergens* is found in Europe. *Babesia MOI*, which is very closely related to *B. divergens*, is found in Missouri, US. Several cases of *Babesia-WA1* have been reported from the West Coastal regions of the US.

The Giemsa-staining of a whole blood smear is the method of choice for diagnosing Babesiosis. However, it is neither sensitive nor specific. Currently IGeneX offers *Babesia* PCR. We have recently developed a highly specific and sensitive PCR based test for detection of *Babesia-WA1*. The diagnostic assay is a four step PCR assay that detects *Babesia-WA1*, directly from blood samples. It is highly specific and sensitive. The first step of the assay specifically removes "common PCR inhibitors" from blood and at the same time selects and purifies the DNA. In the second step, the purified *Babesia* specific fragment is PCR amplified with *Babesia-WA1* primers. In the third step, the PCR amplified *Babesia-WA1* DNA fragment is detected by agarose gel electrophoresis. The fourth step, Dot-Blot analysis, is a confirmation step for the *Babesia-WA1.* The combination of the four steps provide very high specificity (see below) and sensitivity.

The high specificity is provided by: (1) one of the PCR amplification primers, and (2) the probe used for Southern Blot analysis. This PCR primer has 100% homology to *Babesia-WA1* DNA sequence currently in the GENEBANK, but not to non-*Babesia* parasites such as *Plasmodium* species, *Trypanosomes*, and human DNA sequences. Based on the sequence information, the *Babesia-WA1* probe only “hybridizes” or “binds” to *Babesia-WA1* PCR product. Thus, any of the "right size" amplified product detected by agarose gel electrophoresis and positive by Southern Blot analysis with *Babesia-WA1* probe would be *Babesia-WA1* specific.
Seventy-nine EDTA whole blood samples from patients with Babesiosis-like symptoms from Northern California were tested by the *Babesia-WA1* PCR assay. Of the 79 samples tested, three were positive for *Babesia-WA1*. Two have been already confirmed as *Babesia-WA1* by sequencing. None of the *B. microti* positive samples gave a positive result. The limit of detection of the assay is between 10-100 copies of the ribosomal DNA (rDNA) fragments spiked into EDTA whole blood. Assuming that there are between 100-200 copies of rDNA fragments per parasite, this corresponds to less than one organism per sample tested.

Introduction

Babesiosis is a tick-borne disease caused by an intraerythrocytic parasite. At present, four species of Babesia are known to infect man. They are *Babesia microti*, *Babesia divergens*, *Babesia MO1* and *Babesia-WA1*. *B. microti* was thought to be present in NE Coastal regions of the US, but recently cases have been reported from Switzerland too. *B. divergens* is found in Europe. *Babesia MO1*, which is very closely related to *B. divergens*, is found in Missouri, US. Several cases of *Babesia-WA1* have been reported from the West Coastal regions of the US. *Babesia* is transmitted by the same ticks that transmit *B. burgdorferi*, the causative agent of Lyme disease. Symptoms of Babesiosis are similar to some of the symptoms of Lyme disease: fatigue, malaise, myalgia, arthralgia, chills and fever. Usually the fever is high. The disease is particularly life threatening in splenectomized patients.

The nucleic acid based amplified assay is for the detection of *Babesia* in clinical specimens. A fragment of *Babesia* small sub-unit rDNA is selectively purified directly from the specimen using a proprietary technique. The purified small sub-unit rDNA fragment is subsequently amplified with *Babesia*-specific primers (designed from published sequences), using the Polymerase Chain Reaction (PCR)

PCR is a sensitive method used to amplify minute amounts of DNA from clinical samples. Samples need only contain one recoverable (live or dead) organism. The advantage of the PCR is that upon binding, one is able to greatly increase the material for analysis by performing multiple heating and cooling cycles. If one had a system with 100% efficiency, 31 cycles would produce over a billion copies of DNA from a single organism.

The test has clinical significance at any stage in the course of the disease and is particularly useful as an aid in diagnosis in difficult cases when other laboratory tests are negative. Recoverability of an organism is a strong argument for the presence of disease.

Principle

The Polymerase Chain Reaction (PCR) for *Babesia-WA1* genomic DNA is based on the technology licensed from Roche Molecular Systems, Inc. The technology employs amplification and detection of specific DNA sequences.

The following steps take place:

- Denaturation of DNA
- Primers anneal to target DNA sequence ends
- TAQ Polymerase catalyses the extension and incorporation of complimentary nucleotides.
- PCR amplification of target DNA is exponential. Thus PCR cycle 1 makes 2 copies of target sequence, 2 makes 4 copies, 4 makes 16 copies, etc.

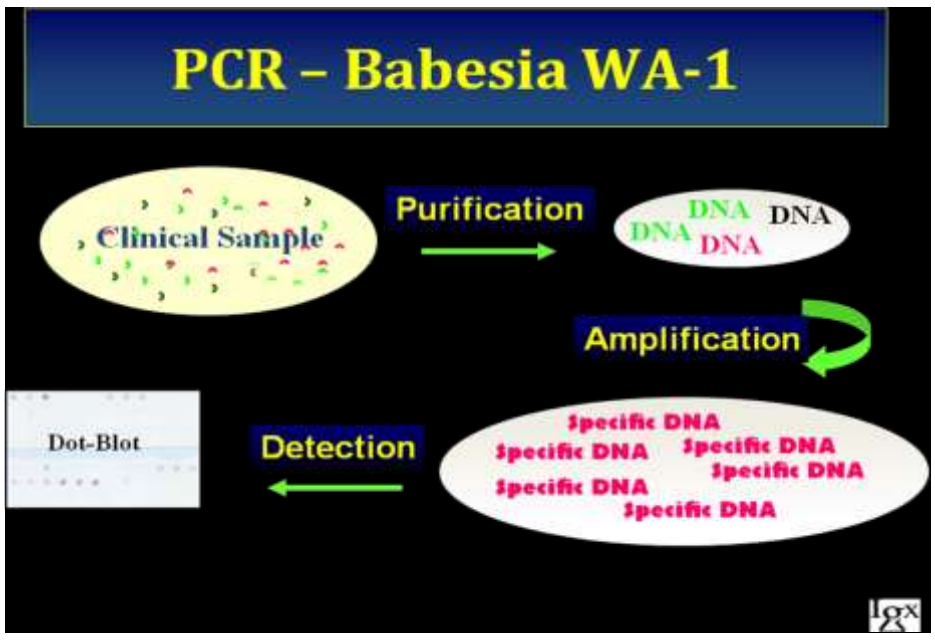
Method

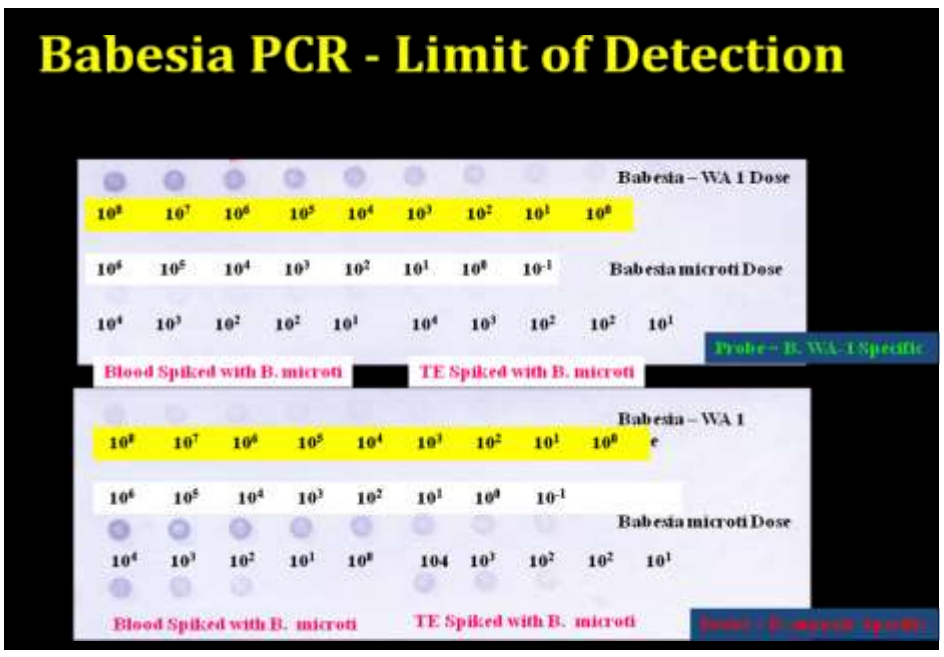
The PCR-based diagnostic assay that detects *Babesia-WA1* specific DNA is a highly sensitive and specific. The four steps, direct from whole blood, serum or Cerebral Spinal Fluid (CSF) is:

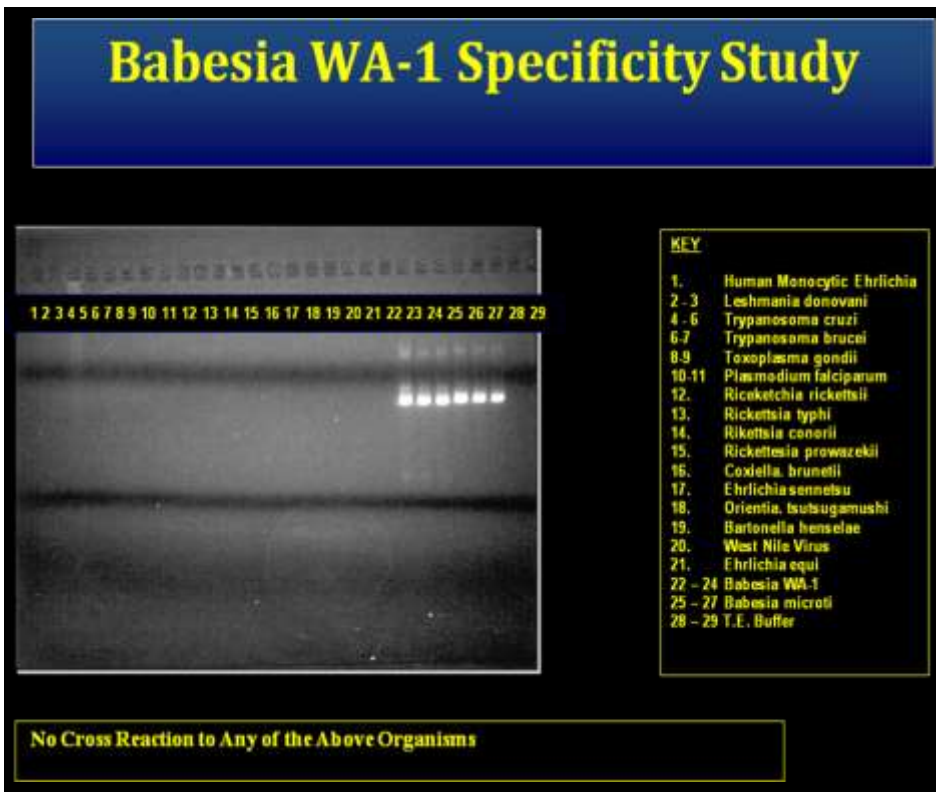
1. Gentra Column DNA Purification Step – This step specifically removes the "common PCR inhibitors" from the clinical sample, and at the same time selects and purifies the DNA.

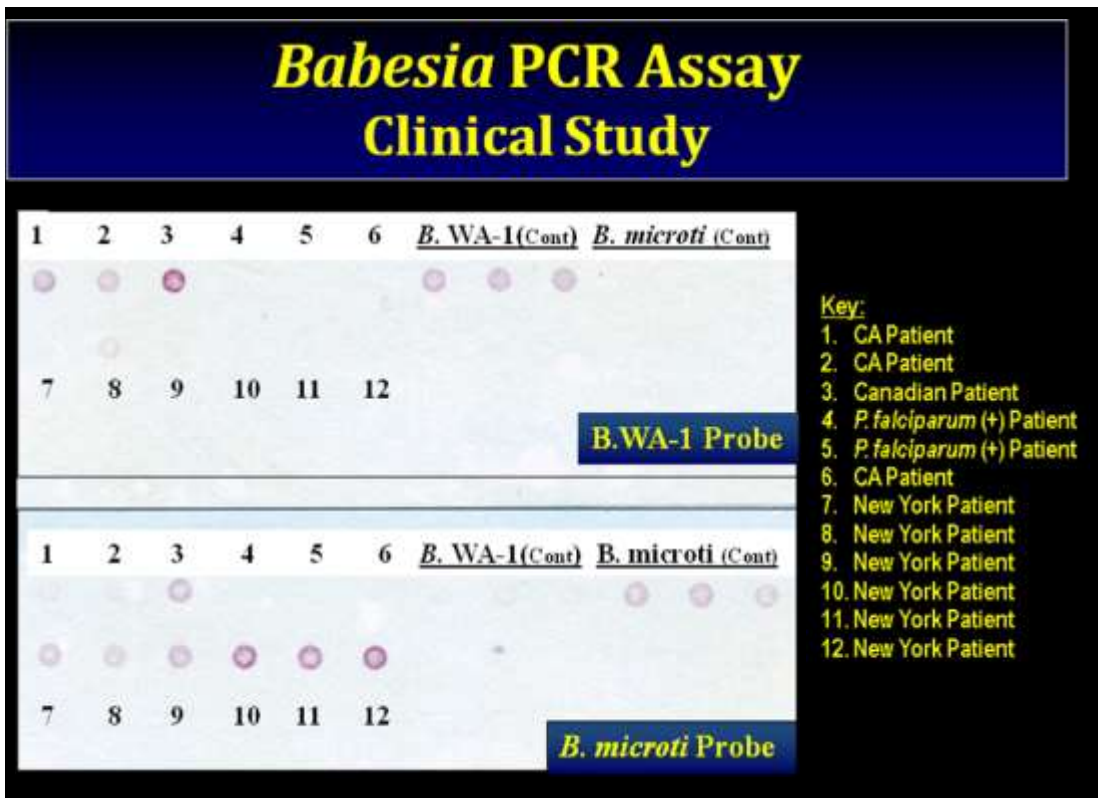
2. PCR Amplification – The purified DNA is PCR amplified with *Babesia-WA1* specific primers. A primer is a synthetically produced nucleic acid sequence, that by design and selection contains *Babesia-WA1* specific nucleotide sequences. The specific sequence "hybridizes" or binds specifically to *Babesia-WA1*, therefore only *Babesia-WA1* specific DNA is amplified.

3. Detection of *Babesia-WA1* Specific Amplified Products – The PCR amplified *Babesia-WA1* DNA fragment is confirmed by Dot- Blot Assay using a highly specific *Babesia-WA1* probe. The PCR product is bound to a nitrocellulose membrane. The membrane bound DNA is hybridized to *Babesia-WA1* probe. Only samples with PCR products complimentary to the *Babesia-WA1* probe shall hybridize and generate a color signal on the membrane.



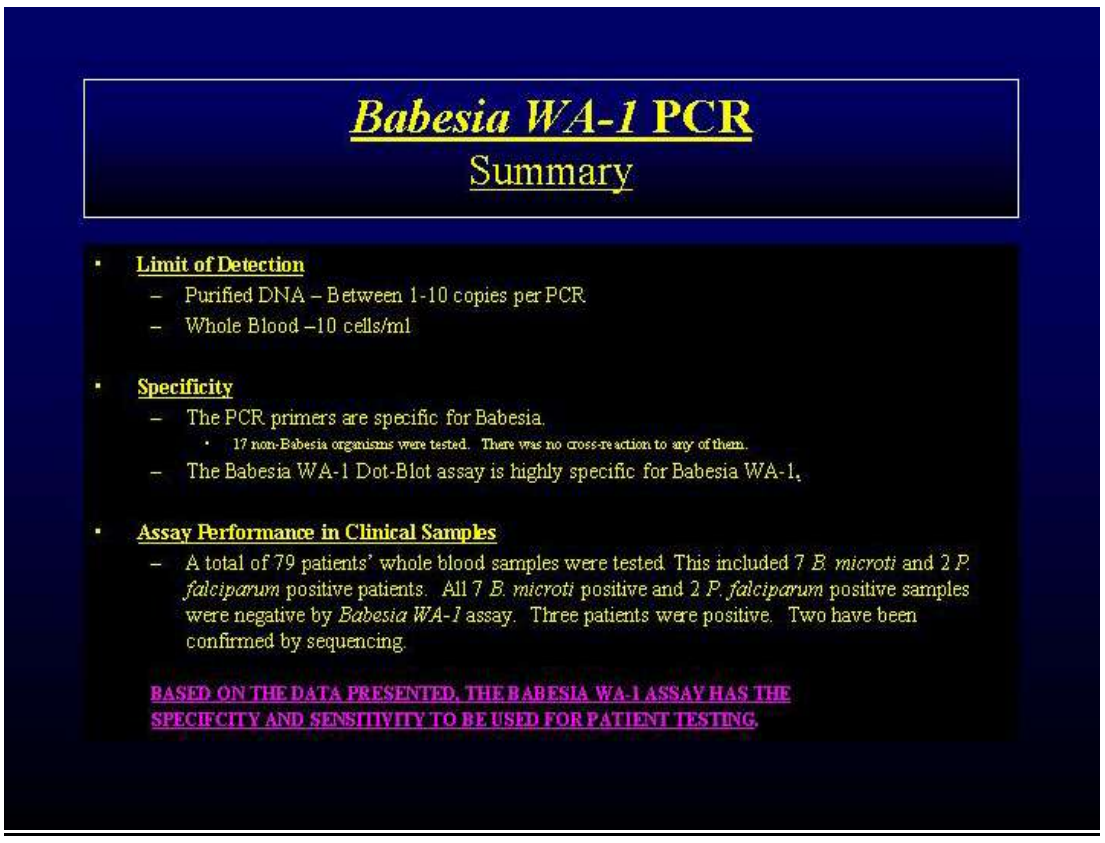






Overall Results

Sample Type	N	*Babesia WA1* Positive	*Babesia WA1* Negative
Clinical Samples **Whole Blood** (suspected of tick-borne diseases)	**71**	**3**	**68**
Clinical Samples **Whole Blood** (Confirmed *Babesia microti* Positive)	**6**	**0**	**6**
Clinical Samples **Whole Blood** (Confirmed *Plasmodium falciparum* Positive)	**2**	**0**	**2**
Non *Babesia-WA1* Organisms (See Specificity Study Chart)	**17**	**0**	**17**
Total	**96**	**3**	**93**



Conclusion

- Limit of Detection of the Purified DNA = Between 10^0 copies of *Babesia WA1* rDNA.
- Limit of Detection in spiked samples (whole blood) = 10 cells per ml of sample assuming that there are 100 to 200 copies of rDNA per cell.
- Highly specific when tested against *B. microti, P. falciparum* and other tick-borne and blood borne pathogens.

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