

REVIEW

DNA QUALITY AND ACCURACY OF AVIAN MALARIA PCR DIAGNOSTICS: A REVIEW

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Abstract. Birds have become increasingly prominent in studies focusing on natural populations and their coevolved pathogens or examining populations under environmental stress from novel and emerging infectious diseases. For either type of study, new DNA-based diagnostic tests, using the polymerase chain reaction (PCR), present challenges in detecting the DNA of pathogens, which exist in low copy number compared with DNA of the host. One example comes from studies of avian malaria: conflicting claims are made by different laboratories about the accuracy of tests using various sets of primers and reagents, especially in relation to blood smears and immunological methods. There is little standardization of protocol or performance among laboratories conducting tests, in contrast to studies of human malaria. This review compares the problems of detecting avian malaria with those of detecting human malaria, and shows definitively that the buffer used to store blood samples following collection is associated with the accuracy of the test. Lower accuracy is associated with use of a lysis buffer, which apparently degrades the DNA in the blood sample and contributes to inhibition of PCR reactions. DNA extraction and purification techniques, and optimization of the PCR reaction, do not appear to be alternative explanations for the effect of storage buffer. Nevertheless, the purest DNA in standard concentrations for PCR is required so that different primers, DNA polymerases, and diagnostic tests can be objectively compared.

Key words: avian malaria, blood cells, DNA quality, human malaria, PCR, primers, storage buffer.