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PEOPLE'S REPUBLIC OF CHINA**

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**Detection of Babesia spp. - Identification of species by nucleic
acid method**

(English Translation)

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed by the National Health Standards Committee Parasitic Diseases Standards Committee.

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Babesia detection of nucleic acid identification

1 Scope

This standard specifies the nucleic acid identification method for the detection of Babesia.

This standard applies to the detection of Babesia in disease prevention and control institutions and medical institutions at all levels.

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only the dated version applies to this article.

Pieces. For undated references, the latest edition (including all amendments) applies to this document.

WS/T 564 Babesia diagnosis

3 Terms and definitions

The following terms and definitions apply to this document.

Babesia spp.

A type of protozoan that is parasitic in human and vertebrate red blood cells can cause babesiosis. Babe species infected with human body the main species are Babesia microti, B.divergens, B.duncani and Orion.

B. venatorum, etc. (see Appendix A).

4.1 High speed centrifuge

The maximum relative centrifugal force is 12 000 x g.

4.2 Vortex

The stepless speed regulation range is from.200 r/min to 3 000 r/min.

4.3 PCR instrument

The temperature range is from 4 ° C to 99 ° C, the temperature is precisely 0.1 ° C, and the hot lid is 105 ° C.

4.5 Electrophoresis

The voltage is 5 V to 5 000 V and the current is 1 mA to 500 mA.

4.6 Gel Imaging System

The lens resolution (H x V) is 1 360 x 1 024 and the light source is transmissive UV.

5.1 Molecular biology reagents

Commercially available products are selected for Taq enzymes, deoxyribonucleoside triphosphates (dNTPs), molecular mass indicators, and nucleic acid extraction kits.

5.2 Chemical reagents

Tris-acetate running buffer (TAE), agarose gel, 6 x loading buffer (see Appendix B).

5.3 Primers

Specific primers for amplifying the 18S ribosomal RNA (18S rRNA) gene and the transcribed spacer (ITS) gene of Babesia (see Appendix C).

5.4 Reference materials

The positive control was genomic DNA of the peabody mjr strain of the hamster.

The blank control was deionized water.

The negative control is whole blood genomic DNA of healthy individuals.

6.1 Sample

The sample is an anticoagulant sample for clinical diagnosis or suspected Babesia case.

6.2.1 Nucleic acid extraction

Genomic DNA extraction uses a commercial whole blood DNA extraction kit (see Appendix B).

6.2.2 18S rRNA gene amplification See Appendix C.

6.2.3 ITS gene amplification

See Appendix C.

6.2.4.1.1 Electrophoresis

Take 5 μ L of the second round of PCR product and mix with 1 μ L of 6x loading buffer and apply to 1.0% agarose gel containing ethidium bromide substitute.

In electrophoresis at 5 V/cm in 1xTAE buffer for about 40 min, stop electrophoresis when bromophenol blue reaches the bottom, using a gel imaging system or UV analyzer analysis.

6.2.4.1.2 Judgment of PCR results

6.2.4.1.2.1 PCR amplification of the 18S rRNA gene of *Babesia*, the amplification fragment with a specificity of about 400 bp, blank pair no bands appeared in the negative and negative controls, and the PCR results were positive. PCR amplification of *Babesia* 18S rRNA gene, no size of 400 bp left the right-specific amplified fragment, the blank control and the negative control did not show a band, and the PCR result was negative.

6.2.4.1.2.2 PCR amplification of the IBS gene of *Babesia*, the emergence of specific amplified fragments ranging from 700 bp to 2 100 bp, blank pair no bands appeared in the negative and negative controls, and the PCR results were positive. PCR amplification of *Babesia* ITS gene, no specific amplified fragment, empty There were no bands in the white control and the negative control, and the PCR results were negative.

6.2.4.2 Sequencing analysis

Positive PCR amplification products were sequenced in both directions and the sequences were subjected to BLAST alignment analysis (see Appendix C).

6.2.4.3 Identification of species

According to the electrophoresis results of the PCR amplification products and the sequencing results, the species are comprehensively determined (see Appendix C).

Appendix A

(informative appendix) Pathogenic biology A.1 Classification *Babesia* spp. belongs to Apicomplexa, Sporozoa, and P.

(Piroplasmata), Piroplasmida, *Babesia* (*Babesia*) *Babesia*. Currently More than 100 species of *Babesia* have been identified that can infect a variety of mammals and birds such as cattle, horses, sheep, and dogs. In more than 100 species of *Babe*, both the main types of *Babesia* known to infect humans are. *B. microti*, *B. divergens*, *Dunckeri*.

B. duncani (also known as WA1) and *B. venatorum* (also known as EU1). Different *Babesia* species See Table A.1 for a comparison of clinical symptoms.

A.2 distribution

The disease is endemic.

In the United States, cases of *Babesia* are mainly found in Massachusetts, New York, Connecticut, California, and Rhode Island.

In Europe, cases of *Babesia* in France, Portugal, Italy, Poland, Norway and other places have occurred. Also, in Egypt, Mexico, Cases of *Babesia* have also been reported in Australia, Japan, South Korea, Pakistan and South Africa.