

PCR DIAGNOSTICS



BASICS

OVERVIEW

- The polymerase chain reaction, or PCR, was first described by Kary Mullis in 1985, for which he won the Nobel Prize in Chemistry. It is a technique whereby the presence of a specific DNA sequence is detected by creating multiple copies of target DNA by using a thermoresistant DNA polymerase enzyme and detection of the product.
- PCR can be used for the detection of microorganisms such as bacteria, viruses, or parasites, or used to identify gene sequences or message RNA that could be involved in a metabolic or developmental abnormality.

AMPLIFICATION

- In conventional PCR, the target for amplification is determined by oligonucleotides, or short pieces of DNA complementary to the DNA sequence in the gene or organism of interest. The oligonucleotides serve to prime the polymerase reaction.
 - During the PCR there are multiple cycles of heating to denature the DNA, cooling to anneal the oligonucleotide primers to their complementary sequences, and extension where the nascent DNA chain is elongated from the primers.
 - Because each DNA strand is replicated during every PCR cycle, even the newly synthesized ones, there is exponential amplification of the DNA product. Thus, starting with only 1 copy of DNA, after 30 cycles there will be more than a billion copies. The product is readily detected by gel electrophoresis (Figure 2) or fluorescence of the PCR product.
 - Real-time PCR (RT-PCR) also utilizes oligonucleotide primers. It has the ability to quantify the amount of DNA amplification and has the potential to quantitate the amount of DNA present in the sample.
 - Real-time PCR also utilizes a probe specific for the sequence to be amplified thus increasing specificity.
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- The product specificity in conventional PCR is determined by size of the resulting DNA product, sequencing, enzyme digestion, and/or probing with DNA specific for the target organism.
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- Theoretically, PCR can detect as little as 1 copy of DNA in a sample; however, this degree of sensitivity is never achieved. Realistically, most conventional PCR tests require 100 to 1,000 copies of the target sequence in the sample to be positive; real-time PCR is generally more sensitive and amplification is faster than conventional PCR.
 - PCR can also be used to detect RNA, such as message RNA or RNA viruses. In these cases, RNA is converted to DNA using an enzyme called reverse transcriptase. The resulting DNA can be amplified by conventional or real-time PCR. The entire process is called reverse-transcription PCR or RT-PCR.

REASONS TO USE PCR FOR MICROBIAL DETECTION

- PCR detection of pathogens can be an important tool for diagnosis of disease; however, it is not ideal for all infectious disease processes. For example, it should not be used on samples from sites which might contain the organism as part of normal flora because these would give a positive result regardless of whether the animal has active disease.
- PCR is best used to detect organisms that are difficult or impossible to isolate by culture, require prolonged incubation, or when the organism is present in very low numbers.
- PCR is useful to detect organisms which would be dangerous to culture. It can also be used to find pathogens in archival tissue where the organism is no longer viable, such as formalin-fixed paraffin-embedded or frozen tissues.

Benefits of PCR

- The PCR is a rapid and sensitive technique that can be used to detect pathogens that might be overlooked or difficult to detect using conventional culture techniques.
- The extraction and amplification of DNA from tissue can be performed in 1 to 2 days, depending on the specific test and laboratory work flow; generally, this is faster than most culture and identification techniques.

Drawbacks of PCR

- The laboratory carrying out testing by PCR must have adequate expertise, equipment, and dedicated facilities to accurately identify pathogens in samples.
- The laboratory must use extreme care and run numerous controls to avoid introduction of DNA that could lead to false positive results. Because PCR is such a sensitive test, it is possible to have false positive results due to small amounts of contaminating DNA present in the sample or inadvertently introduced by the laboratory. Thus, care must be taken to collect samples to prevent contamination with other likely positive materials.
- PCR detects DNA, not viable organisms. Thus, a positive result does not necessarily mean that the animal can spread the organism or that the infection is still active.
- Just as there can be false positive results using PCR, false negative results can occur due to collection of samples that are unlikely to contain the organism, sampling too late in disease when the host animal has cleared the infection, or there is presence of inhibitory substances in the sample that prevents amplification of target DNA.
- The testing laboratory can provide information and assist the practitioner to overcome these difficulties.
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EXAMPLES OF PCR TESTING FOR MICROBIAL PATHOGENS OFFERED BY DIAGNOSTIC LABORATORIES FOR RUMINANTS

- Bovine viral diarrhea virus (RT PCR)
- Bluetongue virus
- Foot and mouth disease virus
- *Mycoplasma* spp.
- *Escherichia coli* (detection of toxins, flagellar genes)
- *Salmonella* spp.
- *Brucella* spp.
- *Mycobacterium avium* ssp. *paratuberculosis*
- Toxigenic *Clostridium* spp. (identifying toxin genes)
- *Coxiella burnetii*
- *Chlamydophila psittaci*

- *Toxoplasma gondii*
- *Neospora caninum*

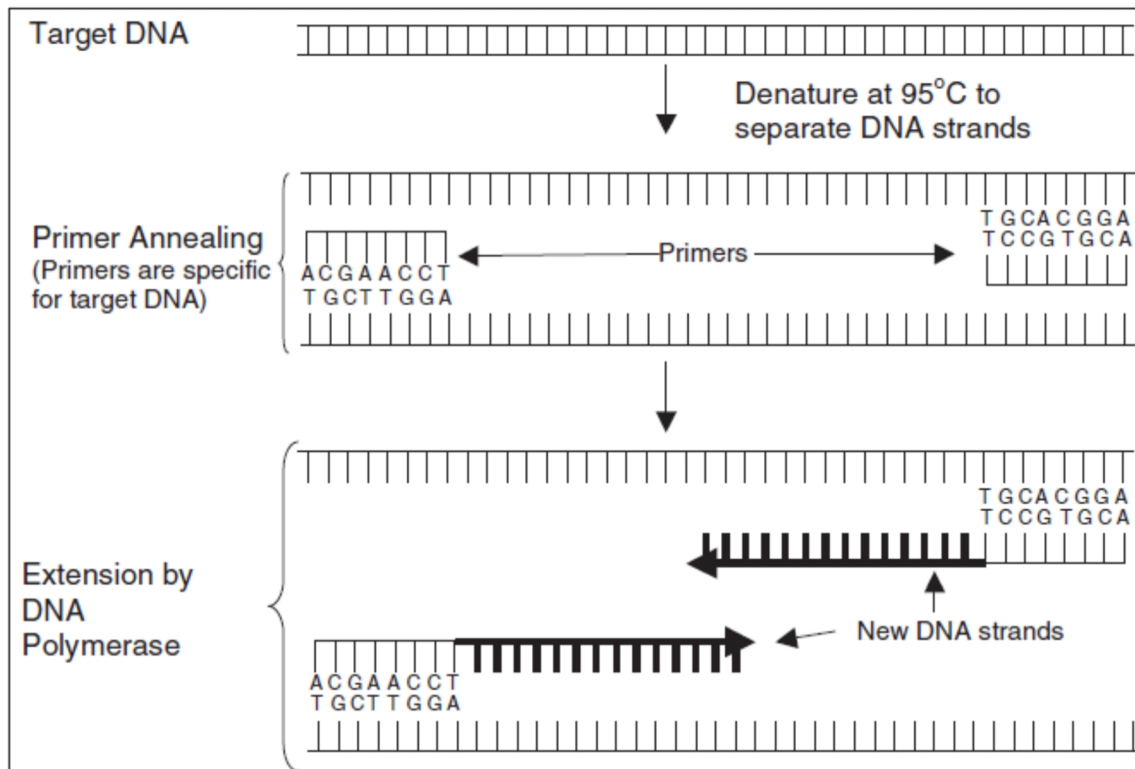


Figure 1. Schematic diagram demonstrating how the PCR generates multiple copies of target DNA.

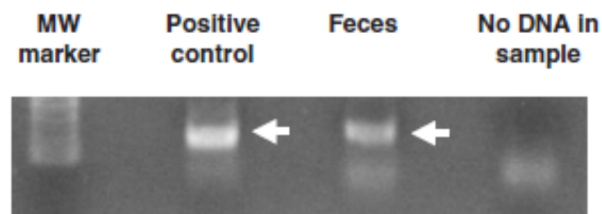


Figure 2. Detection of 16S ribosomal RNA genes from bacteria by using conventional PCR. The arrows indicate the amplified DNA resulting from the PCR in the positive control and feces. The lane on the right has no DNA added and is a negative control.



MISCELLANEOUS

SEE ALSO

Epidemiology: Diagnostic Test Use (www.fiveminutevet.com/ruminant)
Prudent Use of Antimicrobials (www.fiveminutevet.com/ruminant)

ABBREVIATIONS

DNA = deoxyribonucleic acid
PCR = polymerase chain reaction
RNA = ribonucleic acid
RT-PCR = real time-

Suggested Reading

- Khare, S., Ficht, T.A., Santos, R.L., et al. 2004. Rapid and sensitive detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine milk and feces by a combination of immunomagnetic bead separation-conventional PCR and real-time PCR. *J Clin Microbiol.* Mar; 42(3):1075–81.
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- Segura-Alvarez, M., Richter, H., Conraths, F.J., et al. 2003. Evaluation of enzyme-linked immunosorbent assays and a PCR test for detection of shiga toxins for shiga toxin-producing *Escherichia coli* in cattle herds. *J Clin Microbiol.* Dec; 41(12):5760–3.

Author Barbara A. Byrne

Consulting Editor Christopher C.L.. Chase