BioChek

E-BOOK

The basics of PCR:
detecting viruses and bacteria red-handed

In the search for specific bacteria and viruses in poultry and swine livestock, an increasing number of veterinary labs is adopting the usage of the Polymerases Chain Reaction assays (PCR). At the moment, PCR is gaining more popularity in veterinary diagnostics, as it provides specific advantages over other bacteria and virus detection methods.

ONE OF THE MAIN BENEFITS OF USING PCR, IS THAT IT CAN DETECT DISEASES IN AN EARLY STAGE.

PCR is a genetic test capable of detecting the presence of a particular pathogen based on its genetic make-up. The method is designed to detect a part of this genetic make-up that is unique to the pathogen of interest. Through multiplication of the DNA or RNA segment, the presence of the researched pathogen is shown. In this way, PCR can provide a successful detection method for diseases in livestock.

In this eBook you'll learn how PCR works, how it differs from the more frequently used ELISA-method, how to apply the method and, most of all, how it can save hours of your precious time.

ELISA or PCR?

ELISA and PCR are two completely different laboratory techniques, each with its own unique purpose. In the veterinary industry, ELISA is, at the moment, the most frequently used laboratory tool for diagnostic purposes in livestock. In contrast to PCR, ELISA detects the antibodies that are produced to attack the virus or bacteria; not the pathogen itself. This enables you to show whether the researched pathogen was indeed present in the sample at some point in the past, but with an ever present chance that it has already left the host. With PCR, you catch the virus or bacteria while it is still present in the sample. One of the main advantages of using PCR as a detection method, is that it can detect the pathogen causing the disease in an early stage. Very shortly after the infection most pathogens can be detected using a PCR-technique. ELISA, on the other hand, relies on antibody detection. When the animal is infected, it takes a while before the body produces the amount of antibodies an ELISA can detect. This means ELISA detects the pathogen's presence indirectly, with a chance that the pathogen is no longer present in the subject.

LABORATORY CSI: BURGLARS AND FINGERPRINTS

The difference between ELISA and PCR as a detection method can be best explained by the analogy of catching a burglar. Here, the virus or bacteria is represented by the burglar. You have to catch it red-handed to prove it is stealing your valuables: the burglar is only in your house for a short period of time. The antibodies in this case represent the burglar's fingerprints. They remain at the crime scene and prove the burglar was there. To catch the invader red-handed and eliminate the disease (arrest the burglar immediately to prevent it from stealing your belongings, if you will), the PCR method is a good option.

ELISA VS. PCR

Antibody & Antigen

The pathogen is like a burglar - you have to catch it red handed. It can only be detected for a short period of time.

USE A PCR TEST

The antibody is like a fingerprint - it will remain at the crime scene and will prove the burglar was there.

USE AN ELISA TEST KIT

IN SHORT,

PCR offers a more direct detection method when compared to ELISA. PCR is capable of early detection, by detecting the pathogen directly instead of detecting the formed antibodies sometime after the infection. Also, it is less likely to cross-react. PCR and ELISA are two completely different detection methods and they both have their specific purposes. But the results of both methods can also be usefully combined, as proven in the following image.

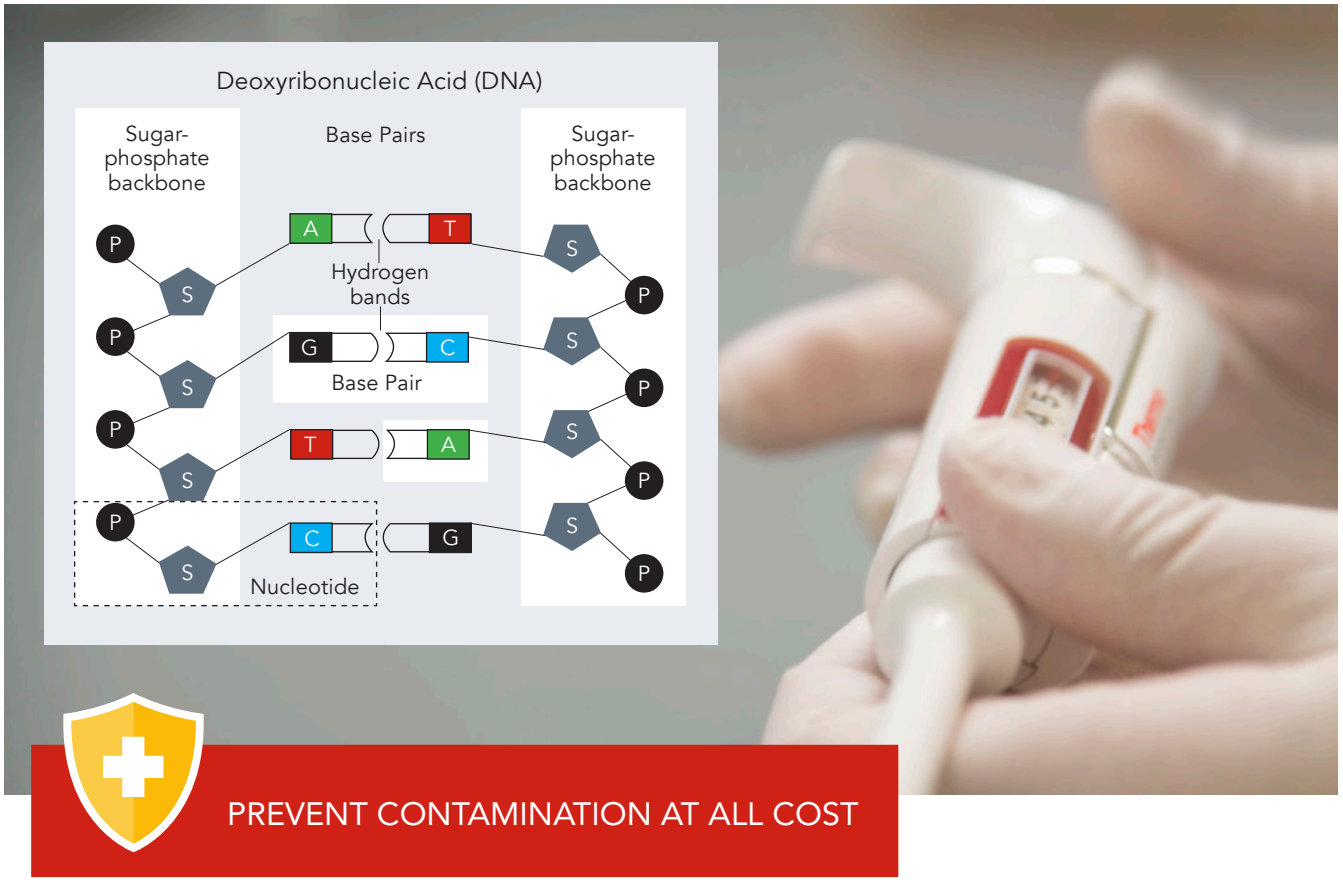
INTERPRETING PCR AND ELISA RESULTS TOGETHER

RESULTS **ELISA**	**PCR**	**Interpretation**
Pos	Pos	• Active viremia, present for longer than 14 days. Animals have begun to build antibody immunity.
Pos	Neg	• Evidence of virus is no longer present, but animals were exposed at some point and mounted immunity.
Neg	Pos	• Early infection - Virus is shed by animals, but there has not been enough time for immunity to develop.
Neg	Neg	• Negative and Naïve - No infection

The PCR basics

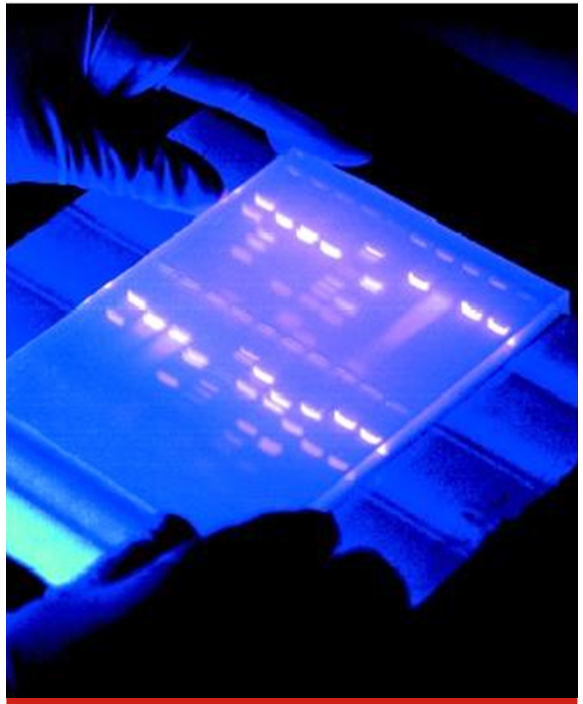
Roughly explained, PCR works by multiplication of a specific segment of the examined DNA- or RNA-material, which is unique for the targeted pathogen. DNA itself consists of four 'building blocks', which are Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). In the 'connection' of two DNA-helixes A only binds to T and C only binds to G. A PCR-test uses this principle to specifically detect pathogens, such as Salmonella and Influenza.

After extraction of DNA or RNA, the first step of PCR is the denaturation of the DNA. In this phase, the double helix structure is melted down at 95 degrees Celsius into two separate strands. Next, the single stranded DNA allows for the binding (annealing) of the primers and the probes if the target pathogen is present in the sample. Primers and probes are the components of the PCR test that detect the specific final genetic part of the pathogen. In the third and last phase, the synthesis of new DNA-strands takes place, as well as the generation of fluorescence by the added probes. This three-phased cycle is repeated 40 times, providing a measurable amount of the targeted DNA-segment. The same process can be used for RNA, with the addition of a reverse transcription step. Here, the present RNA gets converted to cDNA prior to the amplification by PCR. This is executed by a specific enzyme, called reverse transcriptase.



PCR is a very sensitive method: a very small amount of the targeted genetic material is sufficient to detect the pathogen. But at the same time this high sensitivity can be the downside of PCR. If genetic material or PCR-products from previous tests make their way into new reactions, this can lead to false positive signals. That's why lab personnel need to prevent workplace contamination by all means necessary. The usage of qPCR instead of classic PCR can take away a big part of those risks.

With qPCR, there is no need to 'open' the PCR-plate after the amplification reaction is complete. This greatly reduces the risk of contamination. With qPCR, the results are shown by the machine directly as a digital value. For the user, the result is a chart where a curve shows whether a sample is positive of negative for the pathogen. With each PCR-cycle, the amount of DNA doubles. The earlier the curve starts to form, the higher the amount of the targeted genetic material that was present in the sample.



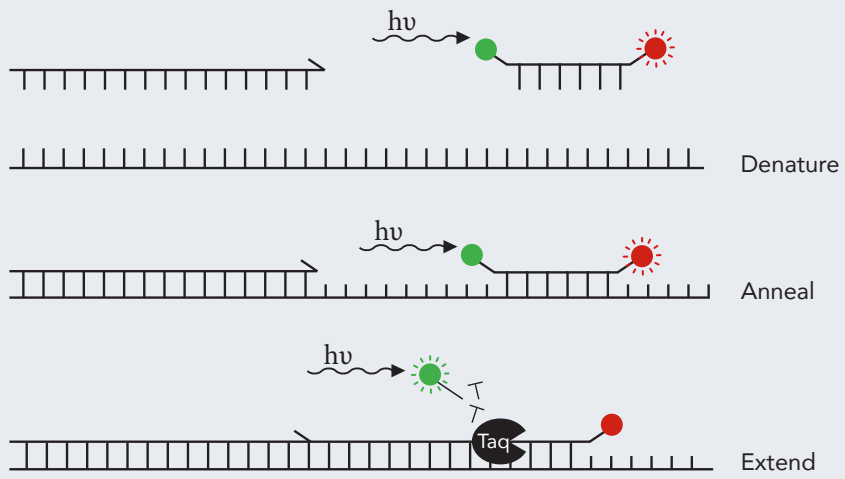
Classic PCR vs. real time PCR

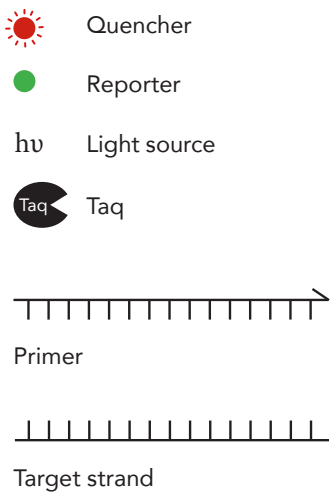
If your company chooses PCR as a pathogen detection method, there is the division between classic and real time PCR (qPCR). With real time PCR you can detect the pathogen's presence without the need for lengthy after-run-procedures. This process is automated for the most part. With classic PCR, you have to detect the PCR product (the result of the reaction) by running it across a gel. This gel has been stained with a dye that will detect PCR-products after exposure to a UV-light. This process is a proven PCR-method, but it is time-consuming. In addition, ethidium bromide, which is the active fluorescent chemical often used to indicate the different PCR-products, is very toxic and hazardous. Where classic PCR starts with the base components mastermix and primers, qPCR adds the usage of 'probes', which allows for real time data acquisition during the PCR-run. Fluorescence is generated by the probes during the PCR-process if the targeted DNA or RNA is present in the sample. If the target is not present, there will be no fluorescence. The following image shows how the addition of a probe to the PCR-reaction allows for real time measurements. An intact probe gives no signal during the PCR reaction. The probe gets destroyed if the pathogen is present, resulting in fluorescent signal.

One of the main advantages of using real time PCR for your research, is that you no longer need to use time-consuming gel electrophoresis. This saves time and minimizes the risk of health hazards. In addition, qPCR allows detection of several different pathogens at the same time, which saves even more time.

The qPCR-process is also fully automatable: the digital results (QC values) can be imported in software or exported to other systems.

Real time PCR is performing PCR while gathering data -probe / beacon used for amplification detection





BEFORE YOU CAN DETECT DNA OR RNA IT SHOULD BE EXTRACTED FROM THE SAMPLE.

Extraction Methods
To extract DNA or RNA from the sample material, there are several methods. Mainly, there is the division between manual and automated methods. One of the advantages of automated methods is that they give reproducible results. It also saves a significant amount of time and allows for high throughput processing of samples. The most frequently used manual method is the Spin Column extraction method. In automated methods, magnetic beads are used (BOOM Method).

Why BioChek?
With the BioChek PCR kits, you can perform all the assays at the same time on one plate. With many PCR providers you can't execute your Salmonella detection at the same time as your Mycoplasma detection. With the BioChek PCR kits, every assay is executed on the same protocol. This way, you can save precious time and work more efficient. In addition, both RNA and DNA assays can be combined with the BioChek kits.

Save time with real time PCR and reduce the health hazards by automating your PCR process and rejecting the usage of hazardous chemicals.

In addition, the BioChek-software helps with sample setup, running PCR, and data collection, analysis and reporting; all in one program.

This way the BioChek PCR-software saves even more time, by streamlining the PCR process in a digital manner (see page 11 for more information) This makes for a good traceability of results in the lab. So say goodbye to inconvenient paper results or spreadsheets!

ARE YOU CURIOUS HOW YOUR COMPANY CAN BENEFIT FROM THE PCR-SOFTWARE?

BioChek is an expert in delivering PCR-kits and automating your processes with the BioChek Monitoring Software

Visit our website at **www.biochek.com**

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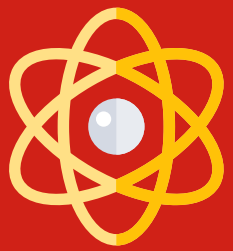
APPENDIX

PCR: from the decision to perform the BioChek PCR assays at your lab, to the first PCR result.



The decision to start performing PCR can have large consequences for your lab. A lot of questions need to be answered and steps need to be taken before the first results will come from the lab. BioChek has highly qualified experts that can help you with all these questions and steps. From lab setup to the needed equipment: BioChek will gladly share their knowledge and assist with the implementation of molecular diagnostics at your lab.

The first question will be if other techniques such as end-point PCR (classic PCR) will be performed at your lab next to the BioChek Real-Time PCR kits. If so there will need to be a separate room to allow this.

How to setup a PCR Lab (classic PCR)

Performing End Point PCR requires gel electrophoresis. This is a step for which it is required to open the PCR plate that contains all the amplified PCR products. This has to be considered when building the lab. As said, contamination is a big risk that must not be ignored. To prevent contamination, you have to maintain strict rules. It is best to physically divide all your reaction stages in your lab-setup.

The reaction stages are:

1. Preparation of reaction components
2. DNA/RNA extraction and PCR setup (adding DNA/RNA to the plate)
3. Running the PCR (amplification)

Room 1

First, you have a room for the preparation of your reagents. This is a clean room often called PCR Room 1, where no positive material should be present anywhere. This includes positive kit controls.

Room 2

In the second room, often called PCR Room 2, the DNA/RNA extraction and PCR setup takes place. You use the reaction material combined with the acquired DNA/RNA, that has to be extracted from the sample material. The positive kit controls should be stored in this PCR room. If your lab will only run Real-Time PCR, the thermocycler can be placed here.

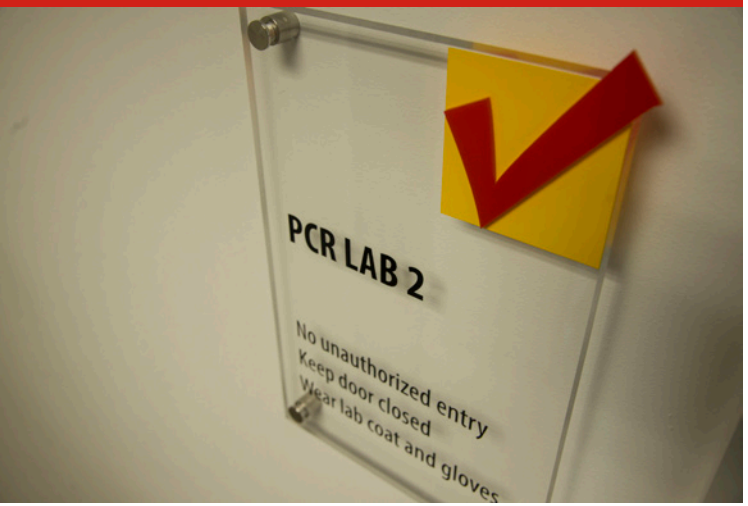
Room 3 (optional)

In the case of end point (classic) PCR, there will have to be a third space, PCR Room 3, where the gel electrophoresis is performed. This isn't needed when performing real time PCR (but it is still nice to have).

THE IDEAL SITUATION FOR A PCR LAB:

- Two (or three for End-Point PCR) separated airspaces
- Use of air locks for all rooms
- Separate airconditioning
- No angular finishes on the walls and the floors, so no dirt can stay behind.

If other activities take place in the lab (serology, culture) this has to be taken into consideration when placing the PCR rooms. Consult the PCR technical staff at BioChek for information and help when designing your PCR labs.



Logistics when running PCR assays every day
When running the same PCR every day there is always the risk of contamination. It is nothing to be worried about if you follow some basic rules and stick to a good cleaning procedure.

Equipment
Make sure every PCR room has its own equipment. Once the equipment has been placed, label it and never move it between the different labs. Every room gets a separate set of pipets, a separate spinner, separate lab coats, and so on.

Workflow
Running PCR as a routine test requires some planning. Staff cannot enter PCR 1 once they have been in room 2 or room 3. BioChek reaction mix is stable for 4-5 hours at 4 degrees Celsius. This allows the tech to prepare the reaction mix in Room 1, extract the DNA in Room 2 and run the PCR after the extraction process is complete.

Separate boxes
To help in the prevention of contamination BioChek has designed an easy to use kit box where the Room 1 and Room 2 components are separated by placing them in separate boxes. This allows the technician to store the components in the correct room without the need to hunt for other boxes and to write down all the batch information.

Training of staff to run PCR assays
If technicians are new to molecular diagnostics some training will be needed for them to run the PCR assays in a routine setting.

The technicians will have to get used to the rules that are in place for a PCR lab and to all the new equipment, especially the thermocycler, and the software that comes with it. BioChek can assist on site with training and assay validation by sending one of our PCR specialists over to help with training of the staff, and running the first tests.

Twice a year BioChek organizes a lab training for both ELISA and PCR at the head office in the Netherlands. These trainings are beginner/intermediate level and a nice opportunity to learn more about lab techniques and the people behind the BioChek assays people use every day all over the world. More advanced training is available on request.

During these trainings emphasis will also be on the BioChek software that can be of great help with running ELISA and PCR tests.
www.biochek.com/lab-trainings

BioChek PCR Software

PCR can be virtually fully automated by the usage of the BioChek II software. It helps with running PCR and sample setup. The BioChek II software automatically checks the PCR run using the PCR controls. There is no need to manually create a plate-lay-out, calculate the amount of reagents or to program the thermocycler. The software collects and analyses the data from the thermocycler and generates easy to read PCR-reports, all in one program. This takes away the struggle of copying results and other information to your Excel-sheets. It makes for good traceability of results, which is convenient for both the technician and the vet.

In a nutshell, performing PCR can be divided into six steps.

1. Creating a worklist/Plate layout
2. Preparation of the reaction mix
3. DNA/RNA extraction
4. Adding DNA/RNA extraction to reaction mix
5. Running PCR on thermocycler
6. Analysing results – creating reports

The software can automate and/or assist step 1, 2, 3, 4 and 6, and will save you lots of time and unfortunate errors in the process.

Note: not all thermocyclers are compatible with the software. Ask your BioChek-representative for more information.

Recap

Once all necessary steps have been taken, equipment has been installed and the staff has been trained, you can start running PCR tests in a routine setting. BioChek is constantly looking for ways to make life easier for the lab. This can be in the form of new assays or new software options. Assays undergo a check on regular basis in order to secure the correct detection of the pathogens.

NEED HELP OR INFORMATION? BIOCHEK IS JUST A PHONE CALL OR E-MAIL AWAY.

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