Ohio Department of Agriculture

ADDL

ANIMAL DISEASE DIAGNOSTIC LABORATORY

Polymerase-Chain-Reaction

PCR is an abbreviation for polymerase-chain-reaction. It is an in vitro method for enzymatic amplification of specific DNA sequences. The advantage of PCR test over the conventional methods is fast turnaround time with high sensitivity and specificity. The turnaround time can be from a few hours to two days including the sample processing time. Another advantage of PCR test is for detection of pathogens that are either difficult to culture or noncultivable. PCR is also useful in the diagnosis of pathogens where the presence of viral nucleic acid may be the only evidence of infection.

There are several types of PCR techniques. Conventionally, the PCR technique is designed to amplify specific sequence of DNA molecules. The modification of the conventional method makes it also possible to detect RNA viruses. This technique is known as Reverse transcriptase polymerase chain reaction, or RT-PCR. The RNA molecule is first transcribed into complementary DNA (cDNA) using reverse trancriptase derived from retroviruses. The cDNA is then amplified by the PCR. Other modifications of the PCR technique include nested polymerase chain reaction, multiplex polymerase polymerase chain reaction, quantitative polymerase chain reaction, and real-time polymerase chain reaction. The nested PCR uses two sets of primers in two sequential amplification reactions. The PCR product from the first round amplification is then used as template in the second PCR reaction. The second set of primers serves to verify the specificity of the first round product, and the transfer of the first round product into a new reaction mixture,which makes the nested PCR highly sensitive. Multiplex PCR uses two or more sets of primer pairs that are specific for different target sequences in the same amplification reaction, which allows coamplifying several target gene fragments. This method is useful where multiple pathogenic genes from the same organism or multiple pathogens are involved. The quantitative PCR quantifys the amount of template DNA or RNA in clinical samples. The real-time PCR is the newest member of the PCR family. The real-time PCR combines amplification and detection in one step in which fluorescence is measured in real time as PCR products are generated. The results are available within 2-3 hours including sample-processing time.