J Eukaryot Microbiol 1994 Jul-Aug;41(4):324-30

Discrimination amongst *Leishmania* by polymerase chain reaction and hybridization with small subunit ribosomal DNA derived oligonucleotides.

Uliana SR, Nelson K, Beverley SM, Camargo EP, Floeter-Winter LM

A method for discriminating among *Leishmania* is described, based upon small subunit ribosomal DNA sequence differences. The method was to amplify the entire 2.2 kb small subunit rDNA by polymerase chain reaction using conserved primers specific for the 5' and 3' termini of the small subunit ribosomal RNA, and then hybridize the product dotted onto nylon membranes with labeled oligonucleotides. The design of the hybridization probes was based upon complete small subunit rDNA sequences from *L. amazonensis*, *L. major* and *L. guyanensis* and partial sequences *of L. mexicana*, *L. braziliensis*, *L. tropica* and *L. chagasi*. A high degree of sequence similarity (> 99%) among species was found. However, sufficient sequence divergence occurred to permit the design of internal oligonucleotide probes specific for species complexes. This procedure successfully discriminated amongst a wide range of *Leishmania* isolates. The method detected as few as 10 cultured organisms and detected parasites in tissue samples from experimentally infected animals. Non-radioactive labeling showed the same specificity and sensitivity as radioactive probes.